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1 Bacteriohopanepolyols in tropical soils and sediments in the Congo

2 River catchment area

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21 ABSTRACT

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

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

34

35 Key words

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

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43 **1. Introduction**

44 Bacteriohopanepolyols (BHPs) are highly functionalised pentacyclic triterpenoids produced
45 by many aerobic bacteria, as well as a number of obligate and facultative anaerobic bacteria
46 (e.g. Rohmer et al., 1984; Sinninghe Damsté et al., 2004; Talbot et al., 2008; Eickhoff et al.,
47 2013). Only bacteria containing the gene encoding for squalene hopane cyclase (*sqhC*;

48 Ochs et al., 1992) are able to biosynthesise hopanoids. Biosynthesis of BHPs is believed to
49 be limited to < 10% of all bacterial species in most communities (Pearson et al., 2007). The
50 initial step in BHP synthesis is the cyclisation of squalene (controlled via the *sqhC* gene) with
51 the addition of the hopanoid side chain (via the *hpnH* gene) and leading to the production of
52 30-(5'-adenosyl)hopane (adenosylhopane; **1a**; Fig. 1; Bradley et al., 2010). It is believed that
53 all hopanoid producing bacteria synthesise adenosylhopane as a BHP precursor compound,
54 however, few hopanoid producers have been observed accumulating adenosylhopane and
55 only one species has been found to contain the related compound adenosylhopane type 2
56 (**1c**) (e.g. Talbot et al., 2007 and references therein; van Winden et al., 2012a). All species in
57 which adenosylhopane has been identified were also found to contain a range of other BHPs
58 including bacteriohopane-32,33,34,35-tetrol (BHT), 35-aminobacteriohopane-32,33,34-triol
59 (aminotriol) or both (Talbot et al., 2007a, 2008; van Winden et al., 2012b). These and other
60 BHPs are formed following cleavage of the adenine moiety (Bradley et al., 2010; Liu et al.,
61 2014), however, it is currently unknown, why accumulation of adenosylhopane only occurs in
62 terrestrial systems (soils in particular), and not in marine sediments. This suggests that the
63 function of adenosylhopane is not restricted to that of a biosynthetic precursor or it would
64 likely accumulate in all settings.

65 While many BHPs have been identified as having multiple bacterial sources, for example
66 BHT (**1b**; Fig 1; e.g. Talbot et al., 2008 and references therein), some have only a few
67 sources and can be linked to specific biogeochemical processes. Adenosylhopane (**1a**) and
68 related compounds, including C-2 methylated homologues (**2a**, **1c**, **2c**, **1d** and **2d**), have
69 been suggested to be biomarkers for soil organic carbon (OC) transport (Cooke et al., 2008b,
70 2009; Zhu et al., 2011; Doğrul Selver et al., 2012, 2015). Another group of diagnostic
71 markers are those produced by aerobic methane oxidising bacteria (methanotrophs)
72 including 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol; **1e**); 35-
73 aminobacteriohopane-30,31,32,33,34-pentol (aminopentol; **1f**), unsaturated aminopentol

74 **(4/5f)** and aminopentol isomer (**1f'**; e.g. Talbot and Farrimond, 2007; Zhu et al., 2010; van
75 Winden et al., 2012b; Berndmeyer et al., 2013; Talbot et al., 2014).

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

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

100 River catchment. However, a recent study of two surface soils and three surface wetland
101 sediments from the Amazon found the highest BHP concentrations and greatest structural
102 diversity within wetland sediments (18 BHPs in sediments vs. 13 in the soils; Wagner et al.,
103 2014), suggesting wetlands as possibly a significant source of BHPs to shelf and fan
104 systems. As tropical wetlands and soils are largely understudied, large uncertainty in BHP
105 end members likely exists.

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

115

116 **2. Material and methods**

117 *2.1. Site location and sample description*

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

121 Details of the soil and Malebo pool sample collection have been reported (Talbot et al.,
122 2014). Briefly, soil samples were collected from 22 sites spanning a wide range of land cover
123 types, ranging from scrub savannah and grasslands, secondary forest and pristine tropical
124 mixed forest, to seasonally flooded and swamp forest environments within the Congo Basin
125 (Fig. 2). Surface soil samples (0-5 cm) were collected in November 2010 and August 2011.

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

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

135 2.2. *pH*

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

142 2.3. *Total OC (TOC)*

143 TOC (%) of the soils and Malebo Pool samples was measured at Newcastle University.
144 Approximately 0.1 g of sample was treated with 4 mol/L HCl (60-70 °C) for removal of
145 inorganic carbon. Following which, HCl was allowed to drain from each sample. Deionised
146 water was added to each sample to neutralise the acid and allowed to drain. The samples
147 were then dried in an oven at 65 °C for between 16 and 24 hours. TOC was measured using
148 a LECO CS244 Carbon/Sulfur Analyser. Precision based on repeat sample analysis was
149 4.5 % (relative standard deviation). Accuracy based on repeated measurements of a
150 standard reference material (Chinese stream sediment, NCS DC 73307; LGC, Teddington,
151 UK) was within the permissible ± 0.05 % TOC. An instrument calibration standard (Carbon in

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

154 2.4. Lipid extraction

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

169 2.5. BHP analysis

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

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

178 mm; 3.0 mm i.d.; 5 μ m particle size) and a security guard column cartridge of the same
179 material coupled to a Finnigan LCQ ion-trap mass spectrometer equipped with an APCI
180 source operated in positive ion mode. Chromatographic separation was accomplished at
181 30 $^{\circ}$ C at 0.5 ml/min with the following solvent gradient: 90% MeOH, 10% H₂O (0 min); 59%
182 MeOH, 1% H₂O, 40% propan-2-ol (at 25 min); isocratic to 45 min returning to the starting
183 conditions in 5 min and stabilising for 10 min. APCI was achieved at 155 $^{\circ}$ C capillary
184 temperature and 490 $^{\circ}$ C APCI vaporiser temperature with a corona discharge current of 8
185 μ A, and sheath and auxiliary gas flow of 40 and 10, respectively (arbitrary units). MSⁿ
186 analysis was carried out in data-dependent mode with three scan events: SCAN 1: full
187 spectrum, m/z 300–1300; SCAN 2: data-dependent MS² spectrum of most intense ion from
188 SCAN 1; SCAN 3: data-dependent MS³ spectrum of most intense ion from SCAN 2.
189 Detection was achieved at an isolation width of m/z 5.0 and fragmentation with normalised
190 collision dissociation energy of 35% and an activation Q value (parameter determining the
191 m/z range of the observed fragment ions) of 0.15. Semi-quantitative estimation of BHP
192 concentration was achieved employing the characteristic base peak ion areas of individual
193 BHPs in mass chromatograms (from SCAN 1) relative to the m/z 345 mass chromatogram
194 base peak area of the acetylated 5 α -pregnane-3 β ,20 β -diol internal standard. Averaged
195 relative response factors relative to the internal standard, determined from a suite of
196 acetylated BHP standards, were used to adjust the BHP peak areas (see van Winden et al.,
197 2012b). Typical error in absolute quantification was \pm 20%, based on selected replicate
198 analyses and BHP standards of known concentration (Cooke, 2010; van Winden et al.,
199 2012b).

200 2.6. *Compound classification and statistics*

201 The abbreviated names of the compounds, characteristic base peak ions (m/z) and structure
202 numbers are given in Table 1. The term tetrafunctionalised compounds refers to BHPs with
203 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. Spearman's rho (r_s) was calculated using IBM SPSS
228 statistics version 21 software. Strong correlation between two variables would result in an r_s

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

233

234 3. Results

235 3.1. TOC and soil pH

236 TOC and soil pH values are presented in Table 2. TOC ranged from 0.23-6.11% in the soils
237 and 1.10-2.68 % in wetland sediment samples; pH ranged from 3.09-5.75 for soils and 4.27-
238 4.8 for wetland sediments (not measured for recently exposed sediment 0-5 and the estuary
239 sample due to insufficient sample material).

240 3.2. BHPs in Congo soils

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

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

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

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

265 3.3. *BHPs in wetland sediments*

266 A total of 19 BHPs were found in the 6 wetland sediments. BHP concentration within the
267 wetland samples ranged between 4300 $\mu\text{g/g}$ TOC (recently exposed surface and sub-
268 surface sample, RE 0-5 and RE 5-15) and 7500 $\mu\text{g/g}$ TOC (permanently submerged sub
269 surface sample, PS 5-15). Aminopentol (**1f**) and adenosylhopane (**1a**) were the dominant
270 BHPs along with BHT cyclitol ether (**1h**) and aminotriol (**1g**) (Table 3). The wetland
271 sediments also contained other CH_4 oxidation markers, including aminotetrol (**1e**),
272 aminopentol isomer (**1f'**) and unsaturated aminopentol (**4/5f**: reported by Talbot et al., 2014;
273 Table 5).

274 Concentration of soil marker BHPs ranged from 620 $\mu\text{g/g}$ TOC (exposed with occasional
275 flooding sub surface sample; EF 5-15) to 1100 $\mu\text{g/g}$ TOC (PS 5-15). Relative abundance of
276 soil marker BHPs ranged from 11-17% of total BHPs. R_{soil} index ranged from 0.61-0.66 (avg.
277 0.63; Fig. 3) and the R'_{soil} index from 0.59-0.62 (avg. 0.60) (Table 2).

278 3.4. *Estuarine sediment*

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

283

284 **4. Discussion**

285 4.1. *BHP distributions*

286 The soils were dominated by non-source specific BHPs (Tables 3-5). Greater BHP diversity
287 was found within soils vs. the wetland and estuarine samples, consistent with other studies
288 (e.g. Pearson et al., 2009; Zhu et al., 2011). BHT cyclitol ether (**1h**) was one of the most
289 dominant BHPs in the soils, wetlands and the estuarine sediments. Studies have shown that,
290 within surface soils where aerobic methane oxidation (AMO; as indicated by aminopentol; **1f**)
291 is not a dominant process, aminotriol (**1g**), BHT cyclitol ether, BHT (**1b**) and adenosylhopane
292 (**1a**) are usually the dominant compounds (Cooke et al., 2008a; Pearson et al., 2009; Cooke,
293 2010; Zhu et al., 2011). Low concentrations of anhydroBHT (**1m**), ribonylhopane (**1k**) and
294 BHT-pseudopentose (methylated, **2l** and non-methylated, **1l**) were also present in the soils
295 (Tables 3 and 4); however, these compounds are not discussed further due to their
296 intermittent occurrence and typically low concentration.

297 4.2. *Soil marker BHPs*

298 A range of soil marker BHP relative abundance was observed for soils (7.9-36% of total
299 BHPs) and wetland sediments (11-17% of total BHPs; Fig. 4). However, the Congo soils had
300 a low mean soil marker BHP abundance of 16% for forest soils (n=16) and 19% for
301 savannah/field soils (n=6) compared with samples from other studies (Table 6). Surface soils
302 from temperate regions show a wider range of soil marker BHP relative abundance (0-66%;
303 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

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

334 The R_{soil} (Zhu et al., 2011; Doğrul Selver et al., 2012) and GDGT based BIT (Hopmans et al.,
335 2004) indices have both been proposed as proxies for soil OC transport. Previous analysis
336 of surface sediments from river-estuary-shelf/ocean transects have identified correlation
337 between R_{soil} and BIT indices (Zhu et al., 2011; Doğrul Selver et al., 2012; 2015. Other
338 studies, however, have not found any correlation between soil marker BHP concentrations or
339 the $R_{\text{soil}} / R'_{\text{soil}}$ and BIT indices (Kim et al., 2011; Wagner et al., 2014). The absence of
340 correlation between these two proxies in terrestrial sources materials (soils, peat) is not
341 unexpected, however, as it is well established that soil BHP distributions contain variable
342 concentrations of the pseudo-marine endmember BHT (e.g. Cooke et al., 2008; Xu et al.,
343 2009; Rethemeyer et al., 2010; Kim et al., 2011) whilst most soils contain little if any
344 crenarchaeol (the marine endmember for the BIT index; Schouten et al., 2013). This is a
345 prominent issue with using BHT as a marine end member in soil OM proxies. Furthermore,
346 relatively little is known about possible marine sources of BHT other than some species of
347 sulfate reducing bacteria (e.g. Blumenberg et al., 2006). Lack of correlation between these
348 two proxies in certain environments could be due to (1) terrestrial end member biomarkers
349 synthesised by microbial organisms living in different environmental niches, for example at
350 different depths in the soil profile (Kim et al., 2011); and (2) variation in (post-depositional)
351 degradation of terrestrial end member biomarkers due to the differences in compound
352 reactivities (e.g. Zhu et al., 2013). As BHT and adenosylhopane have different reactivity and
353 therefore may degrade at different rates upon deposition (e.g. Cooke et al., 2008; Handley et
354 al., 2010), this suggests, at least in some settings, that the R_{soil} could instead be used to
355 describe relative rates of degradation.

356 4.3. *Biomarkers for aerobic methane oxidation*

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

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

391 4.4. BHP reservoirs

392 The Congo River drains the second largest basin in the world (~3.7 x 10⁶ Km²). Soil derived
393 OM is an important component of sediments deposited on the Congo fan (Holtvoeth et al.,
394 2005). The organic fraction of ODP 1075 sediments relates to strongly degraded SOM of old
395 highly developed, Kaolinite-rich feralitic soils (Oxisols) that cover large areas of the Congo
396 river basin (Holtvoeth et al., 2005). The OC from the soils analysed in this study is
397 transported through the Congo River and deposited in Malebo pool (Hughes et al., 2011;
398 Spencer et al., 2012). Previous work has shown that OM exported from Malebo Pool is
399 geochemically similar to OM at the head of the estuary (ca. 350 km downstream) and no
400 major tributaries join the Congo River between this site and the Atlantic Ocean (Spencer et
401 al., 2012). Similarity between the spread in R_{soil} indices for the soils and Malebo pool (Fig. 3)
402 further suggests that BHPs are also subject to this transport mechanism. Due to the position
403 of Malebo pool in the Congo River, OM and therefore BHPs signatures in the wetlands are
404 representative of BHPs from the Congo watershed (Hughes et al., 2011; Spencer et al.,
405 2012). Therefore, a terrestrial R_{soil} endmember of 0.63 (Malebo pool mean; Table 6) is
406 representative of fluvially transported soils within the Congo watershed in combination with
407 BHPs produced in Malebo Pool. Sediments deposited at Malebo pool are flushed into the
408 estuary and then on to the Congo shelf and fan. As only one grab sample from the estuary
409 was analysed in this study, the reported R_{soil} value of 0.2 (Table 6) may not represent the

410 true mean of the Congo estuary. However, BHT and adenosylhopane concentrations for
411 ODP 1075 have previously been reported by Handley et al. (2010). Calculation of the R_{soil}
412 index for sediments between 10 and 100 Ka ($n = 27$; Appendix II) show the R_{soil} index for the
413 estuary is within the range of 0.16-0.54 (interglacial 0.16-0.54; glacial 0.21-0.52) for ODP
414 1075 sediments (Fig. 3). The mean R_{soil} index for ODP 1075 is 0.37 which is approximately
415 half of the terrestrial end-member of Malebo pool, suggesting, that soil OM is a significant
416 contributor to marine OM. This is in accordance with other studies from the Congo deep-sea
417 fan. Holtvoeth et al. (2003) used a binary mixing model approach to determine that between
418 18 and 61% of bulk OM in ODP 1075 is of continental origin. Similarly, Weijers et al. (2009)
419 used a 3 end-member mixing model to determine that between 38 and 52 % of OC within
420 GeoB 6518-1 is of terrestrial (soil) origin.

421 Furthermore, strong similarities are found between the distribution of BHPs identified in the
422 soils, wetlands, estuarine and ODP 1075 samples (Fig. 5 a,b). A suite of common BHPs are
423 identified in the forest and savannah/field soils, and the wetlands, with more than half of the
424 BHPs identified in the hinterland soils also identified in the wetlands. In addition, the
425 common BHPs identified in the hinterland soils and the wetlands represent a major
426 component of the soil BHP profile, contributing an average of 88% (forests) and 94%
427 (savannah/field) of total BHPs (based on concentration; Fig. 5a). Lower BHP diversity is
428 reported for samples from the Congo fan (Handley et al., 2010; Talbot et al., 2014) with
429 many of the methylated and pentose compounds below detection limit. Between 7 and 10 of
430 the 12 BHP compounds identified in ODP 1075 are also found in the wetlands and soils, and
431 are again a major component of all of the BHP profiles representing over 90% of the total
432 BHPs found in the wetland and estuary samples (Fig. 5b). Strong similarities between BHPs
433 identified in the Congo hinterland and wetland samples and those identified in ODP 1075
434 suggests a link between BHP reservoirs. High concentrations of aminotetrol and
435 aminopentol (including aminopentol isomer and unsaturated aminopentol) found in ODP
436 1075 sediments have previously been linked to fluvial transport of these biomarkers to the

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

444 4.5. *Trends in global BHP distribution*

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

460 The differences may suggest that the main factors controlling BHP distributions in tropical
461 climate zones are different from those from temperate and polar climate zones. Kim et al.
462 (2011) found mean annual air temperature (MAAT) and precipitation to influence soil marker

463 BHP distribution in samples from the Mediterranean Têt watershed. Soils from the
464 watershed were collected along a transect with strong environmental contrasts in altitude,
465 MAAT, precipitation and a wide pH range, including some low pH peat samples. Kim et al.
466 (2011) found that the lowest relative abundance of adenosylhopane (the dominant soil
467 marker BHP) occurred at low altitude where MAAT was high, pH more alkaline and
468 precipitation lowest. This could suggest that, during BHP synthesis, adenosylhopane (an
469 intermediate in hopanoid biosynthesis; Bradley et al., 2010) is converted to other BHPs
470 when environmental conditions are favourable for microbial activity (e.g. warmer).

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

478

479 **5. Conclusions**

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

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

489 19% of total BHPs in savannah/field soils) was lower than values for temperate and Arctic
490 surface soils calculated from the available literature data. R_{soil} and R'_{soil} indices for the soils
491 show a large range of 0.58-0.92 and 0.48-0.91, respectively, with savannah/field samples
492 typically showing greater variation than forest soils. This is in accord with other R_{soil} and R'_{soil}
493 values calculated from the literature and reinforces the need for local end members to be
494 determined before any interpretation of the index values is undertaken.

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506

507 **References**

- 508 Berndmeyer, C., Thiel, V., Schmale, O., Blumenberg, M., 2013. Biomarkers for aerobic
509 methanotrophy in the water column of the stratified Gotland Deep (Baltic Sea).
510 *Organic Geochemistry* 55, 103-111.
- 511 Blumenberg, M., Hoppert, M., Krüger, M., Dreier, A., Thiel, V., 2012. Novel findings on
512 hopanoid occurrences among sulfate reducing bacteria: Is there a direct link to
513 nitrogen fixation? *Organic Geochemistry* 49, 1-5.

514 Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H.M., Oppermann, B.I., Seifert, R., Pape,
515 T., Michaelis, W., 2006. Biosynthesis of hopanoids by sulphate-reducing bacteria
516 (genus *Desulfovibrio*). *Environmental Microbiology* 8, 1220-1227.

517 Blumenberg, M., Mollenhauer, G., Zabel, M., Reimer, A., Thiel, V., 2010. Decoupling of bio
518 and geohopanoids in sediments of the Benguela Upwelling System (BUS). *Organic*
519 *Geochemistry* 41, 1119-1129.

520 Bradley, A.S., Pearson, A., Sáenz, J.P., Marx, C.J., 2010. Adenosylhopane: The first
521 intermediate in hopanoid side chain biosynthesis. *Organic Geochemistry* 41, 1075-
522 1081.

523 BS ISO 10390., 2005. Soil quality. Determination of pH. BSI, ISBN 0 580 45659 5.

524 Cooke, M.P., 2010. The Role of Bacteriohopanepolyols as Biomarkers For Soil Bacterial
525 Communities and Soil Derived Organic Matter. PhD Thesis, Newcastle University.

526 Cooke, M.P., Talbot, H.M., Farrimond, P., 2008a. Bacterial populations recorded in
527 bacteriohopanepolyol distributions in soils from Northern England. *Organic*
528 *Geochemistry* 39, 1347-1358.

529 Cooke, M.P., Talbot, H.M., Wagner, T., 2008b. Tracking soil organic carbon transport to
530 continental margin sediments using soil-specific hopanoid biomarkers: A case study
531 from the Congo fan (ODP site 1075). *Organic Geochemistry* 39, 965-971.

532 Cooke, M.P., van Dongen, B.E., Talbot, H.M., Semiletov, I., Shakhova, N., Guo, L.,
533 Gustafsson, Ö., 2009. Bacteriohopanepolyol biomarker composition of organic matter
534 exported to the Arctic Ocean by seven of the major Arctic rivers. *Organic*
535 *Geochemistry* 40, 1151-1159.

536 Coolen, M.J.L., Talbot, H.M., Abbas, B.A., Ward, C., Schouten, S., Volkman, J.K., Sinninghe
537 Damsté, J.S., 2008. Sources for sedimentary bacteriohopanepolyols as revealed by
538 16S rDNA stratigraphy. *Environmental Microbiology* 10, 1783-1803.

- 539 Cvejic, J.H., Putra, S.R., El-Beltagy, A., Hattori, R., Hattori, T., Rohmer, M., 2000. Bacterial
540 triterpenoids of the hopane series as biomarkers for the chemotaxonomy of
541 *Burkholderia*, *Pseudomonas* and *Ralstonia* spp. FEMS Microbiology Letters 183,
542 295-299.
- 543 Doğrul Selver, A., Talbot, H.M., Gustafsson, Ö., Boulton, S., van Dongen, B.E., 2012. Soil
544 organic matter transport along an sub-Arctic river-sea transect. Organic
545 Geochemistry 51, 63-72.
- 546 Doğrul Selver, A., Sparkes, R.B., Bischoff, J., Talbot, H.M., Gustafsson, Ö., Semilitov, I.,
547 Dudarev, O., Boulton, S., van Dongen, B.E., 2015. Distributions of bacterial and
548 archaeal membrane lipids in surface sediments reflect differences in input and loss of
549 terrestrial organic carbon along a cross-shelf Arctic transect. Organic Geochemistry
550 83-84, 16-26.
- 551 Eickhoff, M., Birgel, D., Talbot, H.M., Peckmann, J., Kappler, A., 2013. Bacteriohopanoid
552 inventory of *Geobacter sulfurreducens* and *Geobacter metallireducens*. Organic
553 Geochemistry 58, 107-114.
- 554 Eisma, D., Kalf, J., van Der Gaast, S.J., 1978. Suspended matter in the Zaire estuary and
555 the adjacent Atlantic Ocean. Netherlands Journal of Sea Research 12, 382-406.
- 556 Handley, L., Talbot, H.M., Cooke, M.P., Anderson, K.E., Wagner, T., 2010.
557 Bacteriohopanepolyols as tracers for continental and marine organic matter supply
558 and phases of enhanced nitrogen cycling on the late Quaternary Congo deep sea fan.
559 Organic Geochemistry 41, 910-914.
- 560 Henckel, T., Jackel, U., Conrad, R., 2001. Vertical distribution of the methanotrophic
561 community after drainage of rice field soil. FEMS Microbiology Ecology 34, 279-291.

562 Holtvoeth, J., Kolonic, S., Wagner, T., 2005. Soil organic matter as an important contributor
563 to late Quaternary sediments of the tropical West African continental margin.
564 *Geochimica et Cosmochimica Acta* 69, 2031-2041.

565 Holtvoeth, J., Wagner, T., Schubert, C.J., 2003. Organic matter in river-influenced
566 continental margin sediments: The land-ocean and climate linkage at the Late
567 Quaternary Congo fan (ODP Site 1075). *Geochemistry Geophysics Geosystems*.

568 Hopmans, E.C., Weijers, J.W.H., Schefuß, E., Herfort, L., Sinninghe Damsté, J.S., Schouten,
569 S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched
570 and isoprenoid tetraether lipids. *Earth and Planetary Science Letters* 224, 107-116.

571 Hughes, H.J., Sondag, F., Cocquyt, C., Laraque, A., Pandi, A., Andre, L., Cardinal, D., 2011.
572 Effect of seasonal biogenic silica variations on dissolved silicon fluxes and isotopic
573 signatures in the Congo River. *Limnology and Oceanography* 56, 551-561.

574 Kim, J.H., Talbot, H.M., Zarzycka, B., Bauersachs, T., Wagner, T., 2011. Occurrence and
575 abundance of soil-specific bacterial membrane lipid markers in the Têt watershed
576 (southern France): Soil-specific BHPs and branched GDGTs. *Geochemistry
577 Geophysics Geosystems* 12, DOI 10.1029/2010gc003364.

578 Kulkarni, G., Wu, C.H., Newman, D.K., 2013. The general stress response factor EcfG
579 regulates expression of the C-2 hopanoid methylase HpnP in *Rhodopseudomonas*
580 *palustris* TIE-1. *Journal of Bacteriology* 195, 2490-2498.

581 Liu, W., Sakr, E., Schaeffer, P., Talbot, H.M., Kannenberg, E., Donisi, J., Poralla, K., Takano,
582 E., Rohmer, M., 2014. Ribosylhopane, a novel bacterial hopanoid, as precursor of
583 C35 bacteriohopanepolyols in *Streptomyces coelicolor* A3(2). *ChemBiochem.* 15,
584 2156-2161.

585 Neunlist, S., Rohmer, M., 1985. Novel hopanoids from the methylotrophic bacteria
586 *Methylococcus capsulatus* and *Methylomonas methanica* - (22S)-35-

587 Aminobacteriohopane-30,31,32,33,34-pentol and (22S)-35-amino-3- β -
588 methylbacteriohopane-30,31,32,33,34-pentol. *Biochemical Journal* 231, 635-639.

589 Ochs, D., Kaletta, C., Entian, K.D., Beck-Sickinger, A., Poralla, K., 1992. Cloning, expression,
590 and sequencing of squalene-hopene cyclase, a key enzyme in triterpenoid
591 metabolism. *Journal of Bacteriology* 174, 298-302.

592 Pearson, A., Leavitt, W.D., Sáenz, J.P., Summons, R.E., Tam, M.C.M., Close, H.G., 2009.
593 Diversity of hopanoids and squalene-hopene cyclases across a tropical land-sea
594 gradient. *Environmental Microbiology* 11, 1208-1223.

595 Pearson, A., Page, S.R.F., Jorgenson, T.L., Fischer, W.W., Higgins, M.B., 2007. Novel
596 hopanoid cyclases from the environment. *Environmental Microbiology* 9, 2175-2188.

597 Poger, D., Mark, A.E., 2013. The relative effect of sterols and hopanoids on lipid bilayers:
598 When comparable is not identical. *Journal of Physical Chemistry B* 117, 16129-
599 16140.

600 Poralla, K., Hartner, T., Kannenberg, E., 1984. Effect of temperature and pH on the
601 hopanoid content of *Bacillus acidocaldarius*. *FEMS Microbiology Letters* 23, 253-256.

602 Redshaw, S.E., Cooke, M.P., Talbot, H.M., McGrath, S., Rowland, S.J., 2008. Low
603 biodegradability of fluoxetine HCl, diazepam and their human metabolites in sewage
604 sludge-amended soil. *Journal of Soils and Sediment* 8, 217-230.

605 Rethemeyer, J., Schubotz, F., Talbot, H.M., Cooke, M.P., Hinrichs, K.U., Mollenhauer, G.,
606 2010. Distribution of polar membrane lipids in permafrost soils and sediments of a
607 small high Arctic catchment. *Organic Geochemistry* 41, 1130-1145.

608 Rohmer, M., Bouviernave, P., Ourisson, G., 1984. Distribution of hopanoid triterpenes in
609 prokaryotes. *Journal of General Microbiology* 130, 1137-1150.

610 Rohmer, M., Knani, M., Simonin, P., Sahm, H., 1993. Isoprenoid biosynthesis in bacteria: A
611 novel pathway for the early steps leading to isopentenyl diphosphate. *Biochemical*
612 *Journal* 295, 517-524.

613 Sáenz, J.P., Eglinton, T.I., Summons, R.E., 2011. Abundance and structural diversity of
614 bacteriohopanepolyols in suspended particulate matter along a river to ocean
615 transect. *Organic Geochemistry* 42, 774-780.

616 Schefuß, E., Versteegh, G.J.H., Jansen, J.H.F., Sinninghe Damsté, J.S., 2004. Lipid
617 biomarkers as major source and preservation indicators in SE Atlantic surface
618 sediments. *Deep-Sea Research Part I: Oceanographic Research Papers* 51, 1199-
619 1228.

620 Schmerk, C.L., Bernards, M.A., Valvano, M.A., 2011. Hopanoid production is required for
621 low-pH tolerance, antimicrobial resistance and motility in *Burkholderia cenocepacia*.
622 *Journal of Bacteriology* 193, 6712-6723

623 Schouten, S., Hopmans, E. C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of
624 glycerol dialkyl glycerol tetraether lipids: A review. *Organic Geochemistry* 54, 19-61.

625 Sinninghe Damsté, J.S., Rijpstra, W.I.C., Schouten, S., Fuerst, J.A., Jetten, M.S.M., Strous,
626 M., 2004. The occurrence of hopanoids in planctomycetes: implications for the
627 sedimentary biomarker record. *Organic Geochemistry* 35, 561-566.

628 Sparkes, R.B., Doğrul Selver, A., Bischoff, J., Talbot, H.M., Gustafsson, Ö., Semiletov, I.P.,
629 Dudarev, O.V., van Dongen, B.E., 2015. GDGT distributions on the East Siberian
630 Arctic Shelf: implications for organic carbon export, burial and degradation.
631 *Biogeosciences* 12, 3753-3768.

632 Spencer, R.G.M., Hernes, P.J., Aufdenkampe, A.K., Baker, A., Gulliver, P., Stubbins, A.,
633 Aiken, G.R., Dyda, R.Y., Butler, K.D., Mwamba, V.L., Mangangu, A.M.,

634 Wabakanghanzi, J.N., Six, J., 2012. An initial investigation into the organic matter
635 biogeochemistry of the Congo River. *Geochimica et Cosmochimica Acta* 84, 614-627.

636 Spencer, R.G.M., Stubbins, A., Gaillardet, J., 2014. Geochemistry of the Congo River,
637 estuary and plume. In: Bianchi, T.S., Allison, M.A., Cai, W.J. (Eds.), *Biogeochemical*
638 *Dynamics at Large River-Coastal Interfaces: Linkages with Global Climate Change*.
639 Cambridge University Press. pp. 554-583.

640 Talbot, H.M., Farrimond, P., 2007. Bacterial populations recorded in diverse sedimentary
641 biohopanoid distributions. *Organic Geochemistry* 38, 1212-1225.

642 Talbot, H.M., Watson, D.F., Murrell, J.C., Carter, J.F., Farrimond, P., 2001. Analysis of intact
643 bacteriohopanepolyols from methanotrophic bacteria by reversed-phase high-
644 performance liquid chromatography-atmospheric pressure chemical ionisation mass
645 spectrometry. *Journal of Chromatography A* 921, 175-185.

646 Talbot, H.M., Rohmer, M., Farrimond, P., 2007. Rapid structural elucidation of composite
647 bacterial hopanoids by atmospheric pressure chemical ionisation liquid
648 chromatography/ion trap mass spectrometry. *Rapid Communications in Mass*
649 *Spectrometry* 21, 880-892.

650 Talbot, H.M., Summons, R.E., Jahnke, L.L., Cockell, C.S., Rohmer, M., Farrimond, P., 2008.
651 Cyanobacterial bacteriohopanepolyol signatures from cultures and natural
652 environmental settings. *Organic Geochemistry* 39, 232-263.

653 Talbot, H.M., Handley, L., Spencer-Jones, C.L., Dinga, B.J., Schefuß, E., Mann, P.J.,
654 Poulsen, J.R., Spencer, R.G.M., Wabakanghanzi, J.N., Wagner, T., 2014. Variability
655 in aerobic methane oxidation over the past 1.2 Myrs recorded in microbial biomarker
656 signatures from Congo fan sediments. *Geochimica et Cosmochimica Acta* 133, 387-
657 401.

658 van Winden, J.F., Talbot, H.M., De Vleeschouwer, F., Reichart, G.J., Sinninghe Damsté, J.S.,
659 2012a. Variation in methanotroph-related proxies in peat deposits from Misten Bog,
660 Hautes-Fagnes, Belgium. *Organic Geochemistry* 53, 73-79.

661 van Winden, J.F., Talbot, H.M., Kip, N., Reichart, G.J., Pol, A., McNamara, N.P., Jetten,
662 M.S.M., den Camp, H., Sinninghe Damsté, J.S., 2012b. Bacteriohopanepolyol
663 signatures as markers for methanotrophic bacteria in peat moss. *Geochimica et*
664 *Cosmochimica Acta* 77, 52-61.

665 Weijers, J.W.H., Schouten, S., Schefuss, E., Schneider, R.R., Sinninghe Damsté, J.S., 2009.
666 Disentangling marine, soil and plant organic carbon contributions to continental
667 margin sediments: A multi-proxy approach in a 20 000 year sediment record from the
668 Congo deep-sea fan. *Geochimica et Cosmochimica Acta* 73, 119-132.

669 Welander, P.V., Hunter, R.C., Zhang, L., Sessions, A.L., Summons, R.E., Newman, D.K.,
670 2009. Hopanoids play a role in membrane integrity and pH homeostasis in
671 *Rhodopseudomonas palustris* TIE-1. *Journal of Bacteriology* 191, 6145-6156.

672 Wagner, T., Kallweit, W., Talbot, H.M., Mollenhauer, G., Boom, A., Zabel, M., 2014.
673 Microbial biomarkers support organic carbon transport from methane-rich Amazon
674 wetlands to the shelf and deep sea fan during recent and glacial climate conditions.
675 *Organic Geochemistry* 67, 85-98.

676 Xu, Y.P., Cooke, M.P., Talbot, H.M., Simpson, M.J., 2009. Bacteriohopanepolyol signatures
677 of bacterial populations in Western Canadian soils. *Organic Geochemistry* 40, 79-86.

678 Zhu, C., Talbot, H.M., Wagner, T., Pan, J.M., Pancost, R.D., 2010. Intense aerobic methane
679 oxidation in the Yangtze Estuary: A record from 35-aminobacteriohopanepolyols in
680 surface sediments. *Organic Geochemistry* 41, 1056-1059.

681 Zhu, C., Talbot, H.M., Wagner, T., Pan, J.M., Pancost, R.D., 2011. Distribution of hopanoids
682 along a land to sea transect: Implications for microbial ecology and the use of
683 hopanoids in environmental studies. *Limnology Oceanography* 56, 1850-1865.

684 Zhu, C., Wagner, T., Talbot, H.M., Weijers, J.W.H., Pan, J.-M., Pancost, R.D. 2013.
685 Mechanistic controls on diverse fates of terrestrial organic components in the East
686 China Sea. *Geochimica et Cosmochimica Acta* 117, 129-143.

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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.

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

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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
Anhydrobacteiohopanetetrol	AnhdroBHT	1m	613	[M+H] ⁺
Ribonylhopane	Ribonylhopane	1k	627	[M+H] ⁺
Bacteriohopane-32,33,34,35-tetrol	BHT	1b	655	[M+H-CH ₃ COOH] ⁺
2-methylbacteriohopane-32,33,34,35-tetrol	2-methylBHT	2b	669	[M+H-CH ₃ COOH] ⁺
Bacteriohopane-30,31,32,33,34,35-hexol	Bhhexol	1n	771	[M+H-CH ₃ COOH] ⁺
aminobacteriopenhene-32,33,34-triol	unsaturated aminotriol	4/5g	712	[M+H] ⁺
aminobacteriohopane-32,33,34-triol	aminotriol	1g	714	[M+H] ⁺
2-methylaminobacteriohopane-32,33,34-triol	2-methylaminotriol	2g	728	[M+H] ⁺
3-methylaminobacteriohopane-32,33,34-triol	3-methylaminotriol	3g	728	[M+H] ⁺
35-aminobacteriopenhene-31,32,33,34-tetrol	unsaturated aminotetrol	4/5e	770	[M+H] ⁺
35-aminobacteriohopane-31,32,33,34-tetrol	aminotetrol	1e	772	[M+H] ⁺
35-aminobacteriopenhene-30,31,32,33,34-pentol	unsaturated aminopentol	4/5f	828	[M+H] ⁺
35-aminobacteriohopane-30,31,32,33,34-pentol	aminopentol	1f	830	[M+H] ⁺
35-aminobacteriohopane-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] ⁺
Bacteriopenhene-32,33,34,35-tetrol pseudopentose	unsaturated BHTpentose	4/5l	941	[M+H-CH ₃ COOH] ⁺
Bacteriohopane-32,33,34,35-tetrol pseudopentose	BHTpentose	1l	943	[M+H-CH ₃ COOH] ⁺
2-methylbacteriohopane-32,33,34,35-tetrol pseudopentose	2-methylBHTpentose	2l	957	[M+H-CH ₃ COOH] ⁺
Bacteriohanetetrol carbopseudopentose ether	BHT cyclitol ether	1h	1002	[M+H] ⁺
2-methylbacteriohanetetrol carbopseudopentose ether	BHT cyclitol ether isomer	1h	1002	[M+H] ⁺
Bacteriohanetetrol carbopseudopentose ether	2-methylBHT cyclitol ether	2h	1016	[M+H] ⁺
Bacteriohanetetrol carbopseudopentose ether	3-methylBHT cyclitol ether	3h	1016	[M+H] ⁺
Bacteriohanetetrol carbopseudopentose ether glucosamine	BHT glucosamine	1o	1002	[M+H] ⁺
Bacteriohanepentol carbopseudopentose ether	BHpentol cyclitol ether	1i	1060	[M+H] ⁺
Bacteriohanepentol carbopseudopentose ether (isomer)	BHpentol cyclitol ether (isomer)	1i	1060	[M+H] ⁺
2-methylbacteriohanepentol carbopseudopentose ether (isomer)	2-methylBHpentol cyclitol ether	2i	1074	[M+H] ⁺
3-methylbacteriohanepentol carbopseudopentose ether (isomer)	3-methylBHpentol cyclitol ether	3i	1074	[M+H] ⁺
Bacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether	Bhhexol cyclitol ether	1j	1118	[M+H] ⁺
Bacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether (isomer)	Bhhexol cyclitol ether (isomer)	1j	1118	[M+H] ⁺
2-methylbacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether	2-methylBHhexol cyclitol ether	2j	1132	[M+H] ⁺
3-methylbacteriohopane-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

735

736

737 **Table 4**

738 Concentration ($\mu\text{g/g}$ 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 ($\mu\text{g/g}$ 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