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# Variation in methanotroph-related proxies in peat deposits from Misten Bog, Hautes-Fagnes, Belgium

Julia F. van Winden<sup>a</sup>, Helen M. Talbot<sup>b</sup>, François De Vleeschouwer<sup>c,d</sup>, Gert-Jan Reichart<sup>a</sup>,  
Jaap S. Sinninghe Damsté<sup>a,e,\*</sup>

<sup>a</sup> Department of Earth Sciences, Utrecht University, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands

<sup>b</sup> School of Civil Engineering and Geosciences, Newcastle University, Drummond Building, Newcastle Upon Tyne NE1 7RU, UK

<sup>c</sup> Université de Toulouse, INP, UPS, EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), ENSAT, Avenue de l'Agrobiopole, 31326 Castanet Tolosan, France

<sup>d</sup> CNRS, EcoLab, 31326 Castanet Tolosan, France

<sup>e</sup> Department of Marine Organic Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, The Netherlands

## A B S T R A C T

Methane emissions from peat bogs are strongly reduced by aerobic methane oxidising bacteria (methanotrophs) living in association with *Sphagnum* spp. Field studies and laboratory experiments have revealed that, with increasing water level and temperature, methanotrophic activity increases. To gain a better understanding of how longer term changes in methanotrophic activity are reflected in methanotroph biomarkers, a peat record (0–100 cm) from the Hautes-Fagnes (Belgium) encompassing the past 1500 years, was analysed for methanotroph-specific intact bacteriohopanepolyols (BHPs) and the carbon isotopic composition of diploptene. A predominance of aminobacteriohopanetetrol (aminotetrol) over aminobacteriohopanepentol (aminopentol) indicated the prevalence of type II methanotrophs. Relatively high methanotrophic activity was indicated by all methanotroph markers between 20 and 45 cm depth, around the present oxic–anoxic boundary, most likely representing the currently active methanotrophic community. Comparing methanotrophic markers in the deeper part of the peat profile with environmental variables afforded, however, no clear correlation between change in water level and methanotrophic activity. This is potentially caused by a predominance of type II methanotrophs, a combination of sources for methanotrophic biomarkers or insufficient variation in climatic changes. A proposed way forward would include a study of a core covering a longer timescale, thereby involving greater variability.

## 1. Introduction

Peat bogs are situated at mid to high latitude, primarily in the Northern Hemisphere, and play a large role in the global carbon cycle as they sequester a third of the Earth's soil carbon (Smith et al., 2004). They are also important in the global methane cycle, since they contribute approximately 10% of the total CH<sub>4</sub> flux to the atmosphere (Gorham, 1991). Methane emission from peat bogs depends on a balance between archaeal methane production and subsequent bacterial methane oxidation. Aerobic methane oxidising bacteria (methanotrophs), living in association with *Sphagnum* spp., can retain up to 98% of the diffusive methane flux (van Winden et al., 2012a), but methane cycling strongly depends on environmental factors. Field studies and laboratory experiments have revealed that, with increasing water level and temperature, both methanogenic and methanotrophic activity increase (Frenzel and

Karofeld, 2000; Basiliko et al., 2004; McNamara et al., 2008; Larmola et al., 2010; Kip et al., 2010; van Winden et al., 2012a). Mesocosm experiments suggest, however, that methane oxidation is not able to keep up with increased production rate at increasing temperatures (van Winden et al., 2012a). However, such experiments cannot take into account long term adaptation of the microbial community. Hence, it remains unclear how methane cycling responds to changes in climate on longer timescales.

Insight into the adaptation on longer timescales can potentially be obtained by studying peat bog records when natural climate and environmental changes have resulted in differences in the extent of methane cycling. Variation in past methanotrophic activity is reflected in molecular proxies for methanotrophs (van Winden et al., 2012b), such as compound specific carbon isotopes of the bacterial marker diploptene, and methanotroph-specific intact bacteriohopanepolyols (BHPs, hopanoids with a polyfunctionalized side chain).

Methane is strongly depleted in <sup>13</sup>C, yet diploptene, a lipid derived from methanotrophs (but also from other bacteria; e.g. Rohmer et al., 1984), exhibits only limited <sup>13</sup>C depletion, with δ<sup>13</sup>C

\* Corresponding author at: Department of Marine Organic Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, The Netherlands. Tel.: +31 222369550; fax: +31 222319674.

E-mail address: damste@nioz.nl (J.S. Sinninghe Damsté).

values of ca.  $-30\text{‰}$  and  $-40\text{‰}$  in peat bogs (van Winden et al., 2010). This is probably the result of a mixed origin, since methanotrophs are not the only bacterial source for diploptene. Furthermore, serine cycle methanotrophs (type II), which form part of the methanotrophic community, do not only use methane but also  $\text{CO}_2$  as carbon source, thereby diluting the  $^{13}\text{C}$  depleted, methane-derived signal in methanotroph lipids. Nevertheless, in a previous mesocosm experiment, it was shown that the carbon isotopic depletion of diploptene strongly correlated with methane production and methane consumption (van Winden et al., unpublished data). This may be a result of an increased contribution of methanotroph-derived diploptene with enhanced methane cycling. Alternatively, it is possibly caused by enhanced expression of the enzymatic isotope effect associated with methane oxidation, with increased methane availability.

Methanotroph-specific BHPs may also serve as biomarkers for methanotrophy, potentially containing useful process-specific information.

Methanotrophs in peat bogs comprise two main groups, type I methanotrophs ( $\gamma$ -Proteobacteria) and type II methanotrophs ( $\alpha$ -Proteobacteria). Aminobacteriohopanepentol (aminopentol) is a marker for type I methanotrophs, while aminobacteriohopanetetrol (aminotetrol) is indicative for type II methanotrophs (Neunlist and Rohmer, 1985a,b; Cvejic et al., 2000; Talbot et al., 2001; Coolen et al., 2008; van Winden et al., 2012b). Still, the latter may also be produced to a lesser extent by type I methanotrophs and some sulfate reducing bacteria (Neunlist and Rohmer, 1985a; Cvejic et al., 2000; Talbot et al., 2001; Blumenberg et al., 2006, 2009a,b; van Winden et al., 2012b). *Methylocella*-like methanotrophs, belonging to the *Beijerinckiaceae* family within the type II methanotrophs, have been speculated to be one of the most important methane oxidisers in peat bogs (Dedysh, 2009). They are not closely related to the other type II methanotrophs. The only *Methylocella*-like methanotroph analysed for BHPs did not contain aminotetrol, but mainly aminobacteriohopanetriol (aminotriol) and bacteriohopanetetrol (BHT), which are also produced by many other bacteria (van Winden et al., 2012b). BHPs can thus be used to trace contributions of methanotrophs in peat records but the results require careful interpretation.

To gain a better understanding on how longer term climatic and environmental changes affected methanotrophic activity, a peat record from the Hautes-Fagnes, Belgium, encompassing the past

1500 years, was investigated. The core was recovered from the edge of a hummock, to ensure maximum sensitivity to changes in hydrology (De Vleeschouwer et al., 2012). It was analysed for methanotropic biomarkers including intact bacteriohopanepolyols (BHPs) and for compound specific carbon isotope values of diploptene.

## 2. Experimental

### 2.1. Site description

The peat monolith was obtained using a plain titanium  $15 \times 15 \times 100$  cm Wardenaar corer (Wardenaar, 1986) in February 2008 from Misten Bog, Hautes-Fagnes, East Belgium ( $50^\circ33'50''\text{N}$ ,  $06^\circ09'50''\text{E}$ ; Fig. 1) and has been described in detail elsewhere (De Vleeschouwer et al., 2012 and references therein). Briefly, the bog is ombrotrophic in nature and present day average precipitation rate is 1440 mm/yr at a mean annual air temperature of  $6.7^\circ\text{C}$ . Currently, the surface *Sphagnum* hummocks and lawns are covered with abundant shrubs such as *Erica tetralix*, *Calluna vulgaris* and *Vaccinium* spp., reflecting the ongoing evolution of the bog into a peat moor. At the coring site, some *Ericales* rootlets extended up to ca. 30 cm depth.

### 2.2. Dating and age model

The methods used to establish the chronology of the core are derived from and reported in De Vleeschouwer et al. (2012). Briefly, the dates for the peat horizons were based on  $^{14}\text{C}$  AMS dates of selected plant macrofossils, as reported by De Vleeschouwer et al. (2012).  $^{210}\text{Pb}$  was also used to date the most recent layers (down to 18 cm) and an age model for  $^{210}\text{Pb}$  derived according to the Constant Rate of Supply (CRS) model (Appleby, 2001). An age model was constructed using the  $^{14}\text{C}$  dates and the modelled  $^{210}\text{Pb}$  ages. Radiocarbon dates were translated to calendar ages using the IntCal09 calibration curve (Reimer et al., 2009).

### 2.3. Extraction and derivatisation

The monolith was sliced into ca. 1 cm sections. Fourteen slices were selected from the 1 m profile (see Table 1 for detailed

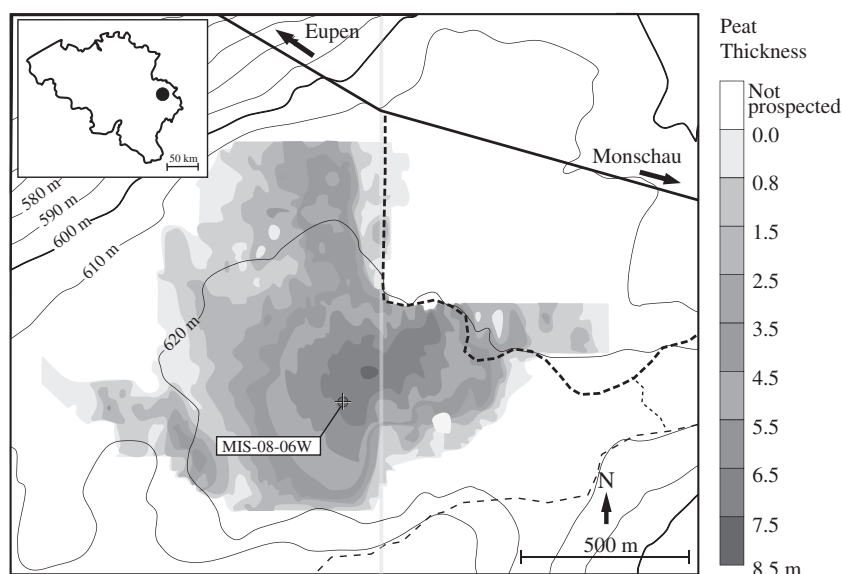


Fig. 1. Misten Bog and core location (MIS-08-06W), after De Vleeschouwer et al. (2012).

**Table 1**Concentrations of BHPs ( $\mu\text{g g}^{-1}$  dry sediment), quantified using HPLC-MS (accuracy  $\pm 20\%$ ), at various depths in the peat core.

Depth (cm)	BHT	2-MethylBHT	Aminotretol	Aminopentol	Aminotriol	Adenosylhopane	BHT cyclitol ether	BHT glucosamine	2-MethylBHT cyclitol ether	BHpentol cyclitol ether	BHhexol cyclitol ether	Total
13.7	45	26	8	0	295	88	215	8	12	6	12	715
21.5	74	21	13	4	366	92	400	10	12	6	8	1007
30.7	80	14	22	5	393	94	403	9	34	11	11	1076
43.4	97	15	35	4	414	65	421	13	26	9	9	1107
50.5	89	22	15	1	310	81	379	8	27	4	3	939
58.5	96	15	10	3	372	42	359	12	19	7	2	938
60.9	81	13	12	3	413	52	337	7	19	6	3	945
63.5	89	18	10	2	454	61	502	5	37	7	5	1190
69.8	161	19	20	3	711	68	511	11	32	9	12	1558
79.7	98	21	14	3	432	46	195	9	14	3	3	837
86.8	81	16	6	1	497	32	317	5	27	6	2	990
93.7	81	9	6	3	388	34	348	5	21	2	3	898
96.4	76	12	7	2	323	33	314	5	24	5	5	808
99.4	61	16	9	2	351	36	441	4	27	5	2	956

depths), freeze-dried and extracted using a modified Bligh and Dyer procedure (Cooke et al., 2008). An aliquot of the extract was used for hopene analysis and separated into an apolar and a polar fraction over an activated  $\text{Al}_2\text{O}_3$  column using hexane:dichloromethane (DCM; 9:1 v/v) and DCM:MeOH (1:1 v/v). To separate hopenes from acyclic and saturated hydrocarbons, the apolar fraction was eluted over an  $\text{AgNO}_3$ -impregnated column using DCM, after elution with hexane, hexane:DCM (9:1), hexane:DCM (1:1) and DCM. The DCM fraction was dried, dissolved in hexane and analysed using gas chromatography (GC), GC-mass spectrometry (GC-MS) and GC-isotope ratio monitoring mass spectrometry (GC-IRMS).

Another aliquot of the extract was used for BHP analysis. After addition of the internal standard (5 $\alpha$ -pregnane-3 $\beta$ ,20 $\beta$ -diol) the aliquot was acetylated with  $\text{Ac}_2\text{O}$  and pyridine (1:1 v/v; 50 °C, 1 h) and left to stand at room temperature overnight. The acetylated extract was dried at 50 °C under a continuous  $\text{N}_2$  flow. After filtration with a 0.45  $\mu\text{m}$  PTFE syringe filter, the extract was dissolved in MeOH/propan-2-ol (60:40 v/v), prior to high performance liquid chromatography (HPLC) MS.

#### 2.4. GC, GC-MS and GC-IRMS

GC was performed with a HP 6890 gas chromatograph equipped with flame ionisation detection (FID) set at constant pressure (100 kPa). A fused silica column (30 m  $\times$  0.32 mm i.d., film thickness 0.1  $\mu\text{m}$ ) coated with CP Sil-5CB was used with He as carrier gas. Samples were injected on-column at 70 °C and temperature was increased at 20 °C per min to 130 °C and 4 °C  $\text{min}^{-1}$  to 320 °C (held 20 min). Quantification was performed by integration of peak areas, using squalane as a co-injected internal standard.

Components were identified using a Thermo Trace GC Ultra (equipped with the same type of column and using the same temperature programme as for GC) coupled to a Finnigan Trace DSQ Single Quadrupole mass spectrometer (GC-MS).

Compound specific  $\delta^{13}\text{C}$  values were determined using GC-IRMS; ThermoFisher Delta V; with the same type of column and using the same GC temperature programme as above. Carbon isotopic ( $\delta$ ) values are reported relative to the Vienna Pee Dee Belemnite (VPDB) standard, with a reproducibility of, on average, 0.5‰.

#### 2.5. HPLC-(atmospheric chemical ionisation (APCI)-MS

Reversed-phase HPLC analysis was carried out using a Surveyor HPLC system (ThermoFinnigan, Hemel Hempstead, UK) fitted with a Gemini (Phenomenex, [Macclesfield, UK]  $\text{C}_{18}$  5  $\mu\text{m}$  column

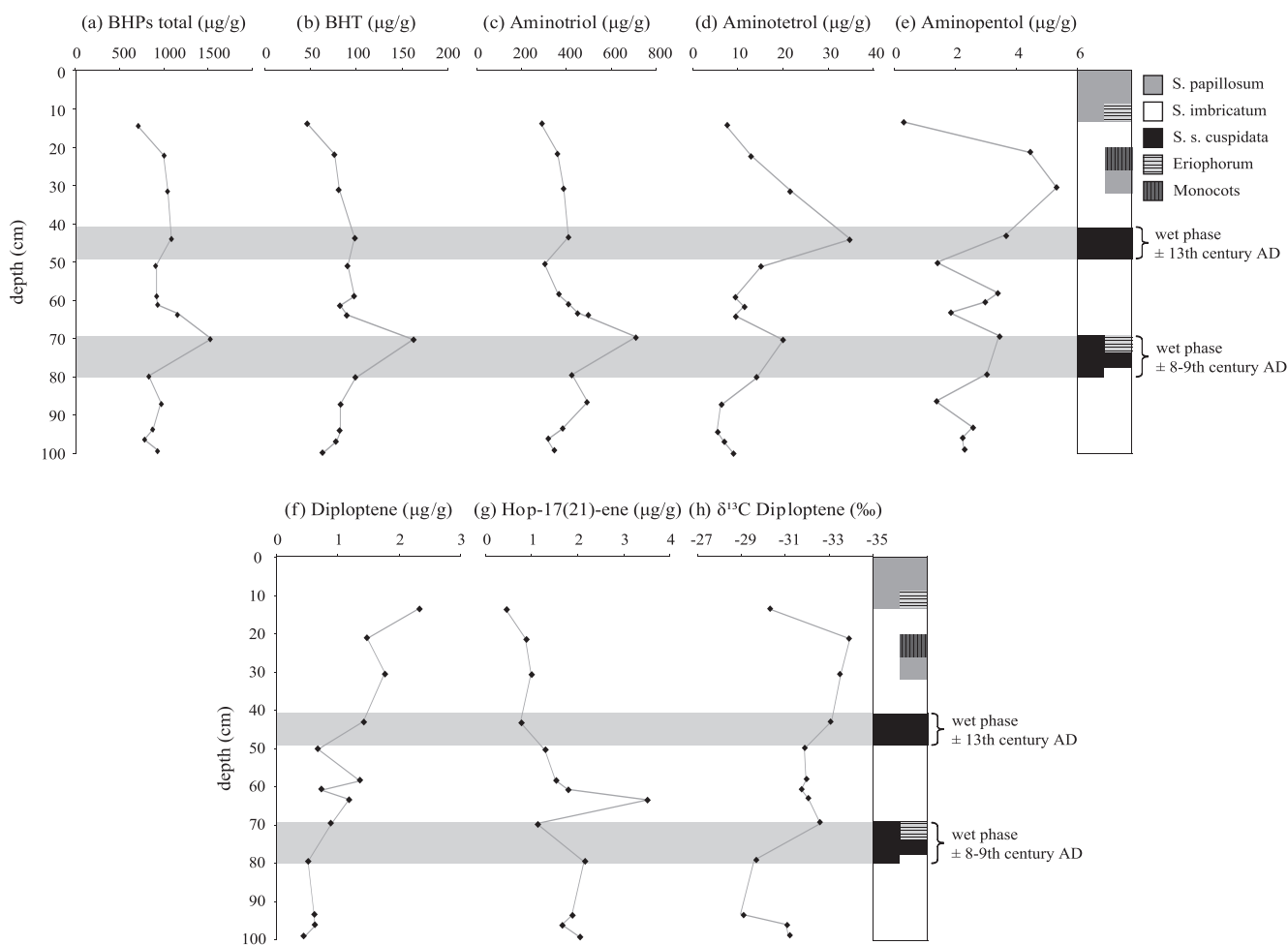
(150 mm  $\times$  3.0 mm i.d.) and a security guard column of the same material. Separation was achieved at a flow rate of 0.5 ml/min at 30 °C with the following gradient profile: 90% A and 10% B (0 min); 59% A, 1% B and 40% C (at 25 min), then isocratic (to 40 min) and returning to the starting conditions over 5 min and finally stabilizing for 15 min before the next injection (A = MeOH, B = water and C = propan-2-ol; all Fisher Scientific HPLC grade). HPLC-MS was performed using a ThermoFinnigan LCQ ion trap mass spectrometer equipped with an APCI source operated in positive ion mode. 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  $\text{MS}^2$  spectrum of most abundant ion from SCAN 1; SCAN 3 – data-dependent  $\text{MS}^3$  spectrum of most abundant ion from SCAN 2.

A semi-quantitative estimate of concentration was obtained based on the comparison of response factors of five authentic per-acetylated BHP standards (provided by M. Rohmer, Strasbourg, France; Cooke et al., 2008) relative to the internal standard 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\beta$ -diol. Bacteriohopanetetrol (BHT) was the only available standard which does not contain N and gives a response 8 $\times$  that of the internal standard. Four N-containing BHPs, i.e. 3 tetrafunctionalised composite structures (aminotriol; BHT cyclitol ether VI and the related BHT glucosamine IX) and adenosylhopane (V) were available. Although these 4 N-containing BHPs individually show some slight variation in response factor over time, taken as an average the response factor remains consistent at 12 $\times$  that of the internal standard (van Winden et al., 2012b). As only a limited number of standards were available relative to the known variety of possible structures, this average value was used for all N-containing BHPs including penta- and hexa-functionalised structures for which no standards were available. Quantification was performed using  $m/z$  traces targeting the characteristic base peak, i.e.  $[\text{M}+\text{H}]^+$ , ion for N-containing structures and  $[\text{M}+\text{H}-\text{CH}_3\text{COOH}]^+$  for those with no N relative to the base peak ion of the internal standard ( $m/z$  345 =  $[\text{M}+\text{H}-\text{CH}_3\text{COOH}]^+$ ). Reproducibility was  $\pm 20\%$  based on replicate injections of a range of samples on different days over time (Cooke, 2010) and was comparable to that reported by other workers (Blumenberg et al., 2009a,b).

### 3. Results and discussion

#### 3.1. BHPs as markers for methanotrophs

BHPs detected across the peat profile include BHT, aminotriol, aminotretol and aminopentol (Fig. 2b–e), known to be produced



**Fig. 2.** Depth distribution of (a) BHPs total, (b) BHT, (c) aminotriol, (d) aminotetrol, (e) aminopentol, (f) diploptene, (g) hop-17(21)-ene and (h) diploptene  $\delta^{13}\text{C}$  (‰) in a 100 cm peat core from Misten Bog, Hautes-Fagnes, Belgium. On the left axis, the current catotelm-acrotelm boundary is indicated and on the right axis, an abstracted vegetation profile is shown, along with the approximate dates of the wet phases (dominated by *S. s. Cuspidata*), adapted from De Vleeschouwer et al. (2012).

by methanotrophs (Rohmer et al., 1984; Neunlist and Rohmer, 1985a,b; Cvejic et al., 2000; Talbot et al., 2001; Coolen et al., 2008; van Winden et al., 2012b). Other BHPs present throughout the profile are 2-methylBHT, adenosylhopane, BHT cyclitol ether, BHT glucosamine, 2-methylBHT cyclitol ether, BHpentol cyclitol ether and BHexol cyclitol ether (Table 1). Aminotriol and BHT cyclitol ether are the most abundant BHPs in all the samples. The depth distribution of the summed BHP concentration is rather constant, with only elevated values around 70 cm (Fig. 2a). The concentration profiles of BHT and aminotriol closely resemble that of the summed BHPs (Fig. 2b, c and a, respectively). The concentrations of aminotetrol and aminopentol are lower than BHT and aminotriol and both show some variability with depth, with peaks in abundance between 20 and 45 cm and at around 70 cm (Fig. 2d and e). High aminotetrol and aminopentol abundances between 20 and 45 cm suggest enhanced methanotrophic activity (Fig. 2d and e). The present oxic-anoxic interface starts around 25 cm (De Vleeschouwer et al., 2012). Since methanotrophic activity is typically highest around the oxic-anoxic interface (Basiliko et al., 2004; van Winden et al., 2012a), the high aminotetrol and aminopentol concentrations between 20 and 45 cm probably reflect ongoing methanotrophic activity.

The presence of aminopentol confirms the presence of type I methanotrophs, yet the abundance of aminotetrol is significantly higher than it (Fig. 2d and e). This indicates a prevalence of type II methanotrophs. Kip et al. (2011) identified two main types of

methanotroph, a type II *Methylocystus* sp. and a type I *Methylomonas* sp. as the dominating methanotrophs in several *Sphagnum* ecosystems. These groups produce primarily aminotetrol and aminopentol, respectively (Talbot et al., 2001; van Winden et al., 2012b), so our results are therefore largely in line with the study of Kip et al. (2011).

The peak abundance in aminopentol is at shallower depth vs. that of aminotetrol (Fig. 2d and e), similar to the pattern observed in another active peat bog (van Winden et al., 2012b). The difference in depth for the peak abundances in the top part of the peat core possibly suggests a slight difference in oxygen and methane affinity between type I and type II methanotrophs, as observed by Crossman et al. (2004). Type I methanotrophs are situated at shallower depth, where oxygen concentration is higher and methane level lower, while type II methanotrophs prefer lower oxygen concentration and higher methane concentration (Crossman et al., 2004).

In the topmost sample, at 14 cm, aminopentol is below detection limit and aminotetrol concentration is low (Fig. 2d and e), in line with low methanotrophic activity. As the top 14 cm of the peat are well above the oxic-anoxic interface, methanotrophic activity should be limited.

Methanotrophs may also produce BHT and aminotriol, which are, however, less diagnostic as they are also rather common among other bacteria (e.g. Talbot et al., 2008). *Methylocella*-like methanotrophs, hypothesised to be one of the most important

methanotrophs in peat bogs (Dedysh, 2009) do not, to the best of our knowledge, produce the diagnostic aminotetrol and aminopentol, but primarily aminotriol (van Winden et al., 2012b; Talbot et al., unpublished data). In the core, BHT and aminotriol largely reflect the pattern of the cumulative BHPs, albeit that levels are slightly elevated between 20 and 45 cm (Fig. 2a–c). Except for the peak abundance at 70 cm, they do not match the curve of aminotetrol or aminopentol. BHT and aminotriol therefore probably have a mixed origin, and most likely do not primarily represent methanotrophs here. This is in line with a low abundance of *Methylocella*-like methanotrophs in *Sphagnum* moss inferred from low or absent PCR product when using PCR primers targeting known *Methylocella* genes (Kip et al., 2010).

The high aminotetrol and aminopentol concentration in the shallow part of the core suggest relatively high present day methanotrophic activity compared with the lower concentration observed downcore. Alternatively, the overall downcore decrease in aminopentol and aminotetrol could be the result of on-going diagenetic decay as the zone of active production by methanotrophs is limited to the oxic (i.e. upper) part of the record.

### 3.2. Hopenes as markers for methanotrophs

Diploptene concentration generally decreases with depth while, the concentration of hop-17(21)-ene increases (Fig. 2f, g). The steady decrease in diploptene suggests that little to none is produced at depth. The decrease in diploptene and the concurrent increase in hop-17(21)-ene may reflect isomerisation of diploptene to hop-17(21)-ene. This could not, unfortunately, be confirmed from the  $\delta^{13}\text{C}$  values of hop-17(21)-ene, since hop-17(21)-ene co-elutes with a 2-methyl analogue, making it difficult to accurately assess its  $\delta^{13}\text{C}$  values. In an experiment where  $^{13}\text{C}$  labelled methane was added to incubated *Sphagnum* moss, hop-17(21)-ene concentration was higher than diploptene concentration (van Winden et al., 2010). Both hopenes were strongly labelled and were therefore considered of (partial) methanotrophic origin. In a follow up field experiment using  $^{13}\text{C}$ -labelled methane, diploptene concentration was relatively high and showed strong label incorporation, while hop-17(21)-ene was below detection limit (van Winden et al., unpublished data). Furthermore, pure cultures of isolated methanotrophs contained diploptene, but no hop-17(21)-ene (van Winden et al., unpublished data). It is likely that hop-17(21)-ene can be indirectly derived from methanotrophs through isomerisation of diploptene. Isomerisation of hopanoids probably occurs rapidly because of the acidic environment (cf. Pancost et al., 2003). The peak in hop-17(21)-ene abundance at 64 cm, superimposed on the gradual increase, is not reflected in the diploptene profile and must therefore reflect another (unknown) origin.

Compound-specific  $\delta^{13}\text{C}$  values of diploptene vary between  $-29\text{‰}$  and  $-34\text{‰}$  (Fig. 2h). These values are relatively heavy vs.  $\delta^{13}\text{C}$  values observed for hopenes from *Sphagnum* from a field study (between ca.  $-32\text{‰}$  and  $-36\text{‰}$ ) or from a mesocosm experiment (between ca.  $-34$  and  $-41\text{‰}$ ) (van Winden et al., 2010; unpublished data). Since  $\delta^{13}\text{C}$  values have been shown to correlate to methanotrophic activity, with lower values associated with higher methanotrophic activity (van Winden et al., unpublished data), the overall relatively high  $\delta^{13}\text{C}$  values here may suggest relatively low methane oxidation rate. Alternatively, the methanotrophic signal may be diluted by other diploptene-producing bacteria compared to fresh *Sphagnum*.

Diploptene  $^{13}\text{C}$  content is relatively depleted in the lowermost samples, subsequently shifting towards higher  $\delta^{13}\text{C}$  values at 94 cm and returning towards lower  $\delta^{13}\text{C}$  values around 70 cm (Fig. 2h). Above this depth, the  $^{13}\text{C}$  content of diploptene remains low, only returning to higher values around 14 cm. At the top part

of the profile, between 20 and 45 cm, diploptene  $\delta^{13}\text{C}$  values are relatively low, in line with a high abundance of aminotetrol and aminopentol, thereby reflecting the currently active methanotrophic community (Fig. 2d, e and h). In the topmost samples, around 14 cm, diploptene is relatively high in  $^{13}\text{C}$ , corresponding to low aminotetrol and aminopentol concentrations, in line with low methanotrophic activity above the oxic–anoxic interface.

The diploptene  $^{13}\text{C}$  content in the deeper parts of the peat is high vs. recent values. Also, aminopentol and aminotetrol suggest relatively high present day methanotrophic activity, with relatively high abundances vs. the lower concentrations downcore. This can be explained in several ways. First, it could indicate that present-day methanotrophic activity is relatively high vs. past activity. Second, it could be the result of (enhanced) contribution of relatively  $^{13}\text{C}$  enriched diploptene originating from a different bacterial source in the deeper – anoxic – part of the profile. Hopanoids have been found in anaerobic bacteria (Sinninghe Damsté et al., 2004; Fischer et al., 2005; Blumenberg et al., 2006) and diploptene is, for example, a major neutral lipid of anammox bacteria (Sinninghe Damsté et al., 2005). However, diploptene concentration indicates little to no diploptene production at depth. Third, the overall downcore decrease in aminopentol and aminotetrol could be a result of on going decay as the zone of active production by methanotrophs is limited to the oxic (i.e. upper) part of the record.

### 3.3. Potential relationship between extent of methane cycling and water level

Over time, methanotrophic activity may have co-varied with water level or temperature. In the Misten Bog profile, two wet phases were recognised, marked by a shift in vegetation towards *Sphagnum* section *Cuspidata* (*S. s. Cuspidata*), which is restricted to wet bog surface conditions (Fig. 2; De Vleeschouwer et al., 2012). These wet phases occurred around the 8th–9th and around the 13th century AD (De Vleeschouwer et al., 2012). These periods are governed by high relative water level, the result of the balance between precipitation and evaporation. Field studies and laboratory experiments have revealed increasing methanotrophic activity with increasing water level (Basiliko et al., 2004; Raghoebarsing et al., 2005; Kip et al., 2010; Larmola et al., 2010).

Indeed, aminotetrol shows elevated values coinciding with the wet phases; however, aminopentol and diploptene  $\delta^{13}\text{C}$  values do not show this (Fig. 2). Therefore, no clear conclusions can be drawn from the combination of different methanotroph biomarkers.

A reason for the discrepancy between the methanotroph markers is possibly the predominance of type II methanotrophs. They use a combination of available  $\text{CH}_4$  and  $\text{CO}_2$ , potentially leading to a dispersed  $\delta^{13}\text{C}$  signature. Additionally, the diploptene  $\delta^{13}\text{C}$  values may be derived from a combination of sources, masking a possible methanotrophic activity related profile. Furthermore, type II methanotrophs do not produce aminopentol, which explains why the aminopentol concentration, close to the detection limit, does not show a clear trend with depth.

Alternatively, the climatic shifts during the formation of the peat bog may have been insufficient to cause significant variation in the methanotroph proxies, explaining the lack of consistency between the proxies. The peaks in aminotetrol may be coincidental, or caused by other bacteria also producing aminotetrol, for instance sulfate reducing bacteria (cf. Blumenberg et al., 2006, 2009a,b), although unlikely in a sulfate-poor environment, or unknown species.

In order to establish how methanotrophic activity correlates with climatic changes, a future study could combine a study of methanotroph biomarkers with additional proxies which may provide information on water level and the methane cycle, such

as testate amoebae, macrofossils and archaeol (cf. Woodland et al., 1998; Pancost et al., 2011). Finally, to ensure sufficient variation in the methanotroph proxies, future research should focus on peat deposits reflecting larger shifts in climate.

#### 4. Conclusions

Relatively high methanotrophic activity was recorded by the investigated methanotrophic markers between 20 and 45 cm, around the present oxic–anoxic boundary, most likely representing a currently active methanotrophic community. A predominance of aminotetrol over aminopentol indicated a prevalence of type II methanotrophs. In the deeper parts of the peat, methanotrophic markers did not show a clear correlation between the past change in water level and methanotrophic activity. This may be related to a predominance of type II methanotrophs, which do not produce aminopentol and which consume both CO<sub>2</sub> and CH<sub>4</sub>, resulting in a lower sensitivity of diplotene δ<sup>13</sup>C values. Furthermore, the lack of correlation could be caused by a mixture of sources for the biomarkers or insufficient variation in climatic change. A proposed way forward would include a study of peat deposits, showing greater climatic variability, with the application of multiple proxies.

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