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 μg g^{-1}_{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

present only at very low levels, supporting previous studies which have shown that the majority of precursor organisms biosynthesising hopanoids in peat environments are heterotrophs. The surface layers also contained a highly unusual signature comprising high concentrations of unsaturated compounds, including unsaturated bacteriohopanetetrol pseudopentose, which has previously only been reported in *Gloeocapsa* cyanobacteria. This genus is known to occur in symbiotic association with host *Sphagnum* species, and has the ability to fix atmospheric nitrogen which is a well known trait amongst members of the peat microbiome and amongst hopanoid producing microorganisms. The apparent capacity for hopanoids to protect organisms from external stresses such as low pH is therefore likely to be a significant factor accounting for the high BHP contributions from heterotrophs, methanotrophs and phototrophic organisms in *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 hibridisation 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 CO_2 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

revealed that they are mainly derived from *Alphaproteobacteria*, a group of highly diverse organisms including phototrophs, heterotrophs and methanotrophs (Bragina et al., 2012b). However, the ability to fix N_2 is also widely distributed in other phyla across the *Sphagnum* peat microbiome; for example Betaproteobacteria of the genus *Burkholderia* sp., are also known to be important sources of fixed N_2 in peatlands (e.g. Belova et al., 2006; Opelt et al., 2007; Bragina et al., 2013; Shcherbakov et al., 2013).

The ability to fix nitrogen and/or oxidise methane is a very common, although not universal trait, amongst organisms which produce hopanoids (e.g. Pearson et al., 2007; Blumenberg et al., 2012; Ricci et al., 2014). Hopanoids are pentacyclic triterpenoid lipids produced by some bacteria (e.g. Rohmer et al., 1984; Ourisson et al., 1987; Ourisson and Rohmer, 1992; Farrimond et al., 1998; Talbot et al., 2008; Pearson et al., 2009). These compounds typically consist of a C_{30} ring system (**I**; see Appendix), although variations such as methylation at C-2 (**II**) or C-3 (**III**) and unsaturation at C-6 and/or C-11 (**IV**-**VI**) do occur; however, the latter are rarely observed in environmental materials. They can also contain an extended polyfunctionalised side chain derived from D-ribose (Flesch and Rohmer, 1988) and are termed bacteriohopanepolyols (BHPs).

Typical structures have functional groups present at C-32, 33, 34 and 35, the most common example being bacteriohopane-32,33,34,35-tetrol (BHT, **Ia**). Structures with additional hydroxyl moieties at C-31 and/or C-30 are also known (e.g. Rohmer, 1993). BHPs can be broadly assigned into one of two categories “non-composite” and “composite” with the former only containing a simple functionality at the C-35 position such as -OH or $–NH_2$ including BHT (**Ia**) and 35-aminobacteriohopane-32,33,34-triol (**Id**) whilst the latter have a more complex functionality such as a sugar or aminosugar at C-35. Some structures are only known to be produced by certain groups of organisms (see summary in Table 1; Rohmer, 1993; Talbot and Farrimond, 2007; Talbot et al., 2008, 2014) providing a potentially powerful tool for profiling the microbial community, as recently demonstrated in

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

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

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

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

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

2. Materials and methods

2.1. Site description and samples

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

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

2.2 Total Organic Carbon (TOC) analysis

Aliquots were analysed in duplicate using a Carlo-Erba EA1108 elemental analyser to determine percentage carbon, nitrogen and hydrogen. Percentage inorganic carbon was determined using a Strohlein Instruments Coulomat 702 carbon analyser adapted to analyse CO_2 liberated from H_3PO_4 digestion. Elemental compositions were determined as percent of the dry weight of peat analysed. Total organic carbon was calculated as the

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

2.3. Extraction and derivatisation,

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

2.4. LC-MS analysis

Reversed-phase HPLC was performed using a Surveyor HPLC system (ThermoFinnigan, Hemel Hempstead, UK) fitted with a Phenomenex (Macclesfield, UK) Gemini C_{18} 5 µm column (150 mm x 3.0 mm i.d.) and a security guard column cartridge of the same material. Separation was achieved at 30ºC with a flow-rate of 0.5 mL min^{-1} and the following gradient profile: 90% A and 10% B (0 min); 59% A, 1% B and 40% C (at 25 min), then isocratic to 45 min, returning to the starting conditions in 5 min and stabilising for 10 min before injecting the next sample (A = MeOH, B = water, C = propan-2-ol; all HPLC grade from Thermo Fisher Scientific).

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

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

A semi-quantitative estimate of BHP abundance (±20%) is calculated from the characteristic base peak ion peak areas of individual BHPs in mass chromatograms (from SCAN 1) relative to the *m/z* 345 ($[M+H-CH_3COOH]^+$) base peak area response of the acetylated 5α-pregnane-3β,20β-diol internal standard. Averaged relative response factors (from a suite of five acetylated authentic BHP standards) are used to adjust the BHP peak areas relative to that of the internal standard where BHPs containing one or more nitrogen 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% ± 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% ± 2.5%, (min 39.1%, max 49.1%, n = 31).

3.2 Bacteriohopanepolyols (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 μg g^{-1}_{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)

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

3.2.1 35-amino functionalised BHPs

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

3.2.2 Composite BHPs

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

3.2.3 Unsaturated BHPs

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

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

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**; **IIg**, **IIh** 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 (**IIa**; Table 2) is present in all samples, and 2-methylaminotriol (**IId**; 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 (**IIg**, **IIh**; Table 2) only present in a few of the surface samples above 22 cm depth. The fifth compound, 2-methylBHT pseudopentose (**IIi**), 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 (**IIIb**); 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

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

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

Amongst the heterotrophs, relatively few species make only non-composite BHPs. The Alphaproteobacterial genera *Beijerinkia* and *Bradyrhizobium* contain relatively simple BHP distributions dominated by aminotriol (**Id**), with additional BHT (**Ia**) in *Beijerinkia indica* (Table 1). Members of the Actinobacteria also only make these less complex compounds (Table 1). However, as these compounds represent two of the three most abundant compounds at BM (Fig. 1; Table 2) contributions from such sources could be important. Unlike any of the other heterotrophs with known BHP compositions, *Bradyrhizobium japonicum* also accumulates adenosylhopane (**Ig**; Table 1), which is present in the highest concentration in the 2-4 cm sample and constituted the greatest relative proportion of the total BHPs (13.4%; Table 2) in the 10-12 cm sample, likely indicating an aerobic source such as *B. japonicum*. Adenosylhopane is a biosynthetic intermediate in the synthesis of other side chain elongated BHPs (Bradley et al., 2010); therefore, there are potentially numerous sources for this compound, although, with the exception of *B. japonicum* few organisms have been shown to accumulate it in detectable 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 *Bradyhizobium* 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 km^2 (>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 CH_4 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 (**IIIf** 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 *Methylomicrobium alcaliphilum* (Banta et al., 2015), an alkaliphilic organism
453 unlikely to be present in *Sphagnum* peat. Type II organisms, including representatives of
454 the generas *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**)

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

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

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

Aminotriol concentrations are over two orders of magnitude greater than aminotetrol concentrations, inconsistent with their relative abundances in previously cultured type II 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* Methylomirabillis oxyfera" have been identified in peat (e.g. Zhu et al., 2012),
519 the diagnostic BHP produced by these organisms (3-Methyl-bacteriohopanehexol, **IIIf;**
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 (Welander 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. Welander et al., 2009).
547 The original identification of this compound, together with its saturated and C-2 methylated
548 homologues (**IIi**) 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 (**Ii**, 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 (**IIi**; 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 $\mu g\ g^{-1}_{TOC}$) and
598 below 400 cm (1100-3000 $\mu g\ g^{-1}_{TOC}$), whilst values throughout the remainder of the core
599 were lower, ranging from 160 $\mu g\ g^{-1}_{TOC}$ (32-34 cm) to 900 $\mu g\ g^{-1}_{TOC}$ (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 (**Ia**), BHT-cyclitol ether (**Ij**)
606 and aminotriol (**Id**). 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,

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

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

Among the defining features of soil BHP distributions are high levels of adenosylhopane (**Ig**), its C-2 methylated homologue (**IIg**; Talbot et al., 2007a) and two pairs of related compounds with as yet unidentified terminal groups (including "adenosylhopane type 2"; **Ih** and **IIh**, Table 1), together comprising 28% of the average total BHP assemblage (Cooke et al., 2008a; Cooke, 2010). Five soils from Canada and permafrost soils from Svalbard and Siberia contained even higher proportions of this BHP group (Xu et al., 2009; Rethemeyer et al., 2010; Höfle et al., 2015). In the peat samples studied here, the distribution of these compounds is rather different, with adenosylhopane and related compounds only present in significant concentration in the shallow sub-surface (up to 15% in 2-4 and 8-12 cm; Fig. 3a) but are otherwise below 10%. In *Sphagnum* peats from 2 other European sites (UK, Belgium; van Winden et al., 2012a and b respectively), the relative abundance of soil marker BHPs is also low, typically less than 10% except in the near surface samples (up to 15%; Fig. 3a). The only exceptions are 4 peat samples 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 (**Ig**) 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 17β,21β(*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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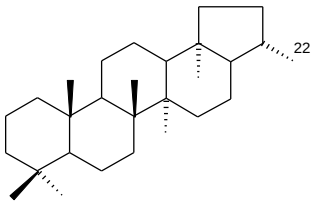
Appendix 1.

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



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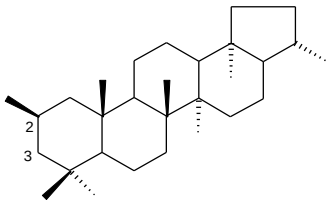

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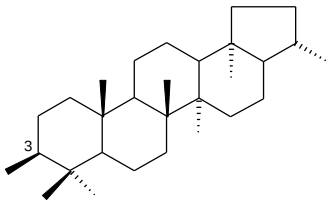

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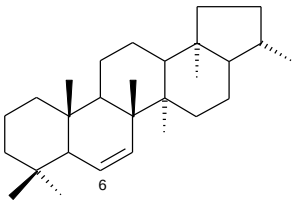

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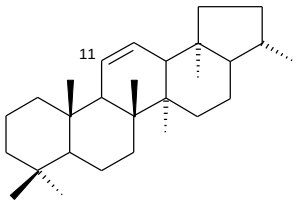

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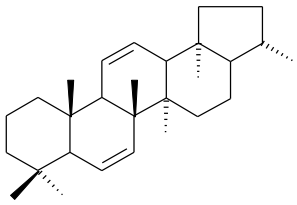

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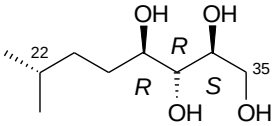

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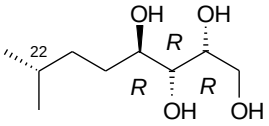

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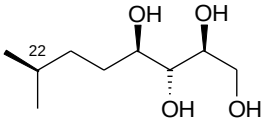

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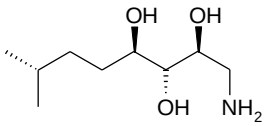

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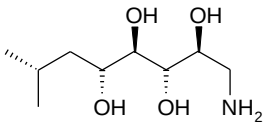

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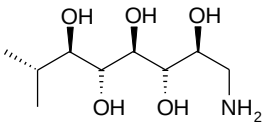

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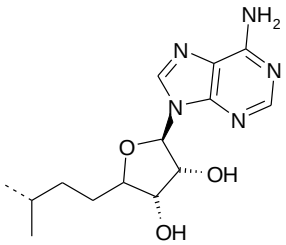

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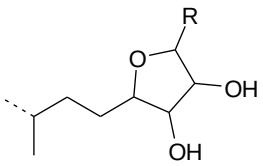

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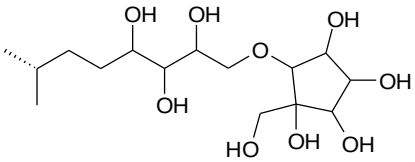

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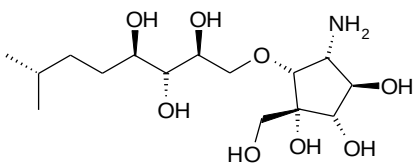

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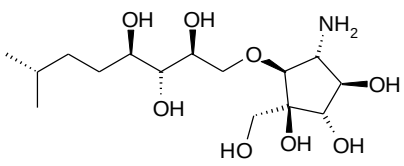

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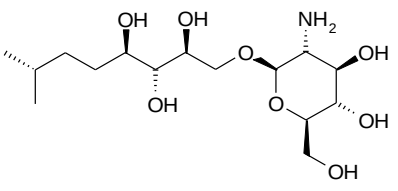

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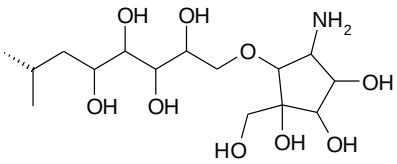

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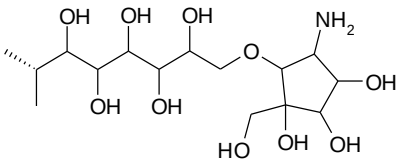

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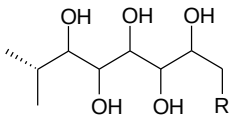

p (R= unknown)

1173

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1175

List of Figures

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

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

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

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

APCI Base Peak ion (*m/z*) **BHP structure number**[b]	**746**[a] **Ig**	**760**[a] **IIg**	**761** **Ih**	**775** **IIh**	**712** **IVd** or **Vd**	**714** **Id**	**728** **IId**	**728** **IIId**	**772** **Ie**	**830** **If**	**653** **IVa,b** or **c**	**655** **Ia**	**669** **IIa**	**1002** **Ij** and/or **Ik**	**1002** **Im**	**1058** **IVn** **or** **Vn**	**1060** **In**	**1118** **Io**	**1132** **Ip**	**941** **IVi** **or** **Vi**	**943** **Ii**	**957** **IIi**	**References**
HETEROTROPHS[c]																							
PROTEOBACTERIA **Alphaproteobacteria**																							
Acetobacter pasteurianus (AAB)[d,e]											+[f]	+											Zundel and Rohmer, 1985
Acetobacter europaeus (AAB) [d,e]											+	+											Simonin et al., 1994
Acetobacter aceti xylinus (AAB) [d,e]											+	+		+	+	+	+						Talbot et al., 2007b
Beijerinckia indica						+						+											Vilcheze et al., 1994
Beijerinckia mobilis						+																	Vilcheze et al., 1994
Bradyrhizobium sp.	+	+				+																	Bravo et al., 2001
Methylobacterium spp. (n = 8)[g,e]												+		+	+								Knani et al., 1994
Zymomonas mobilis												+		+	+								Flesch and Rohmer, 1989
Betaproteobacteria																							
Burkholderia spp. (n = 9)												+		+	+		(+)[h]						Cvejic et al., 2000b
Gammaproteobacteria																							
Azotobacter vinelandii														+			+						Vilcheze et al., 1994
Frateuria aurantia (AAB)												+		+	+		+						Joyeux et al., 2004
Deltaproteobacteria																							
Geobacter spp. (n = 2)[e]												+		+	+								Eickhoff et al., 2013b
Acidobacteria												+		+									Garcia Costas et al., 2012
Actinobacteria																							
Frankia spp.[e]												+											Rosa-Putra et al., 2001
Streptomyces coelicolor A3(2)						+																	Poralla et al., 2000
Firmicutes																							
Alicyclobacillus acidocaldarius[e]												+			+								Poralla et al., 1984
Alicyclobacillus acidoterestris[e]												+			+		+	+					Řezanka et al., 2011
Planctomycetes												(+)		(+)									Sinnighe Damsté et al., 2004; Rush et al., 2014

Table 1. Continued

APCI Base Peak ion (*m/z*) BHP structure number[b]	746[a] Ig	760[a] Iig	761 Ih	775 IIh	712 IVd or Vd	714 Id	728 IId	728 IIId	772 Ie	830 If	653 IVa,b or c	655 Ia	669 IIa	1002 Ij and/or Ik	1002 Im	1058 IVn or Vn	1060 In	1118 Io	1132 Ip	941 IVi or Vi	943 Ii	957 IIi	References
METHANOTROPHS																							
Alphaproteobacteria																							
Methylocella spp.	+					+						+											van Winden et al., 2012a
Methylosinus sp.						+			+			+											Neunlist and Rohmer, 1985b
Methylocystis sp.						+			+														Talbot et al., 2001
Gammaproteobacteria																							
Methylovulum sp.[e]					+	+			+	+													van Winden et al., 2012a
Methylomonas sp.									+	+													Neunlist and Rohmer, 1985a
Methylococcus sp.[e]									+	+													Neunlist and Rohmer, 1985a
Methylocaldum spp.[e]									(+)	+													Cvejic et al., 2000a
Verucomicrobia						+																	van Winden et al., 2012a
PHOTOTROPHS																							
Alphaproteobacteria																							
PNSB[i,e]	+		(+)		(+)	(+)						(+)	(+)	(+)	(+)								Talbot et al., 2007a
Rhodoblastus sp.	+											+		+	+								Talbot et al., 2007a
Cyanobacteria[e]	(+)					(+)	(+)				(+)	(+)	(+)	(+)	(+)		(+)			(+)	(+)	(+)	Talbot et al., 2008
Gloeocapsa 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

[i] PNSB = Purple non-sulphur bacteria.

Table 2. Total organic Carbon (%) and concentration of individual BHPs (μg g^{-1}_{TOC}) and grouped structural types in peat samples from Bissendorfer Moor.

Base peak (m/z)		653	653	655	669	712	714	728	728	772	830	746	760	761	775	941	943	957
Depth		IVa,	IVa,	Ia	IIa	IVd or	Ib	IIb	IIIb	Ie	If	Ig	IIg	Ih	Ih	IVi or	Ii	IIi
cm	% TOC	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

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

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

[a] bdl = below detection limit;

[b] tetra = tetrafunctionalised BHPs with functional groups at C32, 33, 34 and 35, penta = pentafunctionalised with additional functional group at C31, hexa = hexafunctionalised with additional functional groups at C30 and 31, soil = all soil-marker BHPs inclidng adenosylhopane and related structures (**Ig**, **IIg**, **Ih**, **IIh),** unsat = the combined relative % of all unsaturated BHPs (tetra- and pentafunctionalised) with ring systems **IV** and/or **V**;

[c] R_{soil} = (**Ig**+**IIg**+**Ih**+**IIh**)/(**Ia**+**Ig**+**IIg**+**Ih**+**IIh**) (Zhu et al., 2011).

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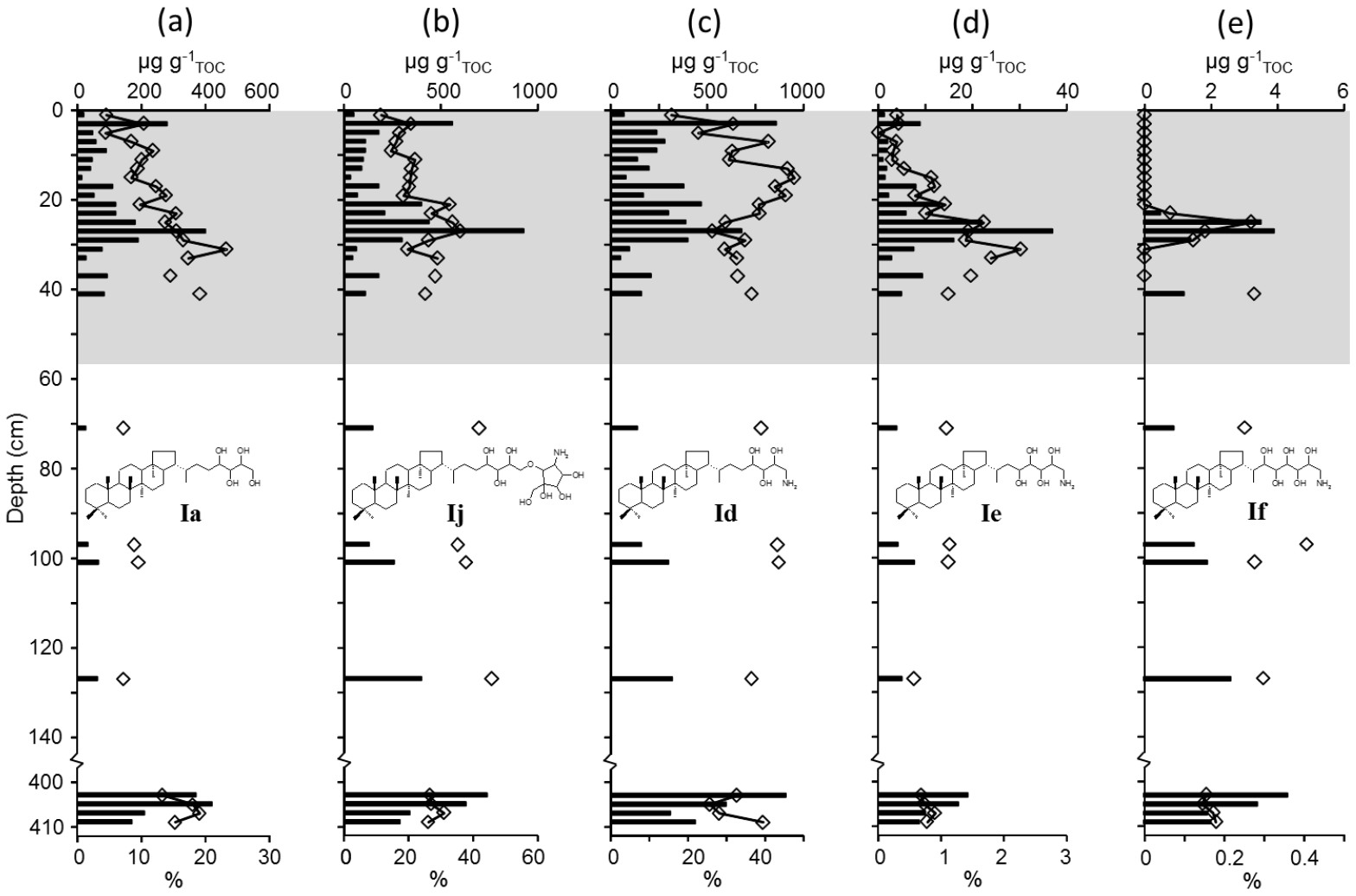

Figure 1

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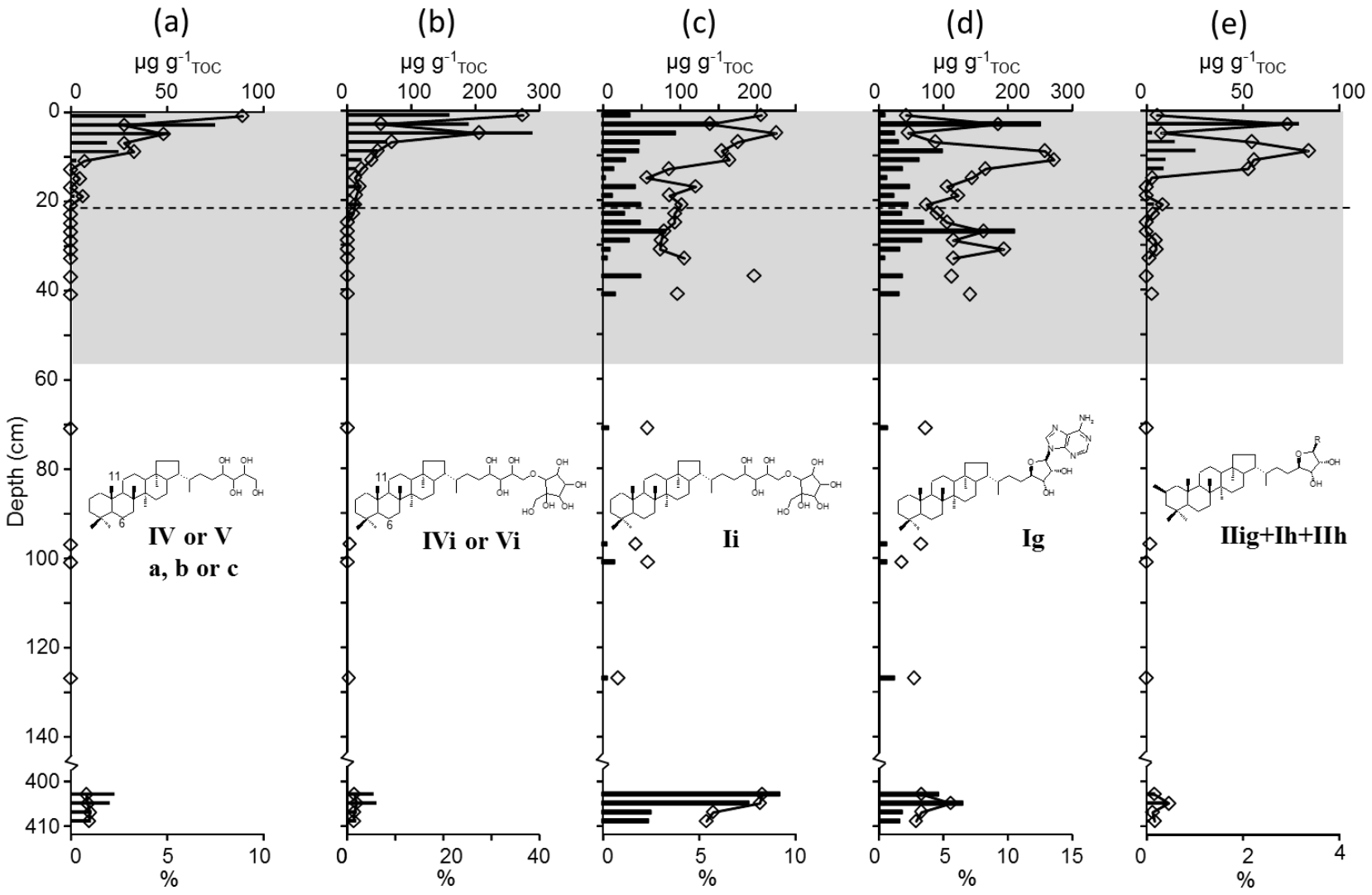

Figure 2

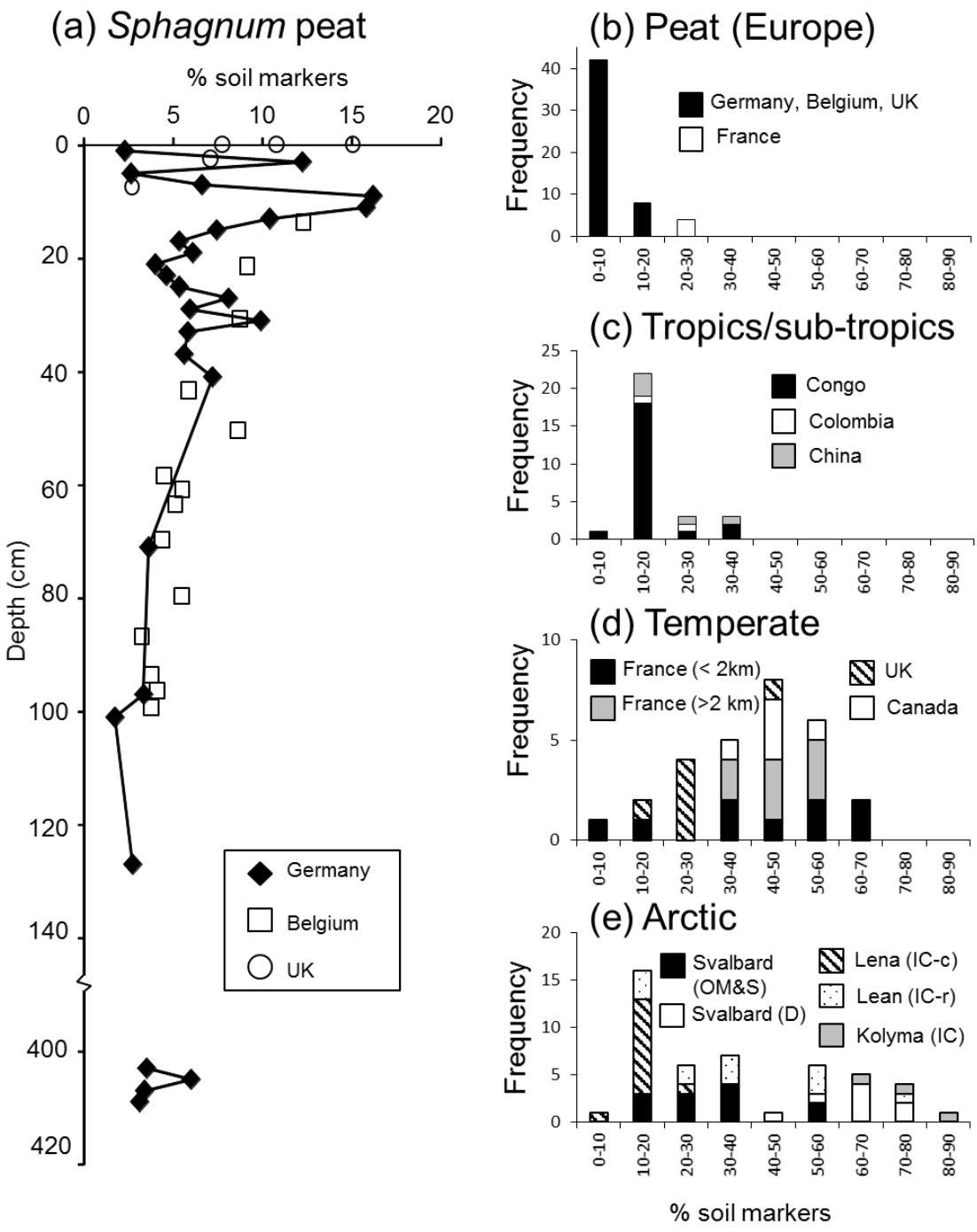

Figure 3

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