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Origin and preservation of bacteriohopanepolyolsignatures in *Sphagnum* peat from Bissendorfer Moor (Germany)

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Abstract

Distributions of bacteriohopanepolyols (BHPs) were investigated in a peat core from the Bissendorfer Moor (Germany) in order to test the utility of BHPs as indicators of microbial processes in peats. Between 13 and 22 BHPs were identified in each sample (23 structures in total), with total concentrations ranging from 160 – 2800 $\mu\text{g g}^{-1}\text{TOC}$. We have tentatively ascribed sources of most BHPs observed at this site via comparison of known BHPs source organisms with recent microbiological studies on the peat microbiome. Members of the Alpha-, Beta- and Gammaproteobacteria and specifically the genera *Burkholderia*, *Bradyrhizobium* and *Rhodoblastus*, as well as other phyla including the cyanobacteria, Acidobacteria and Acetobacteria are amongst the most likely sources. Additionally, BHP signatures which could be assigned directly to methane oxidising bacteria (35-aminobacteriohopanepentol and 35-aminobacteriohopanepentol) were

27 present only at very low levels, supporting previous studies which have shown that the
28 majority of precursor organisms biosynthesising hopanoids in peat environments are
29 heterotrophs. The surface layers also contained a highly unusual signature comprising
30 high concentrations of unsaturated compounds, including unsaturated
31 bacteriohopanetetrol pseudopentose, which has previously only been reported in
32 *Gloeocapsa* cyanobacteria. This genus is known to occur in symbiotic association with
33 host *Sphagnum* species, and has the ability to fix atmospheric nitrogen which is a well
34 known trait amongst members of the peat microbiome and amongst hopanoid producing
35 microorganisms. The apparent capacity for hopanoids to protect organisms from external
36 stresses such as low pH is therefore likely to be a significant factor accounting for the high
37 BHP contributions from heterotrophs, methanotrophs and phototrophic organisms in
38 *Sphagnum* peats.

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1. Introduction

Peatlands contain vast stocks of organic carbon (OC) with Northern areas (including boreal and subarctic peatlands above 45° N) currently storing around 547 Gt OC as waterlogged peat (Yu et al., 2010). Carbon cycling in peats is important in terms of degradation or preservation of organic matter, with the former culminating in methanogenesis (Ciais et al., 2013). These processes are affected by changes in temperature, water table depth and organic matter content (Gorham, 1991; Kotsyurbenko et al., 2007 and references therein). New proxies for unravelling these processes in modern and ancient settings could therefore be useful for elucidating the environmental controls regulating carbon cycling and methane emissions (e.g. Pancost et al., 2011; Chambers et al., 2012; Zheng et al., 2014).

Until recently, microbiological investigations of organisms inhabiting peat bogs, including their adaptation to harsh conditions of low pH and low nutrient input, has largely focussed on organisms with specific metabolisms such as methanogenic archaea and methanotrophic bacteria as they have direct relevance to carbon cycling and climate change (see review by Andersen et al., 2013 and references therein). However, the use of visualisation techniques such as fluorescence in situ hybridisation and confocal laser scanning microscopy have revealed that *Sphagnum* mosses host a wide range of endophytic organisms in their dead hyaline cells (e.g. Opelt and Berg, 2004; Opelt et al., 2007; Bragina et al., 2012a; Shcherbakov et al., 2013). Of the organisms inhabiting the hyaline cells, methanotrophs are of particular interest as they limit the release of methane to the atmosphere and have also been shown to provide CO₂ to the host *Sphagnum* plants (Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010). They may also provide a significant source of fixed nitrogen which is otherwise typically limited in these systems under low atmospheric N deposition (Auman et al., 2001; Larmola et al., 2014). Studies of genes encoding nitrogenase reductase proteins (*nifH*) in *Sphagnum* mosses have

72 revealed that they are mainly derived from *Alphaproteobacteria*, a group of highly diverse
73 organisms including phototrophs, heterotrophs and methanotrophs (Bragina et al., 2012b).
74 However, the ability to fix N₂ is also widely distributed in other phyla across the *Sphagnum*
75 peat microbiome; for example Betaproteobacteria of the genus *Burkholderia* sp., are also
76 known to be important sources of fixed N₂ in peatlands (e.g. Belova et al., 2006; Opelt et
77 al., 2007; Bragina et al., 2013; Shcherbakov et al., 2013).

78 The ability to fix nitrogen and/or oxidise methane is a very common, although not
79 universal trait, amongst organisms which produce hopanoids (e.g. Pearson et al., 2007;
80 Blumenberg et al., 2012; Ricci et al., 2014). Hopanoids are pentacyclic triterpenoid lipids
81 produced by some bacteria (e.g. Rohmer et al., 1984; Ourisson et al., 1987; Ourisson and
82 Rohmer, 1992; Farrimond et al., 1998; Talbot et al., 2008; Pearson et al., 2009). These
83 compounds typically consist of a C₃₀ ring system (**I**; see Appendix), although variations
84 such as methylation at C-2 (**II**) or C-3 (**III**) and unsaturation at C-6 and/or C-11 (**IV-VI**) do
85 occur; however, the latter are rarely observed in environmental materials. They can also
86 contain an extended polyfunctionalised side chain derived from D-ribose (Flesch and
87 Rohmer, 1988) and are termed bacteriohopanepolyols (BHPs).

88 Typical structures have functional groups present at C-32, 33, 34 and 35, the most
89 common example being bacteriohopane-32,33,34,35-tetrol (BHT, **Ia**). Structures with
90 additional hydroxyl moieties at C-31 and/or C-30 are also known (e.g. Rohmer, 1993).
91 BHPs can be broadly assigned into one of two categories “non-composite” and
92 “composite” with the former only containing a simple functionality at the C-35 position such
93 as -OH or -NH₂ including BHT (**Ia**) and 35-aminobacteriohopane-32,33,34-triol (**Id**) whilst
94 the latter have a more complex functionality such as a sugar or aminosugar at C-35. Some
95 structures are only known to be produced by certain groups of organisms (see summary in
96 Table 1; Rohmer, 1993; Talbot and Farrimond, 2007; Talbot et al., 2008, 2014) providing a
97 potentially powerful tool for profiling the microbial community, as recently demonstrated in

98 Siberian permafrost by comparison with genomic profiling (Höfle et al., 2015). Other BHPs
99 have been related to specific environmental settings. For example adenosylhopane (**lg**) is
100 a biosynthetic intermediate in the synthesis of other elongated hopanoids (Bradley et al.,
101 2010), which only seems to accumulate significantly in soils (e.g. Cooke et al., 2008a; Xu
102 et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011). These compounds have been
103 used to trace the transport of terrestrial (soil) organic matter to marine sediments (e.g.
104 Cooke et al., 2008b; Cooke et al., 2009; Handley et al., 2010; Taylor and Harvey, 2011;
105 Zhu et al., 2011; Doğrul Selver et al., 2012, 2015; Wagner et al., 2014).

106 BHPs appear to perform a regulating and rigidifying function similar to that of some
107 sterols in eukaryotes (Kannenberg and Poralla, 1999 and references therein; Sáenz et al.,
108 2012). Although their exact function remains unclear, their regulation has been linked to a
109 variety of environmental factors including temperature, pH, moisture limitation (e.g.
110 Kannenberg and Poralla, 1999; Poralla et al., 2000; Joyeux et al., 2004; Welander et al.
111 2009; Kulkarni et al., 2013) and also growth phase (Joyeux et al., 2004; Doughty et al.,
112 2009; Welander and Summons, 2012).

113 Classically, investigations of bacterial communities in peat bogs using hopanoids
114 have focussed on the geohopanoide degradation products of BHPs, such as hopanoic
115 acids, hopanols and hopanes (e.g. Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995;
116 Pancost et al., 2003; McClymont et al., 2008) and total hopanoide abundance has been
117 used to study relative changes in bacterial biomass in the past (Pancost et al., 2003). In
118 comparison, there have been few previous reports of intact polyfunctionalised BHPs in
119 peat. BHT was first reported by Ries-Kautt and Albrecht (1989). More recently Kim et al.
120 (2011) found between 5 and 16 BHPs in four peat samples from the catchment of the
121 River Têt (France). Van Winden et al. (2012a) also reported the BHP composition of
122 *Sphagnum* moss and underlying peat (to a depth of 10 cm) from a site at Moorhouse (UK),
123 an acidic ombrotrophic blanket bog, and identified up to 13 BHP structures, including

124 markers for methane oxidising bacteria, albeit in low abundance. Similar distributions were
125 also reported from a *Sphagnum* peat core from Belgium between 13 and 100 cm depth
126 (van Winden et al., 2012b).

127 These earlier studies indicate the potential for BHPs to be well preserved in peat
128 but have been limited in age/depth resolution, typically less than 400 years in age and
129 likely within the zone of a thriving bacterial population or focused on intermediate depths
130 without comparable surface samples (van Winden et al., 2012b). The persistence of these
131 signatures to greater depth in peat deposits has not been investigated. Therefore, we
132 report for the first time a full characterisation of intact BHPs in peat samples from
133 Bissendorfer Moor (BM, Germany) to a depth of 410 cm and an age of ~2,900 cal. yr BP
134 (Pancost et al., 2011). We focus in particular on the surface samples within the range of
135 seasonal water table fluctuations. The primary objectives were to explore whether the
136 diversity of BHPs reflects the predominantly heterotrophic microbially-mediated processes
137 that occur in peat, determine whether nitrogen fixation and methane oxidation signatures
138 could be identified against this background, and to explore how well such BHP-based
139 signals are preserved at depth. A secondary objective was to compare peat BHP
140 distributions to those determined for other settings; it is expected that they will be broadly
141 similar to soil distributions, but specific characteristics of the peat environment, including
142 nutrient limitation, a relatively low pH and strongly reducing conditions, could induce
143 profound differences.

144

145 **2. Materials and methods**

146 *2.1. Site description and samples*

147 Peat samples were collected from Bissendorfer Moor, Germany (9.683065 E,
148 52.506028 N; McClymont et al., 2011). This ombrotrophic bog lies 50 metres above sea-
149 level and has been designated as a nature conservation area of 498 ha since 1971. The

150 vegetation comprises a treeless central area with mainly cotton grass and heather.
151 Hollows and hummocks are not sharply differentiated with *Sphagnum magellanicum*, *S.*
152 *rubellum*, and *S. papillosum* growing on hummocks and *S. cuspidatum* and *S. recurvum* in
153 hollows (Pancost et al., 2011). Birch and pinewood dominate in the surrounding dry areas.
154 The average annual temperature at the site is 8.9°C (range 0.6 to 17.2°C) and pH in the
155 range 3.8 to 4.3 (Charman et al., 2007). Water table depth is dynamic, and ranged
156 between 0 and 56 cm (measured in late summer 2003; Charman et al., 2007), with a
157 modern depth of 20 cm indicated by a reconstruction based on testate amoebae
158 assemblages (Charman et al., 2007; Pancost et al., 2011). Despite having conservation
159 status, the surface hydrology has been strongly affected by drainage, meaning that the
160 shallow subsurface microbial assemblages could have been affected by the recent human
161 activity at this peatland (Pancost et al., 2011). The core used in this study was 422 cm
162 long and has been analysed for macrofossils and pollen, testate amoebae and
163 humification indices as part of the ACCROTELM project (e.g. Yeloff et al., 2006; Charman
164 et al., 2007; McClymont et al., 2008; Pancost et al., 2011). Macrofossils in the core have
165 been dated, and calibrated ages are based on 'wiggle matched' AMS radiocarbon dates,
166 using software for Bayesian age-depth modelling (Pancost et al., 2011 and references
167 therein). Samples were stored at -20°C and were freeze-dried prior to analysis.

168

169 2.2 Total Organic Carbon (TOC) analysis

170 Aliquots were analysed in duplicate using a Carlo-Erba EA1108 elemental analyser
171 to determine percentage carbon, nitrogen and hydrogen. Percentage inorganic carbon was
172 determined using a Strohle Instruments Coulomat 702 carbon analyser adapted to
173 analyse CO₂ liberated from H₃PO₄ digestion. Elemental compositions were determined as
174 percent of the dry weight of peat analysed. Total organic carbon was calculated as the

175 difference between total percentage carbon and total inorganic carbon (McClymont et al.,
176 2008).

177

178 2.3. *Extraction and derivatisation,*

179 Lipids were extracted from approximately 0.1 - 0.3 g of freeze-dried, ground peat
180 using repeated ultrasonication (x 3) with 5 ml of dichloromethane/methanol (1:1, v/v). This
181 protocol was originally applied to recover and quantify neutral lipids with a focus on
182 determining palaeoclimate information as part of the ACCROTELM project (McClymont et
183 al., 2011, Pancost et al. 2011). Twenty two sample extracts were selected for BHP analysis
184 via LCMS. After addition of the internal standard (5 β -pregnane-3 α ,20 α -diol), aliquots of the
185 TLE were analysed as acetates, formed by heating with acetic anhydride/pyridine (4 ml;
186 1:1 v/v) at 50°C for 1 h and leaving at room temperature overnight. The derivatised extract
187 was rotary evaporated to dryness and redissolved in 500 μ L MeOH/propan-2-ol (6:4 v/v)
188 for LC-MS analysis.

189

190 2.4. *LC-MS analysis*

191

192 Reversed-phase HPLC was performed using a Surveyor HPLC system
193 (ThermoFinnigan, Hemel Hempstead, UK) fitted with a Phenomenex (Macclesfield, UK)
194 Gemini C₁₈ 5 μ m column (150 mm x 3.0 mm i.d.) and a security guard column cartridge of
195 the same material. Separation was achieved at 30°C with a flow-rate of 0.5 mL min⁻¹ and
196 the following gradient profile: 90% A and 10% B (0 min); 59% A, 1% B and 40% C (at 25
197 min), then isocratic to 45 min, returning to the starting conditions in 5 min and stabilising
198 for 10 min before injecting the next sample (A = MeOH, B = water, C = propan-2-ol; all
199 HPLC grade from Thermo Fisher Scientific).

200 LC-MSⁿ was performed using a Finnigan LCQ ion trap mass spectrometer equipped
201 with an atmospheric pressure chemical ionization (APCI) source operated in positive ion
202 mode. LC-MS settings were as follows: capillary 155°C, APCI vaporiser 490°C, corona
203 discharge current 8 μ A, sheath gas flow 40 and auxiliary gas 10 (arbitrary units). LCQ
204 instrument parameters were selected using an automated tune facility on a direct infusion
205 of an acetylated standard of bacteriohopanetetrol cyclitol ether on the protonated
206 molecular ion, m/z 1002 ([M+H]⁺). LC-MSⁿ analysis was carried out in data-dependent
207 mode with three scan events: SCAN 1 – full mass spectrum, m/z 300-1300; SCAN 2:
208 data-dependent MS² spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent
209 MS³ spectrum of most intense ion from SCAN 2. Detection was achieved at an isolation
210 width of m/z 5.0 and fragmentation with normalised collisional dissociation energy of 35%
211 and an activation Q value (parameter determining the m/z range of the observed fragment
212 ions) of 0.15.

213 Structures assigned are based on comparison with authentic standards and
214 published spectra where possible (Talbot et al., 2003a,b, 2007a,b, 2008) or by comparison
215 of APCI MS² and MS³ spectra with those of known compounds, as indicated below. The
216 location of additional ring system methylation can be determined from relative retention
217 times, with C-2 and C-3 methylated structures eluting ca. 0.7 and 1.3 min, respectively,
218 after non-methylated compounds.

219 A semi-quantitative estimate of BHP abundance ($\pm 20\%$) is calculated from the
220 characteristic base peak ion peak areas of individual BHPs in mass chromatograms (from
221 SCAN 1) relative to the m/z 345 ([M+H-CH₃COOH]⁺) base peak area response of the
222 acetylated 5 α -pregnane-3 β ,20 β -diol internal standard. Averaged relative response factors
223 (from a suite of five acetylated authentic BHP standards) are used to adjust the BHP peak
224 areas relative to that of the internal standard where BHPs containing one or more nitrogen
225 atoms give an averaged response approximately 12 times that of the standard and

compounds with no nitrogen atoms give a response approximately 8 times that of the standard (van Winden et al., 2012a).

3. Results

3.1 TOC

TOC (%) contents in the upper section of the core (0-150 cm) are relatively stable, with a mean value of $43.4\% \pm 1.34$ (± 1 standard deviation; min. 40.2%, max, 45.7%, $n = 73$). In the deeper section (350 – 422 cm), TOC contents are generally higher although more variable with an average of $46.1\% \pm 2.5\%$, (min 39.1%, max 49.1%, $n = 31$).

3.2 Bacterioplanepolyols (BHPs)

A total of 23 different BHPs (Tables 1 and 2) were identified in the 22 peat samples investigated, with each individual sample containing between 13 and 22 BHPs (Table 2). Total BHP abundance ranged from 150 to 2800 $\mu\text{g g}^{-1}\text{TOC}$ (Table 2). The highest concentrations of BHPs were present in the 2-4 cm and 26-28 cm layers, within the region of water table fluctuation (0- 56 cm) and also in the four deepest layers (> 400 cm).

The total BHP concentration (Table 2) and those of major individual BHPs (Fig. 1, 2) show a similar depth profile, with highest values in the 2-4 cm, 26-28 cm and deepest samples (>400 cm) but with consistently low-to-intermediate values throughout the rest of the profile (e.g. Fig. 1, 2). The distributions are dominated by saturated tetrafunctionalised BHPs which account for over 80% of the total BHPs in all samples from below 10 cm. Three compounds in particular comprise the bulk of the total tetrafunctionalised BHPs: BHT (**Ia**; Fig. 1a), BHT cyclitol ether (**Ij** and/or **Ik**; Fig. 1b) and aminotriol (**Id**; Fig. 1c)

251 accounting for over 55% of the total BHPs in all except the surface and 4-6 cm samples.
252 Several other less abundant BHPs do not follow this general trend as discussed below.

253

254 3.2.1 35-amino functionalised BHPs

255 Aminotriol (**Id**; Fig. 1c) was the most abundant compound in 14 of the 22 samples. It
256 always co-occurred with aminotetrol (**Ie**; Fig. 1d), although aminotriol is consistently and
257 significantly more abundant (up to 910 $\mu\text{g g}^{-1}\text{TOC}$ compared to $<40 \mu\text{g g}^{-1}\text{TOC}$ for
258 aminotetrol; Table 2). The related hexafunctionalised aminopentol (**If**; Fig. 1e), was only
259 observed below 22 cm and was the only compound identified which showed this profile.
260 Where present, it only occurred in very low concentrations $<5 \mu\text{g g}^{-1}\text{TOC}$ (Fig. 1e; Table 2)
261 and never represented more than 0.5% of the total BHPs. Two minor methylated
262 aminotriols were also observed, 2-methyl and 3-methyl-aminotriol (**IIf** and **IIIf**; Table 2).

263

264 3.2.2 Composite BHPs

265 With the exception of the surface and 4-6 cm samples, the composite structure BHT
266 cyclitol ether (**Ij** and **Ik**; Fig. 1b) was the most abundant or 2nd most abundant compound in
267 all samples (Table 2). The isomeric compound BHT glycoside (**Im**; Table 2) was also
268 present in all but one sample but at significantly lower abundance, similar to the saturated
269 bacteriohopanepentol- and bacteriohopanehexol-cyclitol ethers (**In** and **Io**, respectively;
270 Table 2). A novel composite non-methylated hexafunctionalised BHP with a previously
271 unrecognised terminal group structure (indicated by ion of m/z 344 in MS^2 spectrum of m/z
272 1132; data not shown) was also observed in the surface samples and below 400 cm, but
273 was only rarely observed in the upper section (0-130 cm; Table 2).

274

275 3.2.3 Unsaturated BHPs

276 The surface and 4-6 cm samples had unusual BHP signatures, dominated by
277 unsaturated compounds. These compounds are also present in significant amounts in the
278 2-4 and 8-10 cm samples (Fig. 2a,b; Table 2). A pair of peaks in the m/z 653 mass
279 chromatograms have mass spectra consistent with the previously reported spectra of
280 monounsaturated tetrols (cf. Talbot et al., 2007b, 2008). An unsaturated aminotriol (**IVb** or
281 **Vb** or with unsaturation in the side chain [van Winden et al., 2012a]; Table 2) was also
282 observed, primarily in the shallower sections. Also present are two unsaturated composite
283 BHP compounds, including an unsaturated bacteriohopanepentol cyclitol ether (**IVn** or **Vn**;
284 Table 2). The second is a composite tetrafunctionalised BHP, previously proposed to
285 contain a pentose terminal group (**IVi** or **Vi**, Fig. 2b; Talbot et al., 2008). The unsaturated
286 BHT pentose is particularly dominant in the surface sample, comprising 37% of total
287 BHPs; however, its abundance, both total and relative to other BHPs, decreases rapidly
288 with depth down core (Fig. 2b; Table 2). The related saturated homologue (**IIi**; Fig. 2c) also
289 had its highest relative abundance in the surface layers. However, unlike the unsaturated
290 structure (**IVi** or **Vi**; Fig. 2b), it was present throughout the core, with highest concentration
291 in the deepest layers (>402 cm).

292 All of the aforementioned unsaturated BHPs exhibit markedly similar depth profiles.
293 Maximum concentrations and relative abundances occur in the three most shallow
294 horizons and then decrease dramatically down core and are not detected throughout most
295 of the intermediate depths down to 120 cm (Fig. 2a,b). However, in the deepest part of the
296 core, at >400 cm, these compounds are again present, although at lower relative
297 abundance than the most shallow horizons. This is due to an even greater increase in
298 concentration of other compounds including BHT, Aminotriol and BHT cyclitol ether (Fig.
299 1), but still indicates good preservation of unsaturated compounds at these depths (Fig. 2).

300

301 3.2.4 Adenosylhopane and related structures

Adenosylhopane (**Ig**; Fig. 2d) is always the most abundant representative of the group containing a cyclised side chain and collectively known as “soil-marker BHPs” (Cooke et al., 2008a; Zhu et al., 2011; Doğrul Selver et al., 2012). Adenosylhopane, a related structure (“adenosylhopane Type 2”) with the same cyclised side chain but an alternative terminal group, and two methylated homologues (**Ih**; **Ilg**, **Ilh** respectively; combined sum in Fig. 2e) all exhibited similar depth profiles, being most abundant in the 2-4 and 8-10 and 26-28 cm samples (Fig. 2e; Table 2). These compounds represent up to a maximum of 16% (Fig. 3a) then fall as low as 1.7% and remain low throughout the rest of the core (Fig. 3a).

3.2.5 Methylated BHPs

Six different methylated BHPs were detected in the peat, five of which were methylated at the C-2 position. These compounds represent between 2 and 12 % of the total BHPs in each sample (Table 2). 2-methyl BHT (**Ila**; Table 2) is present in all samples, and 2-methylaminotriol (**Ild**; Table 2) is present in all but four of the near surface samples. The three other C-2 methylated BHPs had a much more limited occurrence, with the methylated adenosylhopane and the methylated homologue of the related compound (**Ilg**, **Ilh**; Table 2) only present in a few of the surface samples above 22 cm depth. The fifth compound, 2-methylBHT pseudopentose (**Ili**), was only detected in 3 samples below 400 cm and in very low abundance (Table 2). Finally, one C-3 methylated compound was observed, 3-methyl-aminotriol (**IIlb**); however, it was only present below 18 cm depth and always at low levels (Table 2), similar to the concentration profile of aminopentol (**If**; Fig. 1e).

4. Discussion

328

329 4.1 Sources of BHPs in BM peat

330 Microbiological studies of *Sphagnum* and peats reveal both typical bacterial groups
331 but also pronounced variations in distribution that appear to depend largely on abiotic
332 factors such as pH (e.g. Bragina et al., 2012a). Particularly common organisms include
333 Alphaproteobacteria with subordinate contributions from Beta-, Gamma- and in some
334 cases Deltaproteobacteria (e.g. Dedysh et al., 2006; Bragina et al., 2012a, b; Serkebaeva
335 et al., 2013). Many of these bacteria are heterotrophic and some are also capable of
336 dinitrogen fixation. In a recent study of a peat soil targeting the *nifH* gene, one of the main
337 components of the nitrogenase complex, a high diversity of diazotrophic bacteria were
338 detected (Zadorina et al., 2009). These sequences also included, but were not limited to,
339 species of Alpha-, Beta-, Gamma and Deltaproteobacteria. Other important phyla in the
340 peat microbiome include Acidobacteria, Actinobacteria, Planctomycetes and
341 Verrucomicrobia (Dedysh et al., 2006; Bragina et al., 2012b; Serkebaeva et al., 2013).
342 Each of these phyla are known to include hopanoid producers (Table 1; e.g. Rohmer et al.,
343 1984; Pearson et al., 2007), although biosynthesis by members of the proteobacteria has
344 been studied much more extensively than most of the other, more recently described phyla
345 (Rohmer et al., 1984; Ourisson et al., 1987; Farrimond et al., 1998; Kuchta et al., 1998;
346 Talbot et al., 2008; see also references in Table 1).

347

348 4.1.1 Heterotrophs

349 As BHP abundances are high in the shallowest layers (Table 2; Figs 1-3) it is
350 likely that many of the most abundant BHPs derive from aerobic heterotrophs. These
351 organisms are important members of the bacterial community found in peat, consuming a
352 wide range of organic substrates, including organic acids, sugars, polyalcohols and some
353 aromatic compounds as carbon and energy sources (e.g. Belova et al., 2006). This is

354 consistent with previous studies on peats which suggest that ^{13}C -enriched hopanes are an
355 indicator of a heterotrophic bacterial population consuming ^{13}C -enriched carbohydrates
356 (Pancost et al., 2000; Xie et al., 2004).

357 The BHP profile of BM is broadly consistent with a dominantly heterotrophic
358 bacterial community. Most heterotrophs make BHT (**Ia**), and the majority also make
359 composite BHPs, with BHT cyclitol ether (**Ij** and/or **Ik**) the most commonly-occurring
360 structure found in members of the Alpha-, Beta-, Gamma and Deltaproteobacteria (see
361 Table 1). Members of the Betaproteobacterial genus *Burkholderia*, which comprises gram-
362 negative, aerobic and microaerophilic chemoorganotrophic bacteria, have been shown to
363 be particularly important and abundant in the peat microbiome (Opelt and Berg, 2004;
364 Belova et al., 2006; Opelt et al., 2007; Sun et al., 2014) and are likely to be important
365 sources of both BHT and cyclitol ether compounds (Table 1). A high diversity of
366 *Burkholderia* species was found in both the endophytic and ectophytic habitats of different
367 *Sphagnum* moss species and in relation to two species (*S. magellanicum*, Opelt et al.,
368 2007; *S. rubellum*, Opelt and Berg, 2004) that are also present at BM.

369 The *Acetobacter* and *Gluconacetobacter* genera are known as acetic acid bacteria
370 (AAB). They are diazotrophic (e.g. Bragina et al., 2012b) and also produce acetate which
371 is a necessary substrate for acetoclastic methanogenesis which is an important pathway in
372 some shallow peats (Kelley et al., 1992; Popp and Chanton, 1999; Chasar et al., 2000;
373 Metje and Frenzel, 2005). Alphaproteobacteria AAB are prolific sources of a wide range of
374 BHPs with core structures of BHT (**Ia**), BHT cyclitol ether (**Ij**) and BHpentol cyclitol ether
375 (**In**) but also including mono and diunsaturated compounds with double bonds at C-6 (**IV**),
376 C-11(**V**) or both (**VI**) and with ring systems both with and without additional methylation at
377 C-3 (**III**; Rohmer and Ourisson, 1986; Peiseler and Rohmer, 1992; Talbot et al., 2007b).
378 Whilst AAB are likely present in BM and a possible source for the numerous unsaturated

379 BHPs, the absence of any C-3 methylated homologues, either of the tetrol or tetra- or
380 pentafunctionalised cyclitol ethers, suggests contributions from these organisms is minor.

381 Of the species of Deltaproteobacteria investigated, only *Geobacter* species are
382 considered potential sources here (Table 1); however, the absence of guanidine
383 substituted BHT cyclitol ether which co-occurs with BHT, BHT cyclitol ether and BHT
384 glucosamine in *Geobacter* spp. suggests a contribution from these organisms is unlikely or
385 limited at this site. Other genomic studies have shown that *sqhC* diversity related to as yet
386 unknown Deltaproteobacteria is high, especially in soils relative to marine sediments
387 (Pearson et al., 2009), such that contributions from other Deltaproteobacteria cannot be
388 excluded.

389 Amongst the heterotrophs, relatively few species make only non-composite BHPs.
390 The Alphaproteobacterial genera *Beijerinckia* and *Bradyrhizobium* contain relatively simple
391 BHP distributions dominated by aminotriol (**Id**), with additional BHT (**Ia**) in *Beijerinckia*
392 *indica* (Table 1). Members of the Actinobacteria also only make these less complex
393 compounds (Table 1). However, as these compounds represent two of the three most
394 abundant compounds at BM (Fig. 1; Table 2) contributions from such sources could be
395 important. Unlike any of the other heterotrophs with known BHP compositions,
396 *Bradyrhizobium japonicum* also accumulates adenosylhopane (**Ig**; Table 1), which is
397 present in the highest concentration in the 2-4 cm sample and constituted the greatest
398 relative proportion of the total BHPs (13.4%; Table 2) in the 10-12 cm sample, likely
399 indicating an aerobic source such as *B. japonicum*. Adenosylhopane is a biosynthetic
400 intermediate in the synthesis of other side chain elongated BHPs (Bradley et al., 2010);
401 therefore, there are potentially numerous sources for this compound, although, with the
402 exception of *B. japonicum* few organisms have been shown to accumulate it in detectable
403 amounts (Table 1).

404 To summarise, proteobacteria are undoubtedly significant sources of BHPs at BM,
405 with *Burkholderia* spp. considered particularly important sources of BHT (**Ia**) and BHT
406 cyclitol (**Ij** and/or **Ik**) although multiple sources are expected for both compounds (see
407 Table 1). *Beijerinckia* spp. are likely sources of aminotriol (**Id**) with additional contributions
408 from *Bradyrhizobium* sp. and Actinobacteria. *B. Japonicum* likely also contributes to
409 adenosylhopane (**Ig**).

410

411 4.1.2 Aerobic methane oxidising bacteria (methanotrophs)

412 The northern peatlands cover an area of approximately 4 million km² (>45°N; Yu et
413 al., 2010 and references therein), and are a major source of methane release to the
414 atmosphere (e.g. Spahni et al., 2011 and references therein). Recent estimates vary but
415 indicate the flux of methane from northern peatlands is in the range 24 to 58 Tg CH₄ yr⁻¹
416 (Zhang et al., 2016 and references therein). This release is attenuated by methane
417 consuming bacteria (methanotrophs) inhabiting the oxic layers of the peat (e.g. Segers,
418 1998; Dedysh, 2009) and within *Sphagnum* (Raghoebarsing et al., 2005). Culturable
419 methanotrophs are classified phylogenetically as either members of the
420 Alphaproteobacteria (known as Type II) or Gammaproteobacteria (known as Type I;
421 Hanson and Hanson, 1996), with a third group recently described from the phylum
422 Verrucomicrobia (Op Den Camp et al., 2009).

423 Dedysh (2009) described the methanotroph diversity in acidic northern wetlands
424 and found that these settings are mainly colonized by methanotrophic representatives of
425 the Alphaproteobacteria (i.e. Type II methanotrophs). However, studies using functional
426 gene analysis by microarray or ultra-deep pyrosequencing of *pmoA* genes on *Sphagnum*
427 peat samples from a range of environments including but not limited to northern regions
428 (Siberia, Sweden, Canada, Argentina and The Netherlands), revealed Type I organisms
429 including the genera *Methylomonas*, *Methylobacter*, *Methylomicrobium* and

430 *Methylocaldum* (Kip et al., 2010, 2011). Type II organisms related to *Methylocystis* and
431 *Methylosinus* spp. (family Methylocystaceae) were also significant members of the
432 methanotroph community at all sites (Kip et al., 2010, 2011) and were particularly
433 dominant in *Sphagnum magellanicum* dominated habitats from Patagonia (Kip et al.,
434 2012). Whilst *Methylocystis* spp. are a common and abundant group in acidic peats, there
435 are other more recently described genera of Type II methanotrophs from the family
436 *Beijerinckiaceae* including *Methylocella* and *Methylocapsa*, which are also important and
437 would not have been observed in some earlier studies utilising the functional gene *pmoA*
438 (which is not present in these organisms; e.g. Rahman et al., 2011 and references
439 therein). Recent studies have also suggested an apparently symbiotic relationship
440 between methanotrophs and *Sphagnum* moss (Kip et al., 2010), with a bacterium
441 occurring inside cells of *S. cuspidatum* showing 93% 16S rRNA sequence similarity to
442 cultured *Methylocella* and *Methylocapsa* sp. (Raghoebarsing et al., 2005).

443 Previous studies of methane oxidising bacteria have shown that they produce
444 characteristic non-composite BHP distributions, with most Type I species producing high
445 levels of the hexafunctionalised compound aminopentol (**If**) and lower amounts of the
446 related pentafunctionalised compound aminotetrol (**Ie**; Table 1; e.g. Talbot et al., 2001; van
447 Winden et al., 2012a; Talbot et al., 2014 and references therein). Some Type I organisms
448 of the genera *Methylococcus* and *Methylocaldum* also produce homologues of
449 aminopentol and aminotetrol with a methyl group at position C-3 (**IIIIf** and **IIIe** respectively;
450 Neunlist and Rohmer, 1985a; Cvejic et al., 2000a), although the 3-methyl-aminotriol (**IIIb**)
451 observed here (Table 2) has only recently been reported from cultures of the Type I
452 organism *Methyломicrobium alcaliphilum* (Banta et al., 2015), an alkaliphilic organism
453 unlikely to be present in *Sphagnum* peat. Type II organisms, including representatives of
454 the genera *Methylocystis* and *Methylosinus* (Alphaproteobacteria), produce a
455 combination of BHT, aminotriol and aminotetrol (Rohmer et al., 1984; Neunlist and

456 Rohmer, 1985b; Cvejic et al., 2000a; Talbot et al., 2001; van Winden et al., 2012a). It
457 should be noted, however, that there are multiple other sources of aminotriol especially
458 amongst other Alphaproteobacteria (Table 1; e.g. Talbot and Farrimond, 2007 and
459 references therein).

460 Of the other Type II methanotrophs from the family Beijerinckiaceae, no cultured
461 representatives of *Methylocapsa* sp. (e.g. Dedysh et al., 2001a) have been tested for BHP
462 production or composition, but van Winden et al. (2012a) reported the BHP composition of
463 *Methylocella palustris*. *Methylocella* and *Methylocapsa* are closely related to another
464 known BHP producer *Beijerinckia* sp., which makes aminotriol (**Id**) and in some cases BHT
465 (**Ia**; Table 1; Vilcheze et al., 1994) but they were not found to produce aminotetrol (**Ie**). The
466 dominant compounds in *M. palustris* (and also *Methylocella tundrae*; Talbot and Rohmer,
467 unpublished data) were aminotriol and BHT with trace levels of adenosylhopane (**Ig**).
468 Unfortunately, there are numerous different sources for all of these compounds so there is
469 no way to conclusively identify BHP contributions from *Methylocella* spp. using BHP
470 analysis (Table 1). Given the importance of this group as demonstrated by microbiological
471 studies (Kip et al., 2010, 2011; Rahman et al., 2011), we consider it likely that Type II
472 methanotrophs of the Beijerinckiaceae family will be important sources of (at least)
473 aminotriol (**Id**) at this site.

474 Although a methanotroph biomarker might be expected to be most abundant at the
475 redox interface i.e. water table depth, where oxygen is present and methane
476 concentrations are the highest (e.g. Sundh et al., 1995), aminotriol was present at high
477 concentration in all samples from BM (Fig. 1c). Aminotetrol (**Ie**) was also present in all
478 samples, representing up to 2.3% of the total BHP distribution with the highest
479 concentration in the 26-28 cm sample (Fig. 1d). Although this is around the approximate
480 average depth of the oxic-anoxic interface, this region of the core will be exposed to
481 oxygen seasonally as the water table lowers. The highest concentration of aminopentol (**If**)

482 for the entire profile occurred in the same sample (Table 2), and it was only present below
483 22 cm; however, it never represented more than 0.4% of the total BHP distribution (Fig.
484 1e). The occurrence of aminotriol and aminotetrol together, in the absence of aminopentol,
485 in the upper section of the core (0 to 22cm) suggests the presence of Type II
486 methanotrophs *Methylocystis/Methylosinus* (family Methylocystaceae; Table 1) at BM
487 which is consistent with previous studies on methanotroph populations in peat (e.g.
488 Dedysh et al., 2001b; Dedysh, 2009; Kip et al., 2011).

489 Aminopentol (**If**), which is only known to occur in Type I methane oxidising bacteria
490 (see review in Talbot et al., 2014), was only observed below 22 cm depth (Fig. 1e).
491 Although this depth is within the reported range of water table variations (0-56 cm;
492 Charman et al., 2007), it is below the current reconstructed water table level (~20 cm)
493 based on testate amoebae analysis (Pancost et al., 2011 after Charman et al., 2007). This
494 suggests that the occurrence of aminopentol in deeper samples likely reflects the
495 presence of relict BHPs initially formed at the anoxic-oxic interface. As such, aminopentol
496 is most likely a fossil compound recording past methanotrophy. The absence of C-3
497 methylated homologues of aminopentol and aminotetrol suggest a likely source organism
498 would be *Methylomonas* sp., consistent with the identification of this genera in *Sphagnum*
499 peat in other studies (Kip et al., 2010) and the recent isolation of the first acid tolerant
500 *Methylomonas* sp. from and acidic *Sphagnum* peat bog (Danilova et al., 2013).

501 We speculate that the only C-3 methylated BHP observed at BM, 3-
502 methylaminotriol (**IIId**; Table 2), present from 18 cm depth and below is most likely related
503 to a genera of Type I methanotrophs based on the similar depth profile to that of
504 aminopentol (which occurs from 22 cm and below; Fig. 1a; Table 2).

505 Aminotriol concentrations are over two orders of magnitude greater than aminotetrol
506 concentrations, inconsistent with their relative abundances in previously cultured type II
507 methanotrophs (Neunlist and Rohmer, 1985b; Jahnke et al., 1999; Talbot et al., 2001; van

508 Winden et al., 2012a). Instead, the marked similarity of the aminotriol BHP depth profile
509 with most other major BHPs (Fig. 1) suggests a significant non-methanotrophic origin for
510 this compound, or a methanotroph origin from one of these more recently described
511 sources (e.g. *Methylocella*; van Winden et al., 2012a). The similar depth profile of
512 aminotetrol, although far less abundant (Fig. 1c and d), suggests that this might also
513 derive, at least partially, from other sources, although the only known non-methanotroph
514 source are sulphate reducing bacteria of the genus *Desulfovibrio* (Blumenberg al., 2006,
515 2009b, 2012), which is unlikely to occur in this environment. Only aminopentol has a depth
516 profile consistent with being derived predominantly from methanotrophs.

517 Finally, although anaerobic methane-oxidisers related to the novel bacterium
518 “*Candidatus Methyloirabillia oxyfera*” have been identified in peat (e.g. Zhu et al., 2012),
519 the diagnostic BHP produced by these organisms (3-Methyl-bacteriohopanehexol, **III**f;
520 Kool et al., 2014) was not detected.

521

522 4.1.3 Phototrophic bacteria

523 The surface layers of peat bogs host a wide range of phototrophic eukaryotes
524 and prokaryotes. This includes nitrogen fixing cyanobacteria which inhabit *Sphagnum* peat
525 bogs, occurring in both epiphytic and intracellular associations with *Sphagnum*, (e.g.
526 Granhall and Selander, 1973; Krivograd Klemenčič and Vrhovška, 2003; Krivograd
527 Klemenčič et al., 2010; Bragina et al., 2012b). Many different classes of hopanoid
528 producing cyanobacteria, including *Anabaena*, *Calothrix*, *Cyanothece*, *Gloeocapsa*,
529 *Microcystis*, *Nostoc*, *Oscillatoria* and *Phormidium* (Talbot et al., 2008 and references
530 therein), have been observed in peat (Krivograd Klemenčič et al., 2010 and references
531 therein). Until recently, cyanobacteria were considered to be the major source of
532 hopanoids methylated at the C-2 position (Summons et al., 1999); however, recent
533 genomic studies have revealed that the capacity for this synthesis also occurs in other

534 phyla, particularly the Alphaproteobacteria and also Acidobacteria (Welanders et al., 2010)
535 and that it is a particularly common trait in organisms found in close (symbiotic)
536 association with plants (Ricci et al., 2014).

537 Of particular interest to this study are the species of *Gloeocapsa* cyanobacteria
538 which were identified, for example, in the Männikjärve bog (central Estonia) and were
539 particularly abundant on *S. magellanicum* plants (Karofeld and Toom, 1999), one of the
540 species present at the BM site. A further seven *Gloeocapsa* spp. were identified in two
541 Slovenian bogs (Krivograd Klemenčič and Vrhovška, 2003). Crucially, a *Gloeocapsa* sp. is
542 the only known source of the unsaturated compound, identified as a “BHT pentose” (**IVi** or
543 **Vi**), that is highly abundant (over 40%) in the surface layers at the BM site (Fig. 1b). Such
544 a high abundance in surface layers is consistent with an aerobic and/or phototrophic
545 source and suggests a particular ecophysiological role, potentially regulating osmotic
546 pressure or proton gradients in the low pH peat environment (cf. Welanders et al., 2009).
547 The original identification of this compound, together with its saturated and C-2 methylated
548 homologues (**IIIi**) was from a sample of an epilithic colony of *Gloeocapsa* sp. from Devon
549 Island (Arctic; Talbot et al., 2008). The saturated pentose compound, with the non-
550 methylated structure (**IIi**, Table 2, Fig. 2c), was also observed in BM peat, being present at
551 all depths but showing a markedly different depth profile to the unsaturated structure (Fig.
552 2b). The methylated pentose structure (**IIIi**; Table 2) was only observed in the deepest
553 levels, below 400 cm. Therefore, the saturated compounds could: (i) derive from an
554 additional source to the unsaturated compounds; (ii) have a markedly different
555 preservation potential for the methylated vs non-methylated compounds; and/or (iii) have
556 different extraction efficiencies in the fresher, near surface material, given that some other
557 methylated BHPs have exhibited resistance to extraction (Herrmann et al., 1996; Allen et
558 al., 2010).

559 The pair of unsaturated bacteriohopanetetrols (**IVa, b** or **c**; Table 2) also had very
560 similar depth profiles to the unsaturated BHT-carbopseudopentose suggesting a common
561 source (Fig. 2a and b). A single unsaturated BHT with an identical mass spectrum was
562 also observed in the original source of the *Gloeocapsa* enrichment (Talbot et al., 2008).
563 One possibility, therefore, is that a *Gloeocapsa* sp. inhabiting this environment produces
564 both the composite carbopseudopentose and one or both of the observed unsaturated
565 tetrols. An alternative heterotrophic source for the unsaturated tetrols, the
566 Alphaproteobacteria AAB, are discussed above but considered minor at most, again due
567 to the absence of C-3 methylated homologues.

568 Purple non-sulfur bacteria (PNSB) are normally anoxygenic photoheterotrophic
569 organisms. They belong to the Alphaproteobacteria and Betaproteobacteria with many
570 representatives being closely related to non-phototrophic, strictly chemotrophic bacteria
571 (Kulichevskaya et al., 2006 and references therein). PNSB are widely distributed in various
572 aquatic ecosystems as well as in sediments, moist soils and natural wetlands
573 (Kulichevskaya et al., 2006), but peat bogs are rarely reported as sources of PNSB
574 (Kulichevskaya et al., 2006). Few PNSB can tolerate low pH with the exception of
575 members of the genera *Rhodoblastus* (Pfennig, 1969; Imhoff, 2001). Several species of
576 *Rhodoblastus* have been isolated from acidic *Sphagnum* bogs (Kulichevskaya et al.,
577 2006), and *Rhodoblastus acidophilus* (formerly *Rhodopseudomonas acidophila*; Imhoff
578 2001) has been shown to produce a number of common hopanoids including BHT (**Ia**) and
579 BHT cyclitol ether (**Ij**; Talbot et al., 2007a and references therein). The major product,
580 however, was adenosylhopane (**Ig**, Table 1; also seen in other PNSB including
581 *Rhodopseudomonas*; Talbot et al., 2007a and references therein; Eickhoff et al., 2013a)
582 suggesting another possible source of adenosylhopane at BM.

583

584 **4.2 Comparison of BHP distributions at BM to other soils and peat**

585 Biohopanoids were present at high concentration throughout the BM peat core (Table 2). A
586 total of 23 different intact BHP structures were observed (Table 2), comparable to recent
587 reports of BHP distributions in soils (e.g. Cooke et al., 2008a; Xu et al., 2009; Zhu et al.,
588 2011; Spencer-Jones et al., 2015) and considerably more complex than typical
589 distributions in aquatic sediments (e.g. Blumenberg et al, 2006, 2009a, 2010; Talbot and
590 Farrimond, 2007; Coolen et al., 2008; Cooke et al., 2008b, 2009), with the exception of
591 sediments occurring on deep-sea fans receiving high terrigenous inputs (Handley et al.,
592 2010; Wagner et al., 2014). Although the extraction method used here (sonication in
593 DCM:MeOH) was not the typical modified Bligh and Dyer method (e.g. Cooke et al.,
594 2008a), which has been widely applied to BHP extractions in other studies, the BHP
595 distributions are comparable and the concentrations in many cases are similar or higher
596 than those reported for other peats (Kim et al., 2011; van Winden et al., 2012a,b). The
597 highest total BHP concentrations were found in the 2-4 cm sample ($2700 \mu\text{g g}^{-1}\text{TOC}$) and
598 below 400 cm ($1100\text{-}3000 \mu\text{g g}^{-1}\text{TOC}$), whilst values throughout the remainder of the core
599 were lower, ranging from $160 \mu\text{g g}^{-1}\text{TOC}$ (32-34 cm) to $900 \mu\text{g g}^{-1}\text{TOC}$ (Table 2).

600 Although to date BHPs in peat have only been studied by LCMS from 3 other
601 locations (Kim et al., 2011; van Winden et al., 2012a,b), our data collectively suggest that
602 whilst soil and peat derived organic matter generally contain the same BHPs, they have
603 characteristically different relative distributions, as recently suggested for tropical and non-
604 tropical soils (Spencer-Jones et al., 2015). Excluding the surface layers, the three most
605 dominant BHPs in the BM peat at all other depths were BHT (**la**), BHT-cyclitol ether (**lj**)
606 and aminotriol (**ld**). Again, these observations are consistent with other recent studies of
607 BHPs in peat (Kim et al., 2011; van Winden et al., 2012a,b) and also with the BHP
608 composition in soils from the Northern hemisphere (Cooke et al., 2008a; Xu et al., 2009;
609 Cooke, 2010; Rethemeyer et al., 2010; Kim et al., 2011) and tropical settings (Wagner et
610 al., 2014; Spencer-Jones et al., 2015). However, we find that the BHP distribution at BM,

611 and other peats, differs from soils in two fundamental ways: (i) they contain a particularly
612 high proportion of unsaturated BHPs in peat surface layers; and (ii) generally exhibit lower
613 proportions of the 'soil-marker' BHPs.

614 The surface layers of the BM peat core contain exceptionally high concentrations of
615 unsaturated biohopanoids, including unsaturated BHT-pentose (**IVi or Vi**), in agreement
616 with observations by van Winden et al. (2012a) for a UK peat bog. The BM site also
617 uniquely contained two isomers of unsaturated BHT (possibly **IVa** and/or **b** or **c**), whilst
618 only one isomer was found in the UK study (van Winden et al., 2012a). These compounds,
619 together with 2 other minor unsaturated BHPs (**IVd or Vd** and **IVm or Vm**) accounted for
620 up to 46 % in the surface layers, rapidly falling to 3% or less below 12 cm depth (although
621 all unsaturated BHPs were observed again below 400 cm depth; Fig. 2 a and b; Table 2).

622 Among the defining features of soil BHP distributions are high levels of
623 adenosylhopane (**Ig**), its C-2 methylated homologue (**Ilg**; Talbot et al., 2007a) and two
624 pairs of related compounds with as yet unidentified terminal groups (including
625 "adenosylhopane type 2"; **Ih** and **IIh**, Table 1), together comprising 28% of the average
626 total BHP assemblage (Cooke et al., 2008a; Cooke, 2010). Five soils from Canada and
627 permafrost soils from Svalbard and Siberia contained even higher proportions of this BHP
628 group (Xu et al., 2009; Rethemeyer et al., 2010; Höfle et al., 2015). In the peat samples
629 studied here, the distribution of these compounds is rather different, with adenosylhopane
630 and related compounds only present in significant concentration in the shallow sub-surface
631 (up to 15% in 2-4 and 8-12 cm; Fig. 3a) but are otherwise below 10%. In *Sphagnum* peats
632 from 2 other European sites (UK, Belgium; van Winden et al., 2012a and b respectively),
633 the relative abundance of soil marker BHPs is also low, typically less than 10% except in
634 the near surface samples (up to 15%; Fig. 3a). The only exceptions are 4 peat samples
635 from France which have a slightly higher relative abundance of soil biomarkers (20-30%;

636 Fig. 3b), however, no details were provided as to the type of peat in that study so it is
637 possible they were not *Sphagnum* peats (Kim et al., 2011).

638 The low values of soil marker BHPs (typically < 10%; Fig 3a,b) within peats are
639 distinctive from those of soils. Values reported recently for tropical and sub-tropical soils
640 had values in the range 0-40% although the majority were in the range 10-20%, slightly
641 higher than the peat samples (Fig. 3c). Temperate soils from Canada (Alberta), France
642 and the UK, had a wide range (0-70%; Fig. 3d) with the widest range found in permafrost
643 soils from the Siberian Arctic and Svalbard (0-90%; Fig. 3e), possibly reflecting competing
644 influences of low temperature leading to higher values and low pH leading to low values
645 (Höfle et al., 2015; Spencer-Jones et al., 2015). As discussed above, the most likely
646 sources of adenosylhopane (**lg**) and related structures in *Sphagnum* peat environments
647 are nitrogen-fixing Alphaproteobacteria (*Bradyrhizobium* sp.; PNSB; *Methylocella* sp.;
648 Table 1). As adenosylhopane is in itself an intermediate in the biosynthetic pathway to
649 other side chain elongated BHPs (Bradley et al., 2010), this suggests that under the low
650 pH conditions found in *Sphagnum* peat (pH 3.8 – 4.3 at BM; Charman et al., 2007), the
651 species that would normally accumulate adenosylhopane convert this precursor to other
652 BHPs. Alternatively, other nitrogen fixers such as *Burkholderia* sp. (Betaproteobacteria)
653 and other, especially Alphaproteobacterial, BHP producers (e.g. Zadorina et al., 2009)
654 outcompete the adenosylhopane accumulators in these systems. In their study of BHPs in
655 Siberian permafrost, Höfle et al. (2015) reported that concentrations of adenosylhopane
656 (and related compounds) were negatively correlated with pH, the implication being that
657 further modification of the side chain is more important at lower pH than more neutral
658 conditions, hence the greater diversity of structures present in low pH peat and peaty soils
659 (Cooke et al., 2008a; van Winden et al., 2012a). Indeed Gong et al. (2015) recently
660 reported that pH was a key factor controlling the geographical distribution of squalene
661 hopene cyclase (*sqhC*) with Proteobacterial and Acidobacteria the dominant source

organisms in the acidic Dajiuhu peatland (China). Regardless of the reasons for this difference in BHP distributions, this variability could prove useful for tracing the origin and transport of terrestrially derived organic matter in the aquatic realm (Cooke et al., 2008b, 2009; Zhu et al., 2011; Doğrul Selver et al., 2012). Furthermore, these effects would almost certainly lead to variations in the values of the R_{soil} and R'_{soil} proxies with values at BM significantly lower than those reported recently for other soils (Table 2; see review in Spencer-Jones et al., 2015).

4.3 Preservation of hopanoids at BM

This is the first study to investigate the BHP distribution in peat samples to a depth of 410 cm, equivalent to an age of ~3000 cal. yr BP. We observe robust preservation of complex, highly functionalised BHPs at BM although there are reports of similarly complex hopanoids in marine sediments from the Congo deep sea fan dating to over 2.5 Ma (Handley et al., 2010; Talbot et al., 2014; Spencer-Jones, 2016) and the oldest confirmed biohopanoid is BHT, in ~50 Ma sediments from Tanzania (van Dongen et al., 2006).

Earlier studies on lignites, peats and soils have reported the rapid conversion of biohopanoids to geohopanoids (e.g. van Dorselaer et al., 1975; Quirk et al., 1984; Ries-Kautt and Albrecht, 1989; Dehmer, 1993, 1995). That is also true here, with a significant increase in geohopanoid concentrations with depth (data not shown). Nonetheless, at BM we continue to see a full suite of $17\beta,21\beta(H)$ tetra-, penta- and hexafunctionalised BHPs even at >400 cm depth indicating favourable preservation conditions (Table 2). Intriguingly, the concentration of BHPs in the deepest samples (402-410 cm) are equivalent to those in the most concentrated near surface samples (2-4 cm; Table 2).

Although originally ascribed to a purely aerobic source, recent work has shown that there are also a number of potential obligate and facultative anaerobic sources that can produce BHPs, including Planctomycetes (Sinninghe Damsté et al., 2004; Rush et al.,

2014), *Desulfovibrio* sp. (Deltaproteobacteria; Blumenberg et al., 2012),
Rhodopseudomonas palustris (Alphaproteobacteria; Rashby et al., 2007) and *Geobacter*
sp. (Deltaproteobacteria; Fischer et al., 2005; Eickhoff et al., 2013b). The BHPs produced
by members of these phyla and/or species are discussed above (see also Table 1);
however, at this time few species from these groups can be directly related to peat
environments. Therefore, although we cannot rule out some contribution from anaerobes
at depth in the peat core, we propose that the major proportion of the BHPs are produced
by aerobes at or above the water table and are subsequently preserved at depth.

5. Conclusions

A peat core from Bissendorfer Moor (Germany) contained a wide range of
structurally distinct BHPs at high concentrations to a depth of 410 cm (c. 2900 cal. yr BP).
By comparison with literature on *Sphagnum* peat microbiological communities, these lipids
can be linked primarily to heterotrophic but also methanotrophic and phototrophic
members of the peat microbiome. One of the most striking conclusions of this work, but
one that is consistent with previous work, was the relatively small impact of bacterial
methanotrophs on the BHP signature. Aminopentol, a biomarker for Type I methanotrophs,
was only present below 22 cm but never represented more than 0.4% of the total BHP
pool. Similarly aminotetrol, produced by both Type I and II methanotrophs, only accounted
for up to 2% of total BHPs. This suggests that even in peat deposits, methanotrophs
represent a relatively minor component of the bacterial (or at least the hopanoid-
producing) population; settings where much higher proportions occur (e.g. Talbot et al.,
2016) must be characterised by a particularly strong methane cycle. Collectively, the
types of compounds present are similar to those reported from soils whilst the relative
distributions show several distinct differences. The near surface samples contained

714 exceptionally high levels of a number of unsaturated BHPs including two isomers of
715 unsaturated BHT, a feature which has not been reported elsewhere. A second major
716 difference between BHPs in BM peat and those reported for soils was the relative
717 abundance of adenosylhopane and related structures, which was as high as 15% in a few
718 (near) surface horizons but generally below 10% and much lower (~1.7%) in deeper
719 layers. These values are significantly lower than those typically reported for soils from
720 other environments (tropical, temperate, Arctic) and are likely influenced by the low pH of
721 the peat environment.

722 BHP signatures in peat are unique and do appear to record specific peat
723 biogeochemical and ecological features, albeit with complex controls which are not yet
724 fully understood. They also have strong preservation potential such that they could be
725 useful in examining peat paleo-archives.

726

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740 **References**

- 741 Allen, M.A., Neilan, B.A., Burns, B.P., Jahnke, L.L., Summons, R.E., 2010. Lipid biomarkers in
742 Hamelin Pool microbial mats and stromatolites. *Organic Geochemistry* 41, 1207–1218.
- 743 Andersen, R., Chapman, S.J., Artz, R.R.E., 2013. Microbial communities in natural and disturbed
744 peatlands: A review. *Soil Biology and Biochemistry* 57, 979-994.
- 745 Auman, A.J., Speake, C.C., Lidstrom, M.E., 2001. *nifH* sequences and nitrogen fixation in type I
746 and type II methanotrophs. *Applied and Environmental Microbiology* 67, 4009–4016.
- 747 Banta, A., Wei, J.H., Welander, P.V., 2015. A distinct pathway for tetrahymanol synthesis in
748 bacteria. *Proceedings of the National Accademy of Science of the USA* 112, 13478-13483.
- 749 Belova, S.E., Pankratov, T.A., Dedysh, S.N., 2006. Bacteria of the genus *Burkholderia* as a typical
750 component of the microbial community of *Sphagnum* peat bogs. *Microbiology* 75, 90-96.
- 751 Blumenberg, M., Krüger, M., Nauhaus, K., Talbot, H.M., Oppermann, B.I., Seifert, R., Pape, T.,
752 Michaelis, W., 2006. Biosynthesis of hopanoids by sulfate-reducing bacteria (genus
753 *Desulfovibrio*). *Environmental Microbiology* 8, 1220-1227.
- 754 Blumenberg, M., Seifert, R., Kasten, S., Bahlmann, E., Michaelis, W., 2009a. Euphotic zone
755 bacterioplankton sources major sedimentary bacteriohopanepolyols in the Holocene Black
756 Sea. *Geochimica et Cosmochimica Acta* 73, 750-766.
- 757 Blumenberg, M., Oppermann, B.I., Guyoneaud, R., Michaelis, W., 2009b. Hopanoid production by
758 *Desulfovibrio bastinii* isolated from oilfield formation water. *FEMS Microbiology Letters* 293, 73–
759 78.
- 760 Blumenberg, M., Mollenhauer, G., Zabel, M., Reimer, A., Thiel, V., 2010. Decoupling of bio- and
761 geohopanoids in sediments of the Benguela Upwelling System (BUS). *Organic Geochemistry*
762 41, 1119–1129.
- 763 Blumenberg, M., Hoppert, M., Krüger, M., Dreier, A., Thiel, V., 2012. Novel findings on hopanoid
764 occurrences among sulfate reducing bacteria: is there a direct link to nitrogen fixation? *Organic*
765 *Geochemistry* 49, 1–5.
- 766 Bradley, A.S., Pearson, A., Sáenz, J.P., Marx, C.J., 2010. Adenosylhopane: the first intermediate
767 in hopanoid side chain biosynthesis. *Organic Geochemistry* 41, 1075–1081.
- 768 Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., Berg, G., 2012a. *Sphagnum*
769 mosses harbour highly specific bacterial diversity during their whole lifecycle. *The ISME*
770 *Journal* 6, 802-813.
- 771 Bragina, A., Maier, S., Berg, C., Müller, H., Chobot, V., Hadacek, F., Berg, G., 2012b. Similar
772 diversity of Alphaproteobacteria and nitrogenase gene amplicons on two related *Sphagnum*
773 mosses. *Frontiers in Microbiology*. 2, Article 275, doi: 10.3389/fmicb.2011.00275.
- 774 Bragina, A., Berg, C., Müller, H., Moser, D., Berg, G., 2013. Insights into functional bacterial
775 diversity and its effects on Alpine bog ecosystem functioning. *Nature Scientific Reports* 3,
776 1955; DOI:10.1038/srep01955

777 Bravo, J-M., Perzel, M., Härtner, T., Kannenberg, E.L., Rohmer, M., 2001. Novel methylated
778 triterpenoids of the gammacerane series from the nitrogen-fixing bacterium *Bradyrhizobium*
779 *japonicum* USDA 110. European Journal of Biochemistry 268, 1323-1331.

780 Chambers, F.M., Booth, R.K., De Vleeschouwer, F., Lamentowicz, M., Le Roux, G., Mauquoy, D.,
781 Nichols, J.E., van Geel, B., 2012. Development and refinement of proxy-climate indicators from
782 peats. Quaternary International 268, 21-33.

783 Charman, D.J., Blundell, A., and ACCROTELM Members, 2007. A new European testate amoebae
784 transfer function for palaeohydrological reconstruction on ombrotrophic peatlands. Journal of
785 Quaternary Science 22, 209–221.

786 Chasar, L.S., Chanton, J.P., Glaser, P.H., Siegel, D.I., Rivers, J.S., .2000. Radiocarbon and stable
787 carbon isotopic evidence for transport and transformation of dissolved organic carbon,
788 dissolved inorganic carbon, and CH₄ in a northern Minnesota peatland. Global Biogeochemical
789 Cycles 14, 1095-1108.

790 Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., House, J.I., 2013. Carbon and Other
791 Biogeochemical Cycles: Climate Change 2013: The Physical Science Basis. Contribution of
792 Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate
793 Change Change. in O. Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K.
794 Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S.
795 Schlömer, C. von Stechow, Z. T. & M J.C. (eds), Climate Change 2013: The Physical Science
796 Basis. Contribution of Working Group I to the Fifth Assessment Report of the
797 Intergovernmental Panel on Climate Change Change. Cambridge University Press,
798 Cambridge, Cambridge, United Kingdom and New York, NY, USA.

799 Cooke, M.P., 2010. The Role of Bacteriohopanepolyols as Biomarkers for Soil Bacterial
800 Communities and Soil Derived Organic Matter (Ph.D. thesis). Newcastle University.

801 Cooke, M.P., Talbot, H.M., Farrimond, P., 2008a. Bacterial populations recorded in
802 bacteriohopanepolyol distributions in soils from Northern England. Organic Geochemistry 39,
803 1347-1350.

804 Cooke, M.P., Talbot, H.M., Wagner, T., 2008b. Tracking soil organic carbon transport to
805 continental margin sediments using soil-specific hopanoid biomarkers: a case study from the
806 Congo fan area. Organic Geochemistry 39, 958-971.

807 Cooke, M.P., van Dongen, B.E., Talbot, H.M., Semiletov, I., Shakhova, N., Guo, L., Gustafson, Ö.,
808 2009. Transport of terrestrial organic matter to the Arctic Ocean via the great Arctic rivers.
809 Organic Geochemistry 40, 1151-1159.

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

813 Cvejic, J.H., Bodrossy, L., Kovács, K.L., Rohmer, M., 2000a. Bacterial triterpenoids of the hopane
814 series from the methanotrophic bacteria *Methylocaldum* spp.: phylogenetic implications and

815 first evidence for an unsaturated aminobacteriohopanepolyol. FEMS Microbiology Letters 182,
816 361-365.

817 Cvejic, J.H., Rosa Putra, S., El-Beltagy, A., Hattori, R., Hattori, T., Rohmer, M., 2000b. Bacterial
818 triterpenoids of the hopane series as biomarkers for the chemotaxonomy of *Burkholderia*,
819 *Pseudomonas* and *Ralstonia* spp. FEMS Microbiology Letters 183, 295-299.

820 Danilova, O.V., Kulichevskaya, I.S., Rozova, O.N., Detkova, E.N., Bodelier, P.L.E., Trotsenko,
821 Y.A., Dedysh, S.N., 2013. *Methylomonas paludis* sp. nov., the first acid tolerant member of the
822 genus *Methylomonas*, from an acidic wetland. International Journal of Systematic and
823 Evolutionary Biology 63, 2282-2289.

824 Dedysh, S.N., Horz, H.-P., Dunfield, P., Liesack, W., 2001a. A novel pmoA lineage represented by
825 the acidophilic methanotrophic bacterium *Methylocapsa acidiphila* B2. Archives of Microbiology
826 177, 117-121.

827 Dedysh, S.N., Derakshani, M., Liesack, W., 2001b. Detection and enumeration of methanotrophs
828 in acidic *Sphagnum* peat by 16S rRNA fluorescence in situ hybridization, including the use of
829 newly developed oligonucleotide probes for *Methylocella palustris*. Applied and Environmental
830 Microbiology 67, 4850-4857.

831 Dedysh, S.N., Pankratov, T.A., Belova, S.E., Kulichevskaya, I.S., Liesack, W., 2006. Phylogenetic
832 analysis and in situ identification of bacteria community composition in an acidic *Sphagnum*
833 peat bog. Applied and Environmental Microbiology 72, 2110–2117.

834 Dedysh, S.N., 2009. Exploring methanotroph diversity in acidic northern wetlands: molecular and
835 cultivation-based studies. Microbiology 78, 655-669.

836 Dehmer, J., 1993. Petrology and organic geochemistry of peat samples from a raised bog in
837 Kalimantan (Borneo). Organic Geochemistry 20, 349-362.

838 Dehmer, J., 1995. Petrological and organic geochemical investigation of recent peats with known
839 environments of deposition. International Journal of Coal Geology 28, 111-138.

840 Doğrul Selver, A., Talbot, H.M., Gustafsson, Ö, Boulton, S., van Dongen, B.E., 2012. Soil organic
841 matter transport along a sub-Arctic river-sea transect. Organic Geochemistry 51, 63-72.

842 Doğrul Selver, A., Sparkes, R.B., Bischoff, J., Talbot, H.M., Gustafsson, Ö., Semiletov, I., Dudarev,
843 O., Boulton, S., van Dongen, B.E., 2015. Distributions of bacterial and archaeal membrane lipids
844 in surface sediments reflect differences in input and loss of terrestrial organic carbon along a
845 cross-shelf Arctic transect. Organic Geochemistry 83-84, 16-26.

846 Doughty, D.M., Hunter, R.C., Summons, R.E., Newman, D.K., 2009. 2-Methylhopanoids are
847 maximally produced in akinetes of *Nostoc punctiforme*: geobiological implications. Geobiology
848 7, 524-532.

849 Eickhoff, M., Birgel, D., Talbot, H.M., Peckmann, J., Kappler, A., 2013a. Oxidation of Fe(II) leads to
850 increased C-2 methylation of pentacyclic triterpenoids in the anoxygenic phototrophic
851 bacterium *Rhodopseudomonas palustris* strain TIE-1. Geobiology 11, 268-278.

852 Eickhoff, M., Birgel, D., Talbot, H.M., Peckmann, J., Kappler, A., 2013b. Bacteriohopanoid
853 inventory of *Geobacter sulfurreducens* and *Geobacter metallireducens*. *Organic Geochemistry*
854 58, 107-114.

855 Farrimond, P., Fox, P.A., Innes, H.E., Miskin, I.P., Head, I.M., 1998. Bacterial sources of
856 hopanoids in Recent sediments: improving our understanding of Ancient hopane biomarkers.
857 *Ancient Biomolecules* 2, 147–166.

858 Flesch, G., Rohmer, M., 1988. Prokaryotic hopanoids: the biosynthesis of the bacteriohopane
859 skeleton. Formation of isoprenic units from two distinct acetate pools and a novel type of
860 carbon/carbon linkage between a triterpene and D-ribose. *European Journal of Biochemistry*
861 175, 405-411.

862 Flesch, G., Rohmer, M., 1989. Prokaryotic triterpenoids. A novel hopanoid from the ethanol-
863 producing bacterium *Zymomonas mobilis*. *Biochemical Journal* 262, 673-675.

864 Fischer, W.W., Summons, R.E., Pearson, A., 2005. Targeted genomic detection of biosynthetic
865 pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe.
866 *Geobiology* 3, 33–40.

867 Garcia Costas, A.M., Tsukatani, Y., Rijpstra, W.I., Schouten, S., Welander, P.V., Summons, R.E.,
868 Bryant, D.A., 2012. Identification of the Bacteriochlorophylls, carotenoids, quinones, lipids, and
869 hopanoids of “*Candidatus Chloracidobacterium thermophilum*”. *Journal of Bacteriology* 194,
870 1158-1168.

871 Gong, L., Wang, H., Xiang, X., Qiu, X., Liu, Q., Wang, R., Zhao, R., Wang, C., 2015. pH shaping
872 the composition of sqhC-containing bacterial communities. *Geomicrobiology Journal* 32, 433-
873 444.

874 Gorham, E., 1991. Northern peatlands: Role in the carbon cycle and probable responses to
875 climatic warming. *Ecological Applications* 1, 182–195.

876 Granhall, U., Selander, H., 1973. Nitrogen fixation in a subarctic mire. *Oikos* 24, 8-15.

877 Handley, L., Talbot, H.M., Cooke, M.P., Anderson, K.E., Wagner, T., 2010. Diverse fully
878 functionalised bacteriohopanepolyol distributions up to 1.2 Ma in sediments from the Congo
879 deep-sea fan. *Organic Geochemistry* 41, 910-914.

880 Hanson, R.H., Hanson, T.E., 1996. Methanotrophic bacteria. *Microbiological Reviews* 60, 439–471.

881 Herrmann, D., Bissere, P., Connan, J., Rohmer, M., 1996. A non-extractable triterpenoid of the
882 hopanoid series in *Acetobacter xylinum*. *FEMS Microbiology Letters* 135, 323-326.

883 Höfle, S., Kusch, S., Talbot, H.M., Mollenhauer, G., Zubrzycki, S., Burghardt, S., Rethemeyer, J.
884 2015. Characterization of bacterial populations in Arctic permafrost soils using
885 bacteriohopanepolyols. *Organic Geochemistry* 88, 1-16.

886 Imhoff, J.F., 2001. Transfer of *Rhodopseudomonas acidophila* to the new genus *Rhodoblastus* as
887 *Rhodoblastus acidophilus* gen. nov., comb. nov. *International Journal of Systematic and*
888 *Evolutionary Microbiology* 51, 1863-1866.

889 Jahnke, L.L., Summons, R.E., Hope, J.M., Des Marais, D.J., 1999. Carbon isotopic fractionation in
890 lipids from methanotrophic bacteria II: the effects of physiology and environmental parameters
891 on the biosynthesis and isotopic signatures of biomarkers. *Geochimica et Cosmochimica Acta*
892 63, 79–93.

893 Joyeux, C., Fouchard, S., Llopiz, P., Neunlist, S., 2004. Influence of the temperature and the
894 growth phase on the hopanoids and fatty acids content of *Frateuria aurantia* (DSMZ 6220).
895 *FEMS Microbiology Ecology* 47, 371–379.

896 Kannenberg, E.L., Poralla, K., 1999. Hopanoid biosynthesis and function. *Naturwissenschaften* 86,
897 168–176.

898 Karofeld, E., Toom, M., 1999. Mud-bottoms in Männikjärve Bog, Central Estonia. *Proceedings of*
899 *the Estonian Academy of Sciences, Biology and Ecology* 48, 216-235.

900 Kelley, C.A., Dise, N.B., Martens, C.S., 1992. Temporal variations in the stable carbon isotopic
901 composition of methane emitted from Minnesota peatlands. *Global Biogeochemical Cycles* 6,
902 263-269.

903 Kim, J.-J., Talbot, H.M., Zarzycka, B., Bauersach, T., Wagner, T., 2011. Occurrence and
904 abundance of soil-specific bacterial membrane lipid markers in the Têt watershed (Southern
905 France): Soil-specific BHPs and branched GDGTs. *Geochemistry, Geophysics, Geosystems*
906 12 DOI: 10.1029/2010GC003364.

907 Kip, N., van Winden, J.F., Pan, Y., Bodrossy, L., Reichart, G.-J., Smolders, A.J.P., Jetten, M.S.M.,
908 Sinninghe Damsté, J.S., Op den Camp, H.J.M., 2010. Global prevalence of methane oxidation
909 by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience* 3, 617-621.

910 Kip, N., Dutilh, B.E., Pan, Y., Bodrossy, L., Neveling, K., Kwint, M.P., Jetten, M.S.M., Op den
911 Camp, H.J.M., 2011. Ultra-deep pyrosequencing of *pmoA* amplicons confirms the prevalence
912 of *Methylomonas* and *Methylocystis* in *Sphagnum* mosses from a Dutch peat bog.
913 *Environmental Microbiology Reports* 6, 667-673.

914 Kip, N., Fritz, C., Langelaan, E.S., Pan, Y., Bodrossy, L., Pancotto, V., Jetten, M.S.M., Smolders,
915 A.J.P., Op den Camp, H.J.M., 2012. Methanotrophic activity and diversity in different
916 *Sphagnum magellanicum* dominated habitats in the southernmost peat bogs of Patagonia.
917 *Biogeosciences* 9, 47-55.

918 Knani, M., Corpe, W.A., Rohmer, M., 1994. Bacterial hopanoids from the pink-pigmented
919 facultative methylotrophs (PPFMs) and from green plant surfaces. *Microbiology* 140, 2755–
920 2759.

921 Kool, D.M., Talbot, H.M., Rush, D., Ettwig, K., Sinninghe Damsté, J.S., 2014. Rare
922 bacteriohopanepolyols as markers for an autotrophic, intra-aerobic methanotroph. *Geochimica*
923 *et Cosmochimica Acta* 136, 114-125.

924 Kotsyurbenko, O.R., Friedrich, M.W., Simankova, M.V., Nozhevnikova, A.N., Golyshin, P.N.,
925 Timmis, K.N., Conrad, R., 2007. Shift from acetoclastic to H₂-dependent methanogenesis in a

926 West Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium*
927 Strain. Applied and Environmental Microbiology 73, 2344-2348.

928 Krivograd Klemenčič, A., Vrhovšek, D., 2003. Algae in the peat bogs Loverenška Jezera and Šijec
929 in Slovenia. Natura Croatica 12, 141-150.

930 Krivograd Klemenčič, A., Smolar-Žvanut, Istenic, D., Griessler-Bulc, T., 2010. Algal community
931 patterns in Slovenian bogs along environmental gradients. Biologia 65, 422-437.

932 Kuchta, T., Kubinec, R., Farkas, P., 1998. Analysis of hopanoids in bacteria involved in food
933 technology and food contamination. FEMS Microbiology Letters 159, 221-225.

934 Kulichevskaya, I.S., Guzev, V.S., Gorlenko, V.M., Liesack, W., Dedysh, S.V., 2006. *Rhodoblastus*
935 *sphagnicola* sp. nov., a novel acidophilic purple non-sulfur bacterium from *Sphagnum* peat bog.
936 International Journal of Systematic and Evolutionary Biology 56, 1397-1402.

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

940 Larmola, T., Tuittila, E.-S., Thijrola, M., Nykänen, H., Martikainen, P.J., Yrjälä, K., Tuomivirta, T.,
941 Fritze, H., 2010. The role of *Sphagnum* mosses in the methane cycling of a boreal mire.
942 Ecology 91, 2356–2365.

943 Larmola, T., Leppänen, S.M., Tuittila, E.-S., Aarva, M., Merilä, P., Fritze, H., Tirola, M., 2014.
944 Methanotrophy induces nitrogen fixation during peatland development. Proceedings of the
945 National Accademy of Science. USA 111, 734-739.

946 McClymont, E.L., Mauquoy, D., Yeloff, D., Broekens, P., van Geel, B., Charman, D.J., Pancost,
947 R.D., Chambers, F.M., Evershed, R.P., 2008. The disappearance of *Sphagnum imbricatum*
948 from Butterburn Flow, UK. Holocene 18, 991-1002.

949 McClymont, E.L., Bingham, E.M., Nott, C.J., Chambers, F.M., Pancost, R.D., Evershed, R.P.,
950 2011. Pyrolysis GC–MS as a rapid screening tool for determination of peat-forming plant
951 composition in cores from ombrotrophic peat. Organic Geochemistry 42, 1420-1435.

952 Metje, M., Frenzel, P., 2005. Effect of temperature on anaerobic ethanol oxidation and
953 methanogenesis in acidic peat from a northern wetland. Applied and Environmental
954 Microbiology 8191-8200.

955 Neunlist, S., Rohmer, M., 1985a. Novel hopanoids from the methylotrophic bacteria *Methylococcus*
956 *capsulatus* and *Methylomonas methanica* (22S)-35-aminobacteriohopane-30,31,32,33,34-
957 pentol and (22S)-35-amino-3 β -methylbacteriohopane-30,31,32,33,34-pentol. Biochemical
958 Journal 231, 635 - 639.

959 Neunlist, S., Rohmer, M., 1985b. The hopanoids of '*Methylosinus trichosporium*':
960 aminobacteriohopanetriol and aminobacteriohopanetetrol. Journal of General Microbiology
961 131, 1363-1367.

962 Op den Camp, H.J.M., Islam, T., Stott, M.B., Harhangi, H.R., Hynes, A., Schouten, S., Jetten,
963 M.S.M., Birkeland, N.K., Pol, A., Dunfield, P.F., 2009. Environmental, genomic and taxonomic

964 perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports 1, 293–
 965 306.

966 Opelt, K., Berg, G., 2004. Diversity and antagonistic potential of bacteria associated with
 967 bryophytes from nutrient-poor habitats of the Baltic Sea Coast. Applied and Environmental
 968 Microbiology 70, 6569–6579.

969 Opelt, K., Chobot, V., Hadacek, F., Schönmann, S., Eberl, L., Berg, G., 2007. Investigations of the
 970 structure and function of bacterial communities associated with *Sphagnum* mosses.
 971 Environmental Microbiology 9, 2795–2809.

972 Ourisson, G., Rohmer, M., 1992. Hopanoids 2. Biohopanoids: A novel class of bacterial lipids.
 973 Accounts of Chemical Research 25, 403-408.

974 Ourisson, G., Rohmer, M., Poralla, K., 1987. Prokaryotic hopanoids and other polyterpenoid sterol
 975 surrogates. Annual Review of Microbiology 41, 301 - 333.

976 Pancost, R.D., Baas, M., van Geel, B., Sinninghe Damsté, J.S., 2000. $\delta^{13}\text{C}$ values and radiocarbon
 977 dates of microbial biomarkers as tracers for carbon recycling in peat deposits. Geology 28,
 978 663-666.

979 Pancost, R.D., Baas, M., van Geel, B., Sinninghe Damsté, J.S., 2003. Response of an
 980 ombrotrophic bog to a regional climate event revealed by macrofossil, molecular and carbon
 981 isotopic data. The Holocene 13, 921-932.

982 Pancost, R.D., McClymont, E.L., Bingham, E.M., Roberts, Z., Charman, D.J., Hornibrook, E.R.C.,
 983 Blundell, A., Chambers, F.M., Lim, K.L.H., Evershed, R.P., 2011. Archaeol as a methanogen
 984 biomarker in ombrotrophic bogs. Organic Geochemistry 42, 1279-1287.

985 Peiseler, B., Rohmer, M., 1992. Prokaryotic triterpenoids of the hopane series.
 986 Bacteriohopanetetrols of new side-chain configuration from *Acetobacter* species. Journal of
 987 Chemical Research-S 298-299.

988 Pearson, A., Flood Page, S.R., Jorgenson, T.L., Fischer, W.W., Higgins, M.B. 2007. Novel
 989 hopanoid cyclases from the environment. Environmental Microbiology 9, 2175–2188.

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

993 Pfennig, N., 1969. *Rhodopseudomonas acidophilla*, sp. n., a new species of the budding purple
 994 nonsulfur bacteria. Journal of Bacteriology 99, 597-602.

995 Popp, T.J., Chanton, J.P., 1999. Methane stable isotope distribution at a *Carex* dominated fen in
 996 north central Alberta. Global Biogeochemical Cycles 13, 1063-1077.

997 Poralla, K., Härtner, T., Kannenberg, E., 1984. Effect of temperature and pH on the hopanoid
 998 content of *Bacillus acidocaldarius*. FEMS Microbiology Letters 23, 253–256.

999 Poralla, K., Muth, G., Härtner, T., 2000. Hopanoids are formed during transition from substrate to
 1000 aerial hyphae in *Streptomyces coelicolor* A3(2). FEMS Microbiology Letters 189, 93-95.

Quirk, M.M., Wardroper, A.M.K., Wheatley, R.E., Maxwell, J.R., 1984. Extended hopanoids in peat environments. *Chemical Geology* 42, 25-43.

Raghoebarsing, A.A., Smolders, A.J.P., Schmid, M.C., Rijpstra, W.I.C., Wolters-Arts, M., Derksen, J., Jetten, M.S.M., Schouten, S., Sinninghe Damsté, J.S., Lamers, L.P.M., Roelofs, J.G.M., Op den Camp, H.J.M., Strous, M., 2005. Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature* 436, 1153-1156.

Rahman, M.T., Crombie, A., Chen, Y., Stralis-Pavese, N., Bodrossy, L., Meir, P., McNamara, N.P., Murrell, J.C., 2011. Environmental distribution and abundance of the facultative methanotroph *Methylocella*. *ISME Journal* 5, 1061–1066.

Rashby, S.E., Sessions, A.L., Summons, R.E., Newman, D.K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of the National Academy of Sciences USA* 104, 15099-15104.

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

Řezanka, T., Siristova, L., Melzoch, K., Sigler, K., 2011. *N*-acylated bacteriohopanehexol-mannosamides from the thermophilic bacterium *Alicyclobacillus acidoterrestris*. *Lipids* 46, 249–261.

Ricci, J.N., Coleman, M.L., Welander, P.V., Sessions, A.L., Summons, R.E., Spear, J.R., Newman, D.K., 2014. Diverse capacity for 2-methylhopanoid production correlates with a specific ecological niche. *ISME Journal* 8,675–684.

Ries-Kautt, M., Albrecht, P., 1989. Hopane-derived triterpenoids in soils. *Chemical Geology* 76, 143-151.

Rohmer, M., 1993. The biosynthesis of the triterpenoids of the hopane series in the Eubacteria: A mine of new enzyme reactions. *Pure and Applied Chemistry* 65, 1293 - 1298.

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

Rohmer, M., Ourisson, G., 1986. Unsaturated bacteriohopanepolyols from *Acetobacter acetii* ssp. *xylinum*. *Journal of Chemical Research S*, 356-357.

Rosa-Putra, S., Nalin, R., Domenach, A.-M., Rohmer, M., 2001. Novel hopanoids from *Frankia* spp. and related soil bacteria. Squalene cyclization and significance of geological biomarkers revisited. *European Journal of Biochemistry* 268, 4300-4306.

Rush, D., Sinninghe Damsté, J.S., Poulton, S.W., Thamdrup, B., Garside, A.L., Acuña González, J., Schouten, S., Jetten, M.S.M., Talbot, H.M., 2014. Anaerobic ammonium-oxidising bacteria: the only known biological source of the bacteriohopanetetrol stereoisomer in marine sediments. *Geochimica et Cosmochimica Acta* 140, 50-64.

Sáenz, J.P., Sezgin, E., Schwille, P., Simons, K., 2012. Functional convergence of hopanoids and sterols in membrane ordering. *Proceedings of the National Academy of Sciences of the United*

States of America 109, 14236–14240.

Segers, R., 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* 41, 23-51.

Serkebaeva, Y.M., Kim, Y., Liesack, W., Dedysh, S.N., 2013. Pyrosequencing-based assessment of the bacteria diversity in surface and subsurface peat layers of a norther wetland, with focus on poorly studies phyla and candidate divissions. *PLoS One* 8, e63994. doi:10.1371/journal.pone.0063994

Shcherbakov, A.V., Bragina, A.V., Kuzmina, E.Y., Berg, C., Muntyan, A.N., Makarova, N.M., Malfanova, N.V., Cardinale, M., Berg, G., Chebotar, V.K., Tikhonovich, I.A., 2013. Endophytic bacteria of *Sphagnum* mosses as promising objects of agricultural microbiology. *Microbiology* 82, 306-315.

Simonin, P., Tindall, B., Rohmer, M., 1994. Structure elucidation and biosynthesis of 31-methylhopanoids from *Acetobacter europaeus*. *European Journal of Biochemistry* 225, 765-771.

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

Spahni, R., Wania, R., Neef, L., van Weele, M., Pison, I., Bousquet, P., Frankenberg, C., Foster, P.N. Joos., F., Prentice, I.C., van Velthoven, P., 2011. Constraining global methane emissions and uptake by ecosystems. *Biogeosciences* 8, 1643-1665.

Spencer-Jones, C.L., 2016. Novel Concepts Derived from Microbial Biomarkers in the Congo System: Implications for Continental Methane Cycling (PhD thesis). Newcastle University, UK.

Spencer-Jones, C.L., Wagner, T., Dinga, B.J., Schefuß, E., Mann, P.J., Poulsen, J.R., Spencer, R.G.M., Wabakanghanzi, J.N., Talbot, H.M., 2015. Bacteriohopanepolyols in tropical soils and sediments: sources and trends. *Organic Geochemistry* 89-90, 1-13.

Summons, R.E., Jahnke, L.L., Hope, J.M., Logan, G.A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature* 400, 554-557.

Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R., Asiegbu, F.O., 2014. Bacterial diversity and community structure along different peat soils in boreal forest. *Applied Soil Ecology* 74, 37-45.

Sundh, I., Mikkilä, Nilsson, M., Svensson, B.H., 1995. Potential aerobic methane oxidation in a *Sphagnum*-dominated peatland – controlling factors and relation to methane emission. *Soil Biology and Biochemistry* 27, 829-837.

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

Talbot, H.M., Watson, D.F., Murrell, J.C., Carter, J.F., Farrimond, P., 2001. Analysis of intact bacteriohopanepolyols from methanotrophic bacteria by reversed phase high performance

liquid chromatography - atmospheric pressure chemical ionisation - mass spectrometry. Journal of Chromatography A 921, 175-185.

Talbot, H.M., Squier, A.H., Keely, B.J., Farrimond, P., 2003a. Atmospheric pressure chemical ionisation reversed-phase liquid chromatography/ion trap mass spectrometry of intact bacteriohopanepolyols. Rapid Communications in Mass Spectrometry 17, 728-737.

Talbot, H.M., Summons, R., Jahnke, L., Farrimond, P., 2003b. Characteristic fragmentation of bacteriohopanepolyols during atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 17, 2788-2796.

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

Talbot, H.M., Rohmer, M., Farrimond, P., 2007b. Structural characterisation of unsaturated bacterial hopanoids by atmospheric pressure chemical ionisation liquid chromatography/ion trap mass spectrometry. Rapid Communications in Mass Spectrometry 21, 1613-1622.

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

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

Talbot, H.M., Bischof, J., Inglis, G.N., Collinson, M.E., Pancost, R.D., 2016. Polyfunctionalised bio- and geohopanoids in the Eocene Cobham Lignite. Organic Geochemistry. <http://dx.doi.org/10.1016/j.orggeochem.2016.03.006>

Taylor, K.A., Harvey, R.H., 2011. Bacterial hopanoids as tracers of organic carbon sources and processing across the western Arctic continental shelf. Organic Geochemistry 42, 487-497.

van Dongen, B.E., Talbot, H.M., Schouten, S., Pearson, N.P., Pancost, R.D., 2006. Well preserved Paleogene and Cretaceous biomarkers from the Kilwa area, Tanzania. Organic Geochemistry 37, 539-557.

van Dorselaer, A., Albrecht, P., Connan, J., 1975. Changes in composition of polycyclic alkanes by thermal maturation (Yallourn Lignite, Australia). In: R. Campos and J. Goni (Editors), Advances in Organic Geochemistry, 1975. Enadimsa, pp. 53-59.

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

1113 van Winden, J.F., Talbot, H.M., De Vleeschouwer, F., Reichart, G.-J., Sinninghe Damsté, J.S.,
 1114 2012b. Variation in methanotroph-related proxies in peat deposits from Misten Bog, Hautes-
 1115 Fagnes, Belgium. *Organic Geochemistry* 53, 73-79.

1116 Vilcheze, C., Llopiz, P., Neunlist, S., Poralla, K., Rohmer, M., 1994. Prokaryotic triterpenoids: new
 1117 hopanoids from the nitrogen-fixing bacteria *Azotobacter vinelandii*, *Beijerinckia indica* and
 1118 *Beijerinckia mobilis*. *Microbiology* 140, 2749 – 2753.

1119 Wagner, T., Kallweit, W., Talbot, H.M., Mollenhauer, G., Boom, A., Zabel, M., 2014. Microbial
 1120 biomarkers support organic carbon transport from Amazon wetlands to the shelf and deep fan
 1121 during recent and glacial climate conditions. *Organic Geochemistry* 67, 85-98.

1122 Welander, P.V., Summons, R.E., 2012. Discovery, taxonomic distribution, and phenotypic
 1123 characterization of a gene required for 3-methylhopanoid production. *Proceedings of the*
 1124 *National Accademy of Science of the USA* 109, 12905–12910.

1125 Welander, P.V., Hunter, R.C., Zhang, L., Sessions, A.L., Summons, R.E., Newman, D.K., 2009.
 1126 Hopanoids play a role in membrane integrity and pH homeostasis in *Rhodopseudomonas*
 1127 *palustris* TIE-1. *Journal of Bacteriology* 191, 6145–6156.

1128 Welander, P.V., Coleman, M.L., Sessions, A.L., Summons, R.E., Newman, D.K., 2010.
 1129 Identification of a methylase required for 2-methylhopanoid production and implications for the
 1130 interpretation of sedimentary hopanes. *Proceedings of the National Accademy of Science of*
 1131 *the USA*. 107, 8537–8542.

1132 Xie, S., Nott, C.J., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P., 2004. Molecular and
 1133 isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. *Geochimica et*
 1134 *Cosmochimica Acta* 68, 2849-2862.

1135 Xu, Y., Cooke, M.P., Talbot, H.M., Simpson, M.E., 2009. Bacteriohopanepolyol signatures of
 1136 bacterial populations in Western Canadian soils. *Organic Geochemistry* 40, 79-86.

1137 Yeloff, D., Bennett, K.D., Blaauw, M., Mauquoy, D., Sillasoo, U., van der Plicht, J., van Geel, B.,
 1138 2006. High precision ¹⁴C dating of Holocene peat deposits: a comparison of Bayesian
 1139 calibration and wiggle-matching approaches. *Quaternary Geochronology* 1, 222–235.

1140 Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W., Hunt, S.J., 2010. Global peatland dynamics since
 1141 the Last Glacial Maximum. *Geophysical Research Letters* 37, L13402.
 1142 <http://dx.doi.org/10.1029/2010GL043584>.

1143 Zadorina. E.V., Slobodova. N.V., Boulygina. E.S., Kolganova. T.V., Kravchenko. I.K., Kuznetsov,
 1144 B.B., 2009. Diversity of diazotrophic bacteria in peat soil by cloning of the *nifH* gene.
 1145 *Microbiology* 78, 218-226.

1146

1147 Zhang, Z., Zimmermann, N.E., Kaplan, J.O., Poulter, B., 2016. Modeling spatiotemporal dynamics
 1148 of global wetlands: comprehensive evaluation of a new sub-grid TOPMODEL parameterization
 1149 and uncertainties. *Biogeosciences* 13, 1387-1408.

1150 Zheng, Y., Singarayer, J.S., Cheng, P., Yu, X., Liu, Z., Valdes, P.J., Pancost, R.D., 2014. Holocene
1151 variations in peatland methane cycling associated with the Asian summer monsoon system.
1152 Nature Communications 5, DOI: 10.1038/ncomms5631

1153 Zhu, C., Talbot, H.M., Wagner, T., Pan, J.M., Pancost, R.D., 2011. Distribution of hopanoids along
1154 a land to sea transect: Implications for microbial ecology and the use of hopanoids in
1155 environmental studies. Limnology and Oceanography 56, 1850–1865.

1156 Zhu, B., van Dijk, G., Fritz, C., Smolders, A.J.P., Pol, A., Jetten, M.S.M., Ettwig, K.F., 2012.
1157 Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent
1158 methane-oxidising bacteria. Applied and Environmental Microbiology 78, 8657-8665.

1159 Zundel, M., Rohmer, M., 1985. Prokaryotic triterpenoids 3. The biosynthesis of 2 β -
1160 methylhopanoids and 3 β -methylhopanoids of *Methylobacterium organophilum* and *Acetobacter*
1161 *pasteurianus* ssp. *pasteurianus*. European Journal of Biochemistry 150, 35-39.
1162
1163
1164

1165 **Appendix 1.**

1166 Ring system and side chains of BHPs observed in peat samples. Side chain structures h, i,
 1167 n and o are based on LC-MSⁿ analysis only. All other side chain structures shown have
 1168 previously been unambiguously identified by NMR. When identified in this study using LC-
 1169 MS only where stereochemistry can not be confirmed, we have assumed the structure to
 1170 be the same as that previously characterised but the occurrence of additional/alternative
 1171 isomers of the side chain cannot be excluded.
 1172

n

o

p (R= unknown)

1173

1174

1175

1176 List of Figures

1177

1178

1179 **Figure 1.** Concentration (black bars; $\mu\text{g g}^{-1}\text{TOC}$) and relative abundance (open diamonds)
1180 as % of total BHPs of dominant BHPs (a) BHT, (b) BHT-Cyclitol ether, (c) aminotriol, and
1181 methanotroph specific markers (d) aminotetrol and (e) aminopentol. Grey shaded area
1182 indicates region of water table variability (0-56 cm; Charman et al., 2007).

1183

1184 **Figure 2.** Concentration (black bars, $\mu\text{g g}^{-1}\text{TOC}$) and relative abundance (open diamonds)
1185 as % of total BHPs of (a) combined unsaturated BHT [2 isomers], (b) unsaturated BHT-
1186 pentose, (c) BHT-pentose, (d) adenosylhopane (e) combined total 2-
1187 methyladenosylhopane, Adenosylhopane-group 2 and C-2 methylated adenosylhopane-
1188 group 2 (see appendix for individual structures). Grey shaded area indicates region of
1189 water table variability (0-56 cm; Charman et al., 2007).

1190

1191 **Figure 3.** Relative abundance of soil marker BHPs with depth in *Sphagnum* peat samples
1192 as % of total BHPs (a). Frequency of samples with relative abundance of soil marker BHPs
1193 in terrestrial samples in indicated ranges from (b) Peat, (c) Tropical and sub-tropical soils
1194 (d) Temperate soils and (e) Arctic samples (Key: OM and S = Organic matter and surface
1195 permafrost soil; D = deep permafrost soils; IC = Ice complex; c = polygon centre; r =
1196 polygon rim). (Data from this study; Cooke et al., 2008a; Xu et al., 2009; Rethemeyer et
1197 al., 2010; Kim et al., 2011; Zhu et al., 2011; van Winden et al., 2012a,b; Wagner et al.,
1198 2014; Doğrul Selver et al., 2015; Höfle et al., 2015; Spencer-Jones et al., 2015).

1199

1200 **Table 1.** Intact bacteriohopanepolyols identified in BM peat sample and potential sources known to be found in peat including
1201 heterotrophs, methanotrophs and phototrophs. (Note some species also produce other compounds not observed in this study; See
1202 Talbot et al., 2008 for review.)

APCI Base Peak ion (m/z) BHP structure number ^b	746 ^a lg	760 ^a llg	761 lh	775 llh	712 IVd or Vd	714 ld	728 lld	728 llld	772 le	830 lf	653 IVa,b or c	655 la	669 lla	1002 lj and/or lk	1002 lm	1058 IVn or Vn	1060 ln	1118 lo	1132 lp	941 IVi or Vi	943 li	957 lli	References
HETEROTROPHS^c																							
PROTEOBACTERIA																							
Alphaproteobacteria																							
<i>Acetobacter pasteurianus</i> (AAB) ^{d,e}											+	+											Zundel and Rohmer, 1985
<i>Acetobacter europaeus</i> (AAB) ^{d,e}											+	+											Simonin et al., 1994
<i>Acetobacter aceti xylinus</i> (AAB) ^{d,e}											+	+		+	+	+	+						Talbot et al., 2007b
<i>Beijerinckia indica</i>						+						+											Vilcheze et al., 1994
<i>Beijerinckia mobilis</i>						+																	Vilcheze et al., 1994
<i>Bradyrhizobium</i> sp.	+	+				+																	Bravo et al., 2001
<i>Methylobacterium</i> spp. (n = 8) ^{g,e}												+		+	+								Knani et al., 1994
<i>Zymomonas mobilis</i>												+		+	+								Flesch and Rohmer, 1989
Betaproteobacteria																							
<i>Burkholderia</i> spp. (n = 9)												+		+	+		(+) ^h						Cvejic et al., 2000b
Gammaproteobacteria																							
<i>Azotobacter vinelandii</i>														+			+						Vilcheze et al., 1994
<i>Frateuria aurantia</i> (AAB)												+		+	+		+						Joyeux et al., 2004
Deltaproteobacteria																							
<i>Geobacter</i> spp. (n = 2) ^e												+		+	+								Eickhoff et al., 2013b
Acidobacteria																							
Actinobacteria																							
<i>Frankia</i> spp. ^e												+											Rosa-Putra et al., 2001
<i>Streptomyces coelicolor</i> A3(2)						+																	Poralla et al., 2000
Firmicutes																							
<i>Alicyclobacillus acidocaldarius</i> ^e												+			+								Poralla et al., 1984
<i>Alicyclobacillus acidoterrestris</i> ^e												+			+		+	+					Řezanka et al., 2011
Planctomycetes																							
												(+)		(+)									Sinnighe Damsté et al., 2004; Rush et al., 2014

Table 1. Continued

APCI Base Peak ion (<i>m/z</i>) BHP structure number ^b	746 ^a lg	760 ^a lig	761 lh	775 llh	712 IVd or Vd	714 ld	728 lld	728 llld	772 le	830 lf	653 IVa,b or c	655 la	669 lla	1002 lj and/or lk	1002 lm	1058 IVn or Vn	1060 ln	1118 lo	1132 lp	941 IVi or Vi	943 li	957 lli	References
<u>METHANOTROPHS</u>																							
Alphaproteobacteria																							
<i>Methylocella</i> spp.	+					+						+											van Winden et al., 2012a
<i>Methylosinus</i> sp.						+			+			+											Neunlist and Rohmer, 1985b
<i>Methylocystis</i> sp.						+			+														Talbot et al., 2001
Gammaproteobacteria																							
<i>Methylovulum</i> sp. ^e					+	+			+	+													van Winden et al., 2012a
<i>Methylomonas</i> sp.									+	+													Neunlist and Rohmer, 1985a
<i>Methylococcus</i> sp. ^e									+	+													Neunlist and Rohmer, 1985a
<i>Methylocaldum</i> spp. ^e									(+)	+													Cvejic et al., 2000a
Verucomicrobia						+																	van Winden et al., 2012a
<u>PHOTOTROPHS</u>																							
Alphaproteobacteria																							
PNSB ^{i,e}	+		(+)		(+)	(+)						(+)	(+)	(+)	(+)								Talbot et al., 2007a
<i>Rhodoblastus</i> sp.	+											+		+	+								Talbot et al., 2007a
Cyanobacteria ^e	(+)					(+)	(+)				(+)	(+)	(+)	(+)	(+)		(+)			(+)	(+)	(+)	Talbot et al., 2008
<i>Gloeocapsa</i> sp.											+	+	+							+	+	+	Talbot et al., 2008

^a Di, tri- and tetraacetate forms are known and observed here.

^b See Appendix for structures

^c BHP producing organisms are listed by group (Heterotroph, Methanotroph, Phototroph) and within group by Phylum. Specific genera or species are listed only if considered to potentially be present in *Sphagnum* peat (see text for further details); alternatively only BHP biosynthesised by other members of the phylum/sub-group are indicated

^d AAB = acetic acid bacteria

^e indicates organism also produces other BHPs not identified in this study

^f + = present in species (or all members of group tested to date; see Talbot et al., 2008 for review)

^g number of species identified to produce BHPs

^h (+) indicates compound found in some but not all tested members of group/genus

1214 ⁱ PNSB = Purple non-sulphur bacteria.
1215

1216

1217 Table 2. Total organic Carbon (%) and concentration of individual BHPs ($\mu\text{g g}^{-1}\text{TOC}$) and grouped structural types in peat samples from

1218 Bissendorfer Moor.

Base peak (m/z)		653	653	655	669	712	714	728	728	772	830	746	760	761	775	941	943	957
Depth	% TOC	IVa,	IVa,	Ia	Ila	IVd or	Ib	IIb	IIIb	Ie	If	Ig	IIg	Ih	Ih	IVi or	li	lli
cm		b or c	b or c			Vd										Vi		
0-2	41.440	21	18	20	6.4	0.00	69	1.4	bdl ^a	1.3	bdl	9.2	bdl	bdl	1.0	160	36	bdl
2-4	40.155	35	40	280	75	15	860	10	bdl	8.9	bdl	250	15	40	24	190	150	bdl
4-6	43.335	18	33	47	12	12	240	bdl	bdl	bdl	bdl	24	bdl	3.3	bdl	290	95	bdl
6-8	40.885	11	8.0	58	19	4.9	280	bdl	bdl	2.0	bdl	30	bdl	7.8	7.2	63	48	bdl
8-10	42.030	10	15	90	28	4.8	240	5.0	bdl	1.8	bdl	98	5.3	13	7.3	48	47	bdl
10-12	44.910	3.3	bdl	46	18	4.1	140	bdl	bdl	1.0	bdl	62	bdl	6.0	4.2	23	30	bdl
12-14	44.705	bdl	bdl	41	14	bdl	200	bdl	bdl	1.8	bdl	36	bdl	7.9	1.3	13	15	bdl
14-16	45.495	0.8	bdl	14	12	1.1	79	2.1	bdl	1.4	bdl	12	0.2	bdl	bdl	2.7	4	bdl
16-18	42.385	bdl	bdl	110	68	bdl	380	6.5	bdl	8.0	bdl	47	bdl	bdl	bdl	23	43	bdl
18-20	41.620	bdl	2.4	52	24	1.3	170	1.8	1.1	2.2	bdl	23	bdl	bdl	bdl	7.0	13	bdl
20-22	42.095	bdl	bdl	120	42	bdl	470	16	4.4	13	bdl	45	4.2	bdl	bdl	20	50	bdl
22-24	44.305	bdl	bdl	120	31	2.0	300	11	2.9	5.9	0.5	35	1.1	bdl	bdl	10	29	bdl
24-26	41.900	bdl	bdl	180	82	bdl	390	21	7.5	22	3.5	69	bdl	bdl	bdl	bdl	49	bdl
26-28	42.450	bdl	bdl	400	150	bdl	680	46	bdl	37	3.9	210	bdl	bdl	bdl	bdl	82	bdl
28-30	42.985	bdl	bdl	190	90	bdl	400	27	bdl	16	1.4	66	2.2	bdl	bdl	bdl	35	bdl
30-32	43.340	bdl	bdl	77	29	bdl	98	7.3	bdl	7.5	bdl	32	0.7	bdl	bdl	bdl	10	bdl
32-34	43.290	bdl	bdl	27	9.5	bdl	51	2.5	bdl	2.8	bdl	9.0	0.1	bdl	bdl	bdl	7	bdl
36-38	44.485	bdl	bdl	93	28	bdl	210	15	1.9	9.4	bdl	36	bdl	bdl	bdl	bdl	50	bdl
40-42	45.460	bdl	bdl	84	17	bdl	160	7.7	bdl	4.9	1.2	31	0.5	bdl	bdl	bdl	17	bdl
70-72	43.100	bdl	bdl	26	4.3	bdl	140	6.5	bdl	3.9	0.9	13	bdl	bdl	bdl	bdl	8	bdl
96-98	44.100	bdl	bdl	33	5.8	1.1	160	7.2	1.2	4.2	1.5	12	0.3	bdl	bdl	1.9	6	bdl
100-102	44.775	bdl	bdl	66	5.7	bdl	300	9.4	bdl	7.7	1.9	12	bdl	bdl	bdl	bdl	16	bdl
126-128	42.890	bdl	bdl	63	7.5	1.9	320	26	bdl	5.0	2.6	24	bdl	bdl	bdl	2.8	7	bdl
402-404	46.755	13	10	370	79	3.5	910	190	8.6	19	4.3	92	bdl	4.8	bdl	42	230	5.00
404-406	47.720	13	7.6	420	100	3.2	600	91	9.6	17	3.4	130	3.6	7.4	bdl	46	190	11.00
406-408	49.485	6.0	5.1	210	38	2.7	310	30	2.5	10	1.9	36	bdl	1.5	bdl	16	63	4.20

1219

408-410 48.515 4.7 6.1 170 27 1.5 440 33 2.7 8.7 2.0 32 bdl 2.0 bdl 15 60 0.00

1220 Table 2. Continued

Base Peak (m/z) Depth cm	1002 Ij and/ or Ik	1002 Im	1058 IVn or Vn	1060 In	1118 Io	1132 Ip	Total μg g ⁻¹ TOC	% Tetra ^b	% Penta ^b	% Hexa ^b	% Soil ^b	% Unsat ^b	R _{soil} ^c
0-2	50	3.2	3.9	8.9	14	15	438.3	87.8	3.2	6.6	2.3	46.3	0.34
2-4	560	28	4.8	45	30	44	2704.7	82.9	2.2	2.7	12.2	10.5	0.54
4-6	180	14	7.2	26	37	18	1056.5	89.1	3.1	5.2	2.6	34.1	0.37
6-8	110	12	1.2	6.0	12	4.2	684.3	89.7	1.3	2.4	6.6	12.9	0.44
8-10	110	9.0	1.9	11	12	4.3	761.4	79.7	1.9	2.1	16.2	10.5	0.58
10-12	100	7.7	bdl	4.3	3.6	3.7	456.9	81.4	1.2	1.6	15.8	6.7	0.61
12-14	91	2.1	bdl	6.6	4.8	1.4	435.9	86.3	1.9	1.4	10.4	3.0	0.52
14-16	34	0.9	bdl	1.2	0.7	0.0	165.9	90.7	1.6	0.4	7.4	2.8	0.47
16-18	180	8.7	bdl	7.4	7.9	2.5	892.0	91.8	1.7	1.2	5.3	2.6	0.30
18-20	69	2.6	0.2	2.2	2.9	0.6	375.3	91.7	1.2	0.9	6.1	2.9	0.31
20-22	400	18	bdl	13	5.4	2.3	1223.3	93.2	2.1	0.6	4.0	1.6	0.29
22-24	210	4.5	bdl	9.0	4.0	1.2	777.1	92.7	1.9	0.7	4.6	1.5	0.23
24-26	440	15	bdl	19	12	1.4	1311.4	90.3	3.1	1.3	5.3	0.0	0.28
26-28	930	bdl	bdl	28	8.3	5.0	2580.2	88.7	2.5	0.7	8.1	0.0	0.34
28-30	300	12	bdl	2.6	4.5	1.8	1148.5	91.8	1.6	0.7	5.9	0.0	0.26
30-32	65	2.0	bdl	1.7	0.9	bdl	331.0	87.1	2.8	0.3	9.9	0.0	0.30
32-34	45	1.2	bdl	0.7	0.4	bdl	155.8	91.7	2.2	0.3	5.8	0.0	0.25
36-38	180	7.6	bdl	4.3	2.5	bdl	637.7	91.8	2.1	0.4	5.6	0.0	0.28
40-42	110	1.5	bdl	1.9	1.2	bdl	437.9	90.7	1.6	0.5	7.2	0.0	0.27
70-72	150	2.5	bdl	2.1	0.9	bdl	358.4	94.2	1.7	0.5	3.6	0.0	0.33
96-98	130	2.0	bdl	1.0	1.3	0.5	369.3	94.4	1.4	0.9	3.3	0.8	0.27
100-102	260	3.3	bdl	3.3	2.3	bdl	687.6	96.0	1.6	0.6	1.7	0.0	0.15
126-128	400	5.8	0.2	5.0	2.5	1.0	874.2	95.4	1.2	0.7	2.7	0.6	0.28
402-404	740	25	0.7	23	8.3	4.6	2782.8	94.4	1.5	0.6	3.5	2.5	0.21
404-406	630	7.3	0.5	26	11	7.0	2334.6	91.2	1.9	0.9	6.0	3.0	0.25
406-408	340	3.7	0.3	9.0	7.7	2.9	1100.5	93.7	1.8	1.1	3.4	2.7	0.15

408-410	290	3.6	0.5	6.8	5.2	2.8	1113.6	94.6	1.4	0.9	3.1	2.5	0.17
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1221 ^a bdl = below detection limit;

1222 ^b tetra = tetrafunctionalised BHPs with functional groups at C32, 33, 34 and 35, penta = pentafunctionalised with additional functional
 1223 group at C31, hexa = hexafunctionalised with additional functional groups at C30 and 31, soil = all soil-marker BHPs inclidng
 1224 adenosylhopane and related structures (**lg, llg, lh, llh**), unsat = the combined relative % of all unsaturated BHPs (tetra- and
 1225 pentafunctionalised) with ring systems **IV** and/or **V**;

1226 ^c $R_{soil} = (\mathbf{lg+llg+lh+llh})/(\mathbf{la+lg+llg+lh+llh})$ (Zhu et al., 2011).

1227

Figure 2

Figure 3