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-aminobacteriohopanepental and 35-aminobacteriohopanepental) 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 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...
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; Retthemeyer 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; Doğru 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 microbiologically-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 ACCROTEL project (e.g. Yeloff et al., 2006; Charman et al., 2007; McClymont et al., 2008; Pancost et al., 2011). Macropores 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 CO₂ liberated from H₃PO₄ 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 30°C 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\textsuperscript{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 \( \mu \)A, 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\textsuperscript{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\textsuperscript{2} spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent MS\textsuperscript{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., 2003\textsuperscript{a,b}, 2007\textsuperscript{a,b}, 2008) or by comparison of APCI MS\textsuperscript{2} and MS\textsuperscript{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\textsubscript{3}COOH]\(^+\)) base peak area response of the acetylated 5\( \alpha \)-pregnane-3\( \beta \),20\( \beta \)-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 MS² 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; Ilg, 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 (Ila; 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 (Ilg, IIh; Table 2) only present in a few of the surface samples above 22 cm depth. The fifth compound, 2-methylBHT pseudopentose (IIIi), 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
4.1 Sources of BHPs in BM peat

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

4.1.1 Heterotrophs

As BHP abundances are high in the shallowest layers (Table 2; Figs 1-3) it is likely that many of the most abundant BHPs derive from aerobic heterotrophs. These organisms are important members of the bacterial community found in peat, consuming a wide range of organic substrates, including organic acids, sugars, polyalcohols and some aromatic compounds as carbon and energy sources (e.g. Belova et al., 2006). This is
consistent with previous studies on peats which suggest that $^{13}$C-enriched hopanes are an indicator of a heterotrophic bacterial population consuming $^{13}$C-enriched carbohydrates (Pancost et al., 2000; Xie et al., 2004).

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

The Acetobacter and Gluconacetobacter genera are known as acetic acid bacteria (AAB). They are diazotrophic (e.g. Bragina et al., 2012b) and also produce acetate which is a necessary substrate for acetoclastic methanogenesis which is an important pathway in some shallow peats (Kelley et al., 1992; Popp and Chanton, 1999; Chasar et al., 2000; Metje and Frenzel, 2005). Alphaproteobacteria AAB are prolific sources of a wide range of BHPs with core structures of BHT (Ia), BHT cyclitol ether (Ij) and BHpentol cyclitol ether (In) but also including mono and diunsaturated compounds with double bonds at C-6 (IV), C-11 (V) or both (VI) and with ring systems both with and without additional methylation at C-3 (III; Rohmer and Ourisson, 1986; Peiseler and Rohmer, 1992; Talbot et al., 2007b). 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 *Beijerinckia* and *Bradyrhizobium* contain relatively simple BHP distributions dominated by aminotriol (Id), with additional BHT (Ia) in *Beijerinckia 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).
To summarise, proteobacteria are undoubtedly significant sources of BHPs at BM, with *Burkholderia* spp. considered particularly important sources of BHT (Ia) and BHT cyclitol (Ij and/or Ik) although multiple sources are expected for both compounds (see Table 1). *Beijerinckia* spp. are likely sources of aminotriol (Id) with additional contributions from *Bradyhizobium* sp. and Actinobacteria. *B. Japonicum* likely also contributes to adenosylhopane (Ig).

4.1.2 Aerobic methane oxidising bacteria (methanotrophs)

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

Dedysh (2009) described the methanotroph diversity in acidic northern wetlands and found that these settings are mainly colonized by methanotrophic representatives of the Alphaproteobacteria (i.e. Type II methanotrophs). However, studies using functional gene analysis by microarray or ultra-deep pyrosequencing of *pmoA* genes on *Sphagnum* peat samples from a range of environments including but not limited to northern regions (Siberia, Sweden, Canada, Argentina and The Netherlands), revealed Type I organisms including the genera *Methylomonas*, *Methylobacter*, *Methylomicrobium* and
Methylocaldum (Kip et al., 2010, 2011). Type II organisms related to Methylocystis and Methylosinus spp. (family Methylocystaceae) were also significant members of the methanotroph community at all sites (Kip et al., 2010, 2011) and were particularly dominant in Sphagnum magellanicum dominated habitats from Patagonia (Kip et al., 2012). Whilst Methylocystis spp. are a common and abundant group in acidic peats, there are other more recently described genera of Type II methanotrophs from the family Beijerinckiaaceae including Methyllocella and Methylocapsa, which are also important and would not have been observed in some earlier studies utilising the functional gene pmoA (which is not present in these organisms; e.g. Rahman et al., 2011 and references therein). Recent studies have also suggested an apparently symbiotic relationship between methanotrophs and Sphagnum moss (Kip et al., 2010), with a bacterium occurring inside cells of S. cuspidatum showing 93% 16S rRNA sequence similarity to cultured Methyllocella and Methylocapsa sp. (Raghoebarsing et al., 2005).

Previous studies of methane oxidising bacteria have shown that they produce characteristic non-composite BHP distributions, with most Type I species producing high levels of the hexafunctionalised compound aminopentol (If) and lower amounts of the related pentafunctionalised compound aminotetrol (Ie; Table 1; e.g. Talbot et al., 2001; van Winden et al., 2012a; Talbot et al., 2014 and references therein). Some Type I organisms of the genera Methylococcus and Methylocaldum also produce homologues of aminopentol and aminotetrol with a methyl group at position C-3 (IlIIf and Ille respectively; Neunlist and Rohmer, 1985a; Cvejic et al., 2000a), although the 3-methyl-aminotriol (IlIib) observed here (Table 2) has only recently been reported from cultures of the Type I organism Methylomicrobium alcaliphilum (Banta et al., 2015), an alkaliphilic organism unlikely to be present in Sphagnum peat. Type II organisms, including representatives of the genera Methylocystis and Methylosinus (Alphaproteobacteria), produce a combination of BHT, aminotriol and aminotetrol (Rohmer et al., 1984; Neunlist and
Rohmer, 1985b; Cvejic et al., 2000a; Talbot et al., 2001; van Winden et al., 2012a). It should be noted, however, that there are multiple other sources of aminotriol especially amongst other Alphaproteobacteria (Table 1; e.g. Talbot and Farrimond, 2007 and references therein).

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

Although a methanotroph biomarker might be expected to be most abundant at the redox interface i.e. water table depth, where oxygen is present and methane concentrations are the highest (e.g. Sundh et al., 1995), aminotriol was present at high concentration in all samples from BM (Fig. 1c). Aminotetrol (I\text{e}) was also present in all samples, representing up to 2.3% of the total BHP distribution with the highest concentration in the 26-28 cm sample (Fig. 1d). Although this is around the approximate average depth of the oxic-anoxic interface, this region of the core will be exposed to oxygen seasonally as the water table lowers. The highest concentration of aminopentol (I\text{f})
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 22 cm) 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 (**IId**; 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
Winden et al., 2012a). Instead, the marked similarity of the aminotriol BHP depth profile with most other major BHPs (Fig. 1) suggests a significant non-methanotrophic origin for this compound, or a methanotroph origin from one of these more recently described sources (e.g. Methylocella; van Winden et al., 2012a). The similar depth profile of aminotetrol, although far less abundant (Fig. 1c and d), suggests that this might also derive, at least partially, from other sources, although the only known non-methanotroph source are sulphate reducing bacteria of the genus Desulfovibrio (Blumenberg et al., 2006, 2009b, 2012), which is unlikely to occur in this environment. Only aminopentol has a depth profile consistent with being derived predominantly from methanotrophs.

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

4.1.3 Phototrophic bacteria

The surface layers of peat bogs host a wide range of phototrophic eukaryotes and prokaryotes. This includes nitrogen fixing cyanobacteria which inhabit Sphagnum peat bogs, occurring in both epiphytic and intracellular associations with Sphagnum, (e.g. Granhall and Selander, 1973; Krivograd Klemenčič and Vrhovška, 2003; Krivograd Klemenčič et al., 2010; Bragina et al., 2012b). Many different classes of hopanoid producing cyanobacteria, including Anabaena, Calothrix, Cyanothece, Gloecapsa, Microcystis, Nostoc, Oscillatoria and Phormidium (Talbot et al., 2008 and references therein), have been observed in peat (Krivograd Klemenčič et al., 2010 and references therein). Until recently, cyanobacteria were considered to be the major source of hopanoids methylated at the C-2 position (Summons et al., 1999); however, recent genomic studies have revealed that the capacity for this synthesis also occurs in other
phyla, particularly the Alphaproteobacteria and also Acidobacteria (Welander et al., 2010) and that it is a particularly common trait in organisms found in close (symbiotic) association with plants (Ricci et al., 2014).

Of particular interest to this study are the species of Gloeocapsa cyanobacteria which were identified, for example, in the Männikjärve bog (central Estonia) and were particularly abundant on *S. magellanicum* plants (Karofeld and Toom, 1999), one of the species present at the BM site. A further seven *Gloeocapsa* spp. were identified in two Slovenian bogs (Krivograd Klemenčič and Vrhovška, 2003). Crucially, a *Gloeocapsa* sp. is the only known source of the unsaturated compound, identified as a “BHT pentose” (IVi or Vi), that is highly abundant (over 40%) in the surface layers at the BM site (Fig. 1b). Such a high abundance in surface layers is consistent with an aerobic and/or phototrophic source and suggests a particular ecophysiological role, potentially regulating osmotic pressure or proton gradients in the low pH peat environment (cf. Welander et al., 2009). The original identification of this compound, together with its saturated and C-2 methylated homologues (III) was from a sample of an epilithic colony of *Gloeocapsa* sp. from Devon Island (Arctic; Talbot et al., 2008). The saturated pentose compound, with the non-methylated structure (II, Table 2, Fig. 2c), was also observed in BM peat, being present at all depths but showing a markedly different depth profile to the unsaturated structure (Fig. 2b). The methylated pentose structure (III; Table 2) was only observed in the deepest levels, below 400 cm. Therefore, the saturated compounds could: (i) derive from an additional source to the unsaturated compounds; (ii) have a markedly different preservation potential for the methylated vs non-methylated compounds; and/or (iii) have different extraction efficiencies in the fresher, near surface material, given that some other methylated BHPs have exhibited resistance to extraction (Herrmann et al., 1996; Allen et al., 2010).
The pair of unsaturated bacteriohopanetetrols (IVa, b or c; Table 2) also had very similar depth profiles to the unsaturated BHT-carbopseudopentose suggesting a common source (Fig. 2a and b). A single unsaturated BHT with an identical mass spectrum was also observed in the original source of the Gloeocapsa enrichment (Talbot et al., 2008). One possibility, therefore, is that a Gloeocapsa sp. inhabiting this environment produces both the composite carbopseudopentose and one or both of the observed unsaturated tetrols. An alternative heterotrophic source for the unsaturated tetrols, the Alphaproteobacteria AAB, are discussed above but considered minor at most, again due to the absence of C-3 methylated homologues.

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

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

Although to date BHPs in peat have only been studied by LCMS from 3 other locations (Kim et al., 2011; van Winden et al., 2012a,b), our data collectively suggest that whilst soil and peat derived organic matter generally contain the same BHPs, they have characteristically different relative distributions, as recently suggested for tropical and non-tropical soils (Spencer-Jones et al., 2015). Excluding the surface layers, the three most dominant BHPs in the BM peat at all other depths were BHT (Ia), BHT-cyclitol ether (Ij) and aminotriol (Id). Again, these observations are consistent with other recent studies of BHPs in peat (Kim et al., 2011; van Winden et al., 2012a,b) and also with the BHP composition in soils from the Northern hemisphere (Cooke et al., 2008a; Xu et al., 2009; Cooke, 2010; Rethemeyer et al., 2010; Kim et al., 2011) and tropical settings (Wagner et 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. 2a 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%);
Fig. 3b), however, no details were provided as to the type of peat in that study so it is possible they were not *Sphagnum* peats (Kim et al., 2011).

The low values of soil marker BHPs (typically < 10%; Fig 3a,b) within peats are distinctive from those of soils. Values reported recently for tropical and sub-tropical soils had values in the range 0-40% although the majority were in the range 10-20%, slightly higher than the peat samples (Fig. 3c). Temperate soils from Canada (Alberta), France and the UK, had a wide range (0-70%; Fig. 3d) with the widest range found in permafrost soils from the Siberian Arctic and Svalbard (0-90%; Fig. 3e), possibly reflecting competing influences of low temperature leading to higher values and low pH leading to low values (Höfle et al., 2015; Spencer-Jones et al., 2015). As discussed above, the most likely sources of adenosylhopane (Ig) and related structures in *Sphagnum* peat environments are nitrogen-fixing Alphaproteobacteria (*Bradyrhizobium* sp.; PNSB; *Methylocella* sp.; Table 1). As adenosylhopane is in itself an intermediate in the biosynthetic pathway to other side chain elongated BHPs (Bradley et al., 2010), this suggests that under the low pH conditions found in *Sphagnum* peat (pH 3.8 – 4.3 at BM; Charman et al., 2007), the species that would normally accumulate adenosylhopane convert this precursor to other BHPs. Alternatively, other nitrogen fixers such as *Burkholderia* sp. (Betaproteobacteria) and other, especially Alphaproteobacterial, BHP producers (e.g. Zadorina et al., 2009) outcompete the adenosylhopane accumulators in these systems. In their study of BHPs in Siberian permafrost, Höfle et al. (2015) reported that concentrations of adenosylhopane (and related compounds) were negatively correlated with pH, the implication being that further modification of the side chain is more important at lower pH than more neutral conditions, hence the greater diversity of structures present in low pH peat and peaty soils (Cooke et al., 2008a; van Winden et al., 2012a). Indeed Gong et al. (2015) recently reported that pH was a key factor controlling the geographical distribution of squalene 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 $R_{\text{soil}}$ and $R'_{\text{soil}}$ 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
exceptionally high levels of a number of unsaturated BHPs including two isomers of
unsaturated BHT, a feature which has not been reported elsewhere. A second major
difference between BHPs in BM peat and those reported for soils was the relative
abundance of adenosylhopane and related structures, which was as high as 15% in a few
(near) surface horizons but generally below 10% and much lower (~1.7%) in deeper
layers. These values are significantly lower than those typically reported for soils from
other environments (tropical, temperate, Arctic) and are likely influenced by the low pH of
the peat environment.

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

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liquid chromatography - atmospheric pressure chemical ionisation - mass spectrometry.


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\textsuperscript{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 occurrence of additional/alternative isomers of the side chain cannot be excluded.
\[ \text{n} \]

\[ \text{o} \]

\[ \text{p} (R = \text{unknown}) \]

1173

1174

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 permafrost 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) | 746 | 760 | 761 | 775 | 712 | 714 | 728 | 728 | 772 | 830 | 655 | 669 | 1002 | 1002 | 1058 | 1060 | 1118 | 1132 | 941 | 943 | 957 |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|
| BHP structure number b  | Ig  | Ilg | Ih  | Ihh | IVd | Iv  | Ild | Ild | llf | lla | lla | lla  | lla   | lla   | lla   | lla   | lla   | lla   | lla   | lla   |
| References              |      |     |     |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |      |      |

**HETEROTROPHS**

**PROTEOBACTERIA**

**Alphaproteobacteria**

- *Acetobacter pasteurianus* (AAB) a, c, e
- *Acetobacter europaeus* (AAB) a, c
- *Acetobacter acetil xylinus* (AAB) a, c
- *Beijerinckia indica*
- *Beijerinckia mobilis*
- *Bradyrhizobium sp.*
- *Methylobacterium spp.* (n = 8) a, c
- *Zymomonas mobilis*

**Betaproteobacteria**

- *Burkholderia spp.* (n = 9)
- *Azotobacter vinelandii*
- *Frateuria aurantia* (AAB)
- *Geobacter spp.* (n = 2) a
- *Acidobacteria*
- *Frankia spp.*
- *Streptomyces coelicolor K3(2)*

**Gammaproteobacteria**

- *Acidobacteria*

**Deltaproteobacteria**

- *Acidobacteria*

**Firmicutes**

- *Alicyclobacillus acidocaldarius*
- *Alicyclobacillus acidoterestris*
- *Planctomycetes*

**References**

Zundel and Rohmer, 1985
Simonin et al., 1994
Talbot et al., 2007b
Vilcheze et al., 1994
Bravo et al., 2001
Knani et al., 1994
Flesch and Rohmer, 1989
Cvejic et al., 2000b
Vilcheze et al., 1994
Joyeux et al., 2004
Eickhoff et al., 2013b
Garcia Costas et al., 2012
Rosa-Putra et al., 2001
Poralla et al., 2000
Poralla et al., 1984
Poralla et al., 2011
Sinnighe Damsté et al., 2004; Rush et al., 2014
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<sup>a</sup> Di, tri- and tetraacetate forms are known and observed here.

<sup>b</sup> See Appendix for structures

<sup>c</sup> 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

<sup>d</sup> AAB = acetic acid bacteria

<sup>e</sup> Indicates organism also produces other BHPs not identified in this study

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

<sup>g</sup> Number of species identified to produce BHPs

<sup>h</sup> (+) indicates compound found in some but not all tested members of group/genus
1 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.

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Note: % Tetra, % Penta, % Hexa, % Soil, % Unsat, Rˢoil are recalculated from data in Table 2.
a bdl = below detection limit;
b tetra = tetrafuctionalised BHPs with functional groups at C32, 33, 34 and 35, penta = pentafunctionalised with additional functional group at C31, hexa = hexafuctionalised with additional functional groups at C30 and 31, soil = all soil-marker BHPs inclidng adenosylhopane and related structures (Ig, Ilg, Ih, Ilh), unsat = the combined relative % of all unsaturated BHPs (tetra- and pentafunctionalised) with ring systems IV and/or V;
c $R_{soil} = (Ig+Ilg+Ilh)/(Ia+Ig+Ilg+Ilh)$ (Zhu et al., 2011).
Figure 1
Figure 2
Figure 3

(a) *Sphagnum* peat

(b) Peat (Europe)
- Germany, Belgium, UK
- France

(c) Tropics/sub-tropics
- Congo
- Colombia
- China

(d) Temperate
- France (<2 km)
- France (>2 km)
- UK
- Canada

(e) Arctic
- Svalbard (OM&S)
- Lena (IC-c)
- Lean (IC-r)
- Svalbard (D)
- Kolyma (IC)

Legend:
- Germany
- Belgium
- UK

Depth (cm)

% soil markers