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Abstract

Methane and CO$_2$ are climatically active greenhouse gases (GHG) and are powerful drivers of rapid global warming. Comparable to the Arctic, the tropics store large volumes of labile sedimentary carbon that is vulnerable to climate change. However, little is known about this labile carbon reservoir, in particular the behaviour of high methane–producing environments (e.g. wetlands), and their role in driving or responding to past periods of global climate change. In this study, we use a microbial biomarker approach that traces continental aerobic methane oxidation (AMO) from sedimentary organic matter in deep-sea fan sediments off the Congo River to reconstruct the link between central African methane cycling and continental export during key periods of global Pleistocene warmth. We use 35-amino bacteriohopanepolysols (BHPs), specifically aminobacteriohopane-$31,32,33,34$-tetrol (aminotetrol) and $35$-aminobacteriohopane-$30,31,32,33,34$-pentol (aminopentol) as diagnostic molecular markers for AMO (CH$_4$ oxidation markers) and the prevalence of continental wetland environments. BHPs were analysed in sediments from the Congo fan (ODP 1075) dated to 2.5 Ma. High resolution studies of key warm marine isotope stages (MIS) 5, 11 and 13 are included to test the relationship between CH$_4$ oxidation markers in sediments at different levels of elevated global atmospheric GHG.

This study presents the oldest reported occurrence, to date, of 35-amino BHPs up to 200 meters below sea floor (~2.5 Ma) with no strong degradation signature observed. Low concentrations of CH$_4$ oxidation markers identified between 1.7 Ma and 1 Ma suggest a reduction in wetland extent in tropical Africa in response to more arid environmental conditions. Correlation of high resolution CH$_4$ oxidation marker signatures with global atmospheric GHG concentrations during MIS 5, 11 and 13...
further emphasize periods of enhanced tropical C cycling. However, subsequent
analysis would be required to further extrapolate the relative importance of tropical
methane sources as a driver of global methane concentrations during the
Pleistocene.

Key words
Pleistocene, Congo, wetland, methane cycle, methanotrophic bacteria,
bacteriohopanepolyols
1. INTRODUCTION

During the past century, Earth has experienced a rapid rise in surface temperature
with instrumental records showing the past 30 years to have been successively
warmer than the previous decades (IPCC, 2013). In parallel with this rise, significant
increase in the atmospheric concentrations of greenhouse gases (GHG) have been
recorded, with methane (CH₄) and carbon dioxide (CO₂) being particularly potent
GHG and potential drivers of climate change (IPCC, 2013). Changes in atmospheric
temperatures and the hydrological cycle have a coupled impact on biogeochemical
cycles, including the C cycle. Perturbations in the C cycle could lead to the
degradation of vulnerable C sources such as wetlands (IPCC, 2013). While modern
elevated atmospheric GHG concentrations are of natural and anthropogenic origins,
the source of them in the palaeo-record as a climate and ecosystem regulator
remains unclear. Within the modern terrestrial system, wetlands are the largest
natural source of CH₄ and are estimated to account for ~70% of all natural emissions
(Wuebbles and Hayhoe, 2002) with tropical wetlands (20°N to 30°S) identified as the
largest CH₄ producers (Bartlett and Harriss, 1993; Sjogersten et al., 2014). Changes
in the extent and volume of tropical methane sources and sinks (i.e. wetlands and
atmospheric oxidation), modulated by fluctuations in the hydrological cycle and
vegetation feedbacks, have been shown to exert a significant control on atmospheric
methane concentrations over the past 800 ka (e.g. Blunier et al., 1995; Loulergue et
al., 2008; Singarayer et al., 2011).

Paleoclimate analysis of West African marine sedimentary archives suggests
significant variations in continental aridity and humidity during the Pleistocene
leading to the destabilisation of vegetation zones (Schefuß et al., 2003). Biomarker
and pollen analysis of deep-sea sedimentary archives from North West Africa
reveals major changes in the balance between C_3 and C_4 vegetation, reflecting
significant changes in climate during the past 160 ka (Zhao et al., 2003).
Furthermore, biomarker and pollen analysis from southwest African margin
sediments along a transect of 9 sites from Congo (4°S) to Cape Bush (30°S) reveal
an expansion of C_4 plant indicators during Holocene glacial periods, thus indicating a
northward expansion of arid zones favouring grass vegetation (Rommerskirchen et
al., 2006). While the quantitative impact of habitat expansion on C cycling remains to
be resolved, the expansion of mangroves and wetland environments during these
warm periods may have resulted in an increased flux in CH_4 and other GHG to the
atmosphere. Indeed, ice core records suggest that changes in the strength of tropical
CH_4 reservoirs had an important control on the global atmospheric CH_4 budget
during the past 800 ka (Petit et al., 1999; Loulergue et al., 2008). However, direct
measurements of atmospheric CH_4 concentration beyond 800 ka are not available
from ice cores, requiring an independent proxy approach to explore CH_4 dynamics
further back in time, and in non-glacial environments. It has been shown that specific
biomarker records are powerful proxies for reconstructing C cycling beyond this time
period of direct measurements (Talbot et al., 2014).

Bacteriohopanepolyols (BHPs) are highly functionalised pentacyclic triterpenoids
produced by many aerobic as well as a number of obligate and facultative anaerobic
bacteria (e.g. Rohmer et al., 1984; Talbot et al., 2008; Eickhoff et al., 2013; and
references therein). Some BHPs with an amino group at the C-35 position (35-amino
BHPs) are thought to be specific indicators of aerobic methane oxidation (AMO) with
35-aminobacteriohopane-31,32,33,34–tetrol (aminotetrol; II; Table 1); 35-
aminobacteriohopane-30,31,32,33,34-pentol (aminopentol; III), unsaturated
aminopentol (IV/V) and aminopentol isomer (III”; van Winden et al., 2012b) being
almost exclusively produced by aerobic methanotrophs (Talbot and Farrimond, 2007; Zhu et al., 2010; van Winden et al., 2012b; Berndmeyer et al., 2013; hereafter referred to as CH₄ oxidation markers). CH₄ oxidation markers have been identified in both terrestrial and marine settings, including but not limited to; peatlands (van Winden et al., 2012a; 2012b; Talbot et al., 2016), a stratified post glacial lake in Antarctica (Coolen et al., 2008), and within suspended particulate matter in ocean settings (Blumenberg et al., 2007; Wakeham et al., 2007; Sáenz et al., 2011), modern soils from the Amazon (Wagner et al., 2014) and Congo hinterland (Spencer-Jones et al., 2015), and ancient sediments from the Amazon (Wagner et al., 2014) and Congo (Talbot et al., 2014) fans. However, despite the variety of terrestrial and marine environments where these biomarkers have been found, current research suggests that when found in marine sediments they are primarily of terrestrial origin (Talbot et al., 2014; Wagner et al., 2014; Schefuß et al., 2016), specifically from catchment wetlands (Spencer-Jones et al., 2015). Large scale fluctuations in the concentration of these compounds during warm – humid interglacial periods have been associated with hydrological changes and corresponding fluctuations in wetland extent (Talbot et al., 2014; Schefuß et al., 2016). Furthermore, hydrologically induced variations in Congo wetland systems during the Holocene resulted in the export of pre-aged OM during arid conditions and younger-OM during humid environmental conditions (Schefuß et al., 2016) potentially leading to lags in biomarker export during past glacial (dry) and at the transition to interglacial (humid) Termination conditions. A previous low-resolution study was limited to the first ~1.2 Ma (115.65 meters below sea floor, m.b.s.f) of a Congo deep-sea fan core (ODP 1075) and, therefore, could not resolve some of the short term variability in 35-amino BHP distributions and, thus, AMO cycling during the earlier parts of the Pleistocene (Talbot et al., 2014).
This study expands on previous research by addressing the causes and implications of short- and long term variability in CH$_4$ oxidation markers in the Congo fan record (Talbot et al., 2014). This study also explores the fluctuations in CH$_4$ oxidation markers under different, elevated atmospheric GHG concentrations. We first test the stratigraphic suitability of 35-amino BHPs for palaeoclimate reconstructions in ODP 1075 sediments by expanding the record to the core’s maximum depth of 201.25 m.b.s.f (~2.5 Ma). Building on this assessment, we introduce high resolution records of CH$_4$ oxidation marker concentration for marine isotope stages (MIS) 2-6 and 10-13 to address changes in short term variability in terrestrial CH$_4$ cycling. These intervals include MIS 5 and 11, which are both considered particularly warm stages in the geologic record with approximate durations of 58 ka and 64 ka, respectively (Howard, 1997; Kukla et al., 1997; Spahni et al., 2005). MIS 5 and 11 are characterised as periods of reduced ice volume with an interglacial and multiple interstadials and stadials (Loutre et al., 2003) and may serve as potential analogues for Earth’s future climate. BHP distributions of MIS 5 and 11 are contrasted with the intermediate warm period MIS 13 and cool MIS 2, 3, 4, 6, 10, and 12, to further test the sensitivity of the biomarkers to global GHG levels and improve characterisation of short-term transitions in AMO variation during the Pleistocene.

2. MATERIALS AND METHODS

2.1. SITE LOCATION AND SAMPLE DESCRIPTION

The Congo River is the largest river in Africa and the second largest river in the world in terms of drainage basin size (~3.7 x 10$^6$ Km$^2$; Runge, 2007; Laraque et al., 2009) and supplies freshwater, nutrients (including large amounts of SiO$_2$) and sediment to the ocean. The Congo River plume extends 800 Km offshore and can be
detected during austral summer when monsoon/precipitation reach their maximum seasonal intensity (Anka and Séranne, 2004). The rapid outflow of the Congo River is caused by the relatively small river mouth and a large canyon head (Berger et al., 2002). As a result of coastal, oceanic and river induced upwelling, modern primary production is very high in the surface waters off the Congo continental margin (Anka and Séranne, 2004).

Sedimentation within the Congo fan is dominated by rainout of suspended clays derived from the Congo River and by pelagic settling of biogenic debris (Berger et al., 2002). The lower Congo basin sediments lack a significant river borne sand and silt fraction, due to most of the coarse debris being deposited before it reaches the ocean (Spencer et al., 2012).

The Congo catchment hosts extensive wetland and water dependent ecosystems and supports one of the world’s largest swamp forests (360 000 Km²; Bwangoy et al., 2010). One prominent example is Malebo Pool, located on the main stem of the Congo River (Fig. 1). Organic matter (OM) exported from this region has been shown to be geochemically very similar to OM at the head of the estuary (~ 350 km downstream), consistent with no major tributaries joining the Congo River between Malebo Pool and the Atlantic Ocean (Spencer et al., 2012). Annual water level change in Malebo Pool is ~3 m and average river flow is ~30 000 m³ s⁻¹ during dry periods and 60 000 m³ s⁻¹ during the wet season (Thieme et al., 2005).

During the Ocean Drilling Program (ODP) leg 175, 13 sites were drilled off the West African coast (Aug-1997; Shipboard Scientific Party, 1998). ODP site 1075 is a deep water drill site on a depth transect in the lower Congo basin located at 2995 m water depth. ODP 1075 is dominated by (1) freshwater input from the Congo River, (2)
seasonal coastal upwelling activity and associated filaments and eddies moving offshore, and (3) incursions of open-ocean waters from the South Equatorial Countercurrent (Berger et al., 2002). Sediments from ODP 1075 and neighboring cores (e.g. ODP 1077) have been closely correlated with climatic signaling (Jahn et al., 2005) and large scale shifts in terrestrial vegetation relating to humidity – aridity cycles (Dupont et al., 2000; Schefuß et al., 2003; 2004). ODP 1075 is approximately 200 m.b.s.f. with an average sedimentation rate of 100 m/Ma. OM in ODP 1075 is of mixed terrestrial and marine origin, with soil OM (SOM) being an important but variable contributor (Holtvoeth et al., 2001, 2003).

Sediment cores from ODP 1075 were stored at the International Ocean Discovery Program (IODP) Bremen Core Repository (MARUM, Bremen University) at 4°C. Samples between 1.65 and 115.65 m.b.s.f were previously collected by Holtvoeth et al. (2001) and made available to this study. The lower section between 115.7-201.25 m.b.s.f of ODP 1075 (core A) were re-sampled at 1 m intervals, resolving on average 13 to 18 ka. The samples were stored in polypropylene bags and shipped to Newcastle University (UK) where they were frozen immediately upon arrival.

2.2. STRATIGRAPHY AND AGE MODEL

Stratigraphy and the age model of ODP 1075 was previously determined by Dupont et al. (2001). Due to low amounts of calcareous foraminifera in ODP 1075 for oxygen isotope stratigraphy, the age model was established by correlating magnetic susceptibility of 1075 with the neighboring site 1077. Both ODP sites 1075 and 1077 represent the same hydrographic conditions and show similar variations in magnetic susceptibility (see Fig. 1 in Dupont et al., 2001). The age model for site 1077 is based on a correlation of the δ¹⁸O curve for Globigerinoides ruber (pink) with the benthic isotope record of ODP site 677 in the deep Pacific (Shackleton et al., 1990).
Sedimentation rates were calculated by linear interpolation between age control points (Jahn, 2002). The error associated with the age model of ODP 1075 has been roughly estimated by Berger et al. (2002) to be approximately 0.01 Ma. The age assignment of MIS are according to Lisiecki and Raymo (2005).

2.3. TOTAL ORGANIC CARBON (TOC)

Total OC (%) content of ODP 1075 samples between 115.7-201.25 m.b.s.f were measured at Newcastle University using a LECO CS244 Carbon/Sulfur Analyser as detailed in Spencer-Jones et al. (2015). TOC of samples between 1.65-115.65 m.b.s.f were obtained from Holtvoeth et al. (2003).

2.4. LIPID EXTRACTION

Total lipids were extracted using an adaptation of the method published in Talbot et al. (2007) and further modified by Osborne (2016), which is based on the Kates modification (Kates, 1972) of the Bligh and Dyer extraction (Bligh and Dyer, 1959).

Total lipids were extracted from 1-3 g of freeze-dried and homogenised sediment. Sediment was extracted in a Teflon centrifuge tube (50 ml) with a monophasic mixture of bi-distilled water (4 ml), methanol (10 ml) and chloroform (5 ml). The sample was agitated vigorously via sonication for 15 min at 40°C followed by centrifugation for 15 min at 12000 rpm. The supernatant was collected and added to a second 50 ml centrifuge tube. This extraction was repeated three times with the supernatant collected at the end of each extraction cycle.

The monophasic extracts were phase separated via addition of chloroform (5 ml) and bi-distilled water (5 ml) to each of the supernatants. The sample was gently inverted and centrifuged for 5 minutes (12000 rpm) to break the emulsion. The organic layer from each tube was transferred to a round bottom flask (100 ml) and concentrated.
using a rotary evaporator. The total lipid extract (TLE) was transferred to a glass vial using chloroform/methanol at a ratio of 2:1 (v/v). A 5α-pregnane-3β,20β-diol internal standard was added to the TLE. One third of the TLE was acetylated using acetic anhydride (250 µl) and pyridine (250 µl). The TLE was heated at 50°C for one hour and then left at room temperature overnight. Samples were dried under a stream of N₂ with heating from below (40°C). BHP extracts were then stored at 4°C prior to further processing.

2.5. BACTERIOHOPANEPOLYOL ANALYSIS

BHP analysis was performed by HPLC-APCI-MS" using a ThermoFinnigan surveyor HPLC system fitted with a Phenomenex Gemini C₁₈ column (150 mm; 3.0 mm i.d.; 5 µm particle size) and a security guard column cartridge of the same material coupled to a Finnigan LCQ ion-trap mass spectrometer equipped with an APCI source operated in positive ion mode, as described in Talbot et al. (2003). The error in absolute quantification was ± 20%, based on selected replicate analyses and BHP standards of known concentration (Cooke, 2010; van Winden et al., 2012b). The abbreviated names of the compounds identified, characteristic base peak ions (m/z) and structure numbers are given (Table 1). All concentrations are given to 2 significant figures with raw data presented in Appendix A. Statistical analysis was performed using Minitab 17.1.0. Spearmans Rho (Rₚ) correlation index was performed on BHP abundances. “CH₄ oxidation markers” is the sum of aminotetrol, aminopentol, aminopentol isomer, and unsaturated aminopentol (II, III, III’, IV/V). In this study, we report 182 new BHP data points from the Congo deep-sea fan (ODP site 1075) which are complemented by 122 data points published by Talbot et al. (2014).
3. RESULTS

Consistent with Talbot et al. (2014), aminotriol (I), aminotetrol (II), aminopentol (III), aminopentol isomer (III'), and unsaturated aminopentol (IV or V) are identified within ODP 1075 sediments (Fig. 2a, b, c, and e). Aminotriol is the most abundant 35-amino BHP with concentrations between 4.6 and 360 µg gTOC−1. This is followed by aminopentol as the second most abundant 35-amino BHP with concentrations ranging between 0 and 280 µg gTOC−1. Aminotetrol is identified at lower concentrations, compared with aminotriol and aminopentol and is intermittently present throughout ODP 1075. Despite differences in concentration, aminotriol, aminotetrol, and aminopentol show similarities in distributions and persist to the bottom of the core at 201.25 m.b.s.f (~2.5 Ma). In contrast, unsaturated aminopentol (0 and 12 µg gTOC−1) and aminopentol isomer (0 and 39 µg gTOC−1) are present at much lower concentrations and do not show a similar distribution to aminotriol, aminotetrol and aminopentol (Fig. 2b and c). Significant correlation is found between aminopentol and aminotriol (Rs 0.891, p<0.05; Fig. 2e), aminopentol and aminotetrol (Rs 0.908, p<0.05; Fig. 2e), and aminotriol and aminotetrol (Rs 0.904, p<0.05, not shown), supporting a common response of all compounds to changing environmental and/or depositional conditions. No statistically relevant correlation is observed between TOC and aminotriol (Rs 0.101, p 0.079), aminotetrol (Rs -0.042, p 0.463), or aminopentol (Rs 0.006, p 0.920) concentrations (µg g sediment−1; figures not shown). Total CH₄ oxidation marker concentrations (sum II, III, III', IV/V) are highly variable down core (Fig. 2d). Peak concentrations in CH₄ oxidation markers occur at around 1686 ka (270 µg gTOC−1), 1271 ka (330 µg gTOC−1) and 491 ka (270 µg gTOC−1). Starting at the Pliocene/Pleistocene transition (~2.5 Ma), a steady increase in CH₄ oxidation marker concentration is observed. Between 1865 and
1713 ka (hereafter referred to as interval ‘a’; Fig. 2d) a clear decrease in CH₄ oxidation marker concentration is observed. A similar reduction in CH₄ oxidation markers (µg gTOC⁻¹) is also observed between 1099 ka and 826 ka (hereafter referred to as interval ‘b’; Fig. 2d).

35-Amino BHPs show a similar distribution within the high resolution sections to the full ODP 1075 record (Fig. 3 and Fig. 4). Aminotriol is the most abundant BHP (4.6 and 270 µg gTOC⁻¹) followed by aminopentol (0 and 210 µg gTOC⁻¹). Unsaturated aminopentol and aminopentol isomer have an intermittent presence between MIS 2-6 and MIS 10-13. Peak concentrations in aminotriol, aminotetrol, and aminopentol are observed during MIS 5, 11, and 13, reaching comparable maximum levels for all three warm intervals (Fig. 3 and Fig. 4). Similar to the overall BHP distribution in ODP 1075 (Fig. 2), CH₄ oxidation marker concentrations are highly variable during the investigated high resolution MIS intervals (Fig. 3 and Fig. 4). High concentrations of total CH₄ oxidation markers are observed during MIS 13 (range 0 and 270 µg gTOC⁻¹, mean 130 µg gTOC⁻¹), followed by a reduction during MIS 12 (range 0 and 210 µg gTOC⁻¹, mean 51 µg gTOC⁻¹), and a marked increase in CH₄ oxidation markers during MIS 11 (range 0 and 260 µg gTOC⁻¹, mean 96 µg gTOC⁻¹). Peak concentration in CH₄ oxidation marker is similar during MIS 11 and 13 (Fig. 3). Low concentrations of CH₄ oxidation markers are observed during MIS 6 (range 0 and 39 µg gTOC⁻¹, mean 14 µg gTOC⁻¹) followed by an increase in concentration during MIS 5 (range 5.7 and 190 µg gTOC⁻¹, mean 66 µg gTOC⁻¹). Following MIS 5, CH₄ oxidation marker concentration remains low during MIS 2-4 (range 0 and 96 µg gTOC⁻¹, mean 14 µg gTOC⁻¹). Overall higher mean CH₄ oxidation markers are observed during MIS 11 and 13 compared with MIS 5 (Fig. 5).
4. DISCUSSION

4.1. DIAGENETIC AND ENVIRONMENTAL CONTROLS ON 35-AMINO BHPS IN ODP 1075

Aminotriol (I), aminotetrol (II) and aminopentol (III) down core profiles do not show clear diagenetic trends, with all of these compounds present within ODP 1075 sediments dated to ~ 2.5 Ma (Fig. 2). The absence of any correlation between TOC and 35-amino BHP suggests that variations in BHPs are not driven by variations in TOC. One previous study exists discussing the preservation of 35-amino BHPs in ancient sediments, where Wagner et al. (2014) identified CH₄ oxidation markers in Amazon fan and shelf sediments to a maximum core depth of 708 cm, dated to approximately 30 ka. The results shown here from the Congo deep-sea fan represent the oldest and longest continuous record of CH₄ oxidation markers in sediments to date. As no clear diagenetic trend in CH₄ oxidation markers is evident we anticipate that these biomarkers can be examined within much deeper sediments, without any reasonable justification for a specific sub-surface limit.

Furthermore, we also report the occurrence of unsaturated aminopentol and aminopentol isomer within sediments dating to 2.2 Ma and 2.4 Ma, respectively (Fig. 2). The observation of unsaturated aminopentol and aminopentol isomer in ODP 1075, again, is the oldest reported occurrence of these two compounds, building confidence that the high resolution 35-amino BHP records presented here represent primary signals, with minimal or no diagenetic alteration.

Previous analysis of 35-amino BHPs within Congo fan sediments suggest that these compounds are of allochthonous origins, likely derived from Congo hinterland wetlands and similar environments (Talbot et al., 2014). In agreement with previous studies, BHP concentrations and proportions of aminotriol:aminopentol:aminotetrol in
our extended ODP 1075 record show strong correlation to each other (Fig. 2e; Cvejic et al., 2000; Talbot et al., 2001; van Winden et al., 2012b; Osborne, 2016) supporting a common methanotroph source.

4.2. A 2.5 MA RECORD OF CH$_4$ CYCLING

As discussed in Talbot et al. (2014), analysis of Congo fan sediments suggests long term fluctuations in CH$_4$ oxidation markers during the Pleistocene with enhanced production and preservation of CH$_4$ oxidation markers during warm-humid interglacial MIS. The data presented here, support and further expand this concept. Intervals ‘a’ and ‘b’ (Fig. 2d), suggest a shift in the terrestrial BHP producing community and thus widespread change in African ecology and hydrology that coincides with increased African climate variability and aridity between 1.7 Ma and 1 Ma (deMenocal, 2004; Trauth et al., 2007). We propose that increased continental African aridity would have reduced wetland extent and, subsequently, reduced the production and supply of CH$_4$ oxidation markers to the Congo fan. These arid intervals of low CH$_4$ oxidation marker concentrations are consistent with the onset and amplification of high latitude glacial conditions (deMenocal et al., 1993, 1995; Tiedemann et al., 1994; Clemens et al., 1996). Furthermore, CH$_4$ oxidation markers have been shown to follow a systematic pattern of elevated concentrations during warm MIS 5, 11, 13, 17, 21, and 33 (Talbot et al., 2014). Our study further supports this trend and shows that this relationship persists beyond 1.2 Ma to 2.5 Ma with high concentrations of CH$_4$ oxidation markers identified during MIS 39, 47, 49, 59, 75, and 83 (Fig. 2). This new data further suggests enhanced methane cycling during warm-humid time periods of the Pleistocene.
4.3. SHORT TERM TRENDS IN CH₄ CYCLING

Throughout the Pleistocene, subtropical African climate periodically oscillated between wet and dry climate conditions, which drove largescale ecological change (Schefu ß et al., 2003, 2005; deMenocal, 2004). The high resolution timeseries of MIS 5, 11, and 13 show high concentrations of CH₄ oxidation markers, consistent with high global GHG concentrations (Fig. 3 and 4) and suggest enhanced C cycling during these intervals. Furthermore, high concentrations of these biomarkers coincide with the expansion of tropical rainforests and water-dependent ecosystems (Miller and Gosling, 2014) supporting the concept that CH₄ oxidation markers may actually document widespread ecological change in the Congo.

Global methane concentrations were high during MIS 5 and MIS 11 (Fig. 3 and 4) and slightly lower during MIS 13, largely owing to a cooler climate (Spahni et al., 2005). However, the range of CH₄ oxidation marker concentrations in the Congo core are comparable for all three time slices (Fig. 3 and 4), suggesting either similarities in methanotrophy/ecosystem and/or a decoupling between BHP synthesis and aerobic methanotrophy.

4.3.1. WEST AFRICAN ECOSYSTEM DURING THE PLEISTOCENE

Similarities in methanotrophy/ecosystem during MIS 5, 11, and 13 are supported by integrated palynological evidence from various sediment cores from the wider West African coastal area suggesting strong similarities in vegetation, habitat types, and interpreted temperatures and precipitation (Miller and Gosling, 2014). Furthermore, fluctuations in global atmospheric CH₄ concentrations may have been due to changes in Northern hemisphere methane sources (Froese et al., 2008; Vaks et al., 2010; Vázquez Riveiros et al., 2013; Reyes et al., 2014), therefore not directly affecting tropical wetland sources and thus BHP signatures in deep-sea fan settings.
Low concentrations of CH$_4$ oxidation marker are observed during MIS 2, 3, 4, 6, 10, 12, which also coincides with globally low concentrations of atmospheric GHG and would support the development of dry-arid conditions (Spahni et al., 2005), with restricted extent of water dependent ecosystems (Dalibard et al., 2014). While wetland ecosystems are not well represented within the palynological archive, pollen records can indicate ecosystem community structure and potential environmental conditions (for example, Miller and Gosling, 2014). Limited evidence from pollen records from the tropical Atlantic off West Africa indeed indicates that vegetation assemblages of the Congo basin and surrounding mountains were susceptible to changes in precipitation during the Pleistocene, with extensive rainforest and mangrove ecosystems common during humid stages (Dalibard et al., 2014). The development of more open, savannah-type ecosystems is characteristic of glacial periods (Dupont et al., 2000; Versteegh et al., 2004; Dupont, 2009), supporting our conclusion of a reduction in wetland habitats during these overall dryer periods.

Progressively lower mean CH$_4$ oxidation marker concentrations are observed during MIS 5 compared with the older warm isotope stages (i.e. MIS 11 and 13; Fig. 5). This is despite MIS 5 being considered a very warm interglacial with high global atmospheric methane concentrations (Spahni et al., 2005; Loulergue et al., 2008). This disparity may suggest more extended wetlands during MIS 11, 13, and potentially earlier interglacial climate stages of the Pleistocene, compared to MIS 5. Terrestrial and marine paleoclimate records from Africa suggest a trend towards greater aridity during the Pleistocene (Schefuß et al., 2003; deMenocal, 2004; Ségalen et al., 2007; Trauth et al., 2009) consistent with shifts in vegetation from C3 (trees and shrubs) to C4 (tropical grasses). However, despite the global trend towards greater aridity, regional/local trends may have diverged showing more
pronounced variability and higher frequency alternating between dry and humid periods (e.g. Johnson et al., 2016).

### 4.3.2. DECOUPLING OF BHP SYNTHESIS AND GLOBAL GREENHOUSE GAS CONCENTRATIONS

In addition to climatological and hydrological driven changes in Congo carbon cycling, CH$_4$ oxidation marker concentrations could also show a potential decoupling between aerobic methanotrophy, BHP synthesis, and atmospheric GHG concentrations. Support for this concept comes from incubations of enrichment cultures that show temperature variations having a strong control on AMO intensity, with peak methane oxidation occurring between 20 and 40°C (van Winden, 2011; Sherry et al., 2016) and resulting in a shift in methanotroph community structure (e.g. Sherry et al., 2016). Furthermore, aerobic methanotroph activity appears closely controlled by the heterogeneity in the soil environment, the solubility and bioavailability of methane - a direct variable linked to methane production and methane flux - and other environmental parameters (e.g. pH, salinity; Sherry et al., 2016). Climate and hydrology cycles were highly variable during the Pleistocene, with MIS 13 relatively cool and arid and MIS 5 and 11 relatively warm and humid (Spahni et al., 2005). These differences could have resulted in differences in carbon cycling and resulted in the BHP concentrations observed in Fig. 2, 3, and 4. Under the warm and humid environmental conditions of MIS 5 and 11, the ecosystem would have supported extended wetlands with intense methanogenesis. Aerobic methanotrophy could have decreased through a potential reduction in the oxic-anoxic boundary or through the methanotroph community being bypassed due to the sudden release of methane bubbles (ebullition, e.g. Bastviken et al., 2004) and/or plant mediated transport of methane gas to the atmosphere (Whalen, 2005). The
degree to which these parameters control BHP synthesis as well as preservation within marine sediments is still largely unknown (Jahnke et al., 1999; Poralla et al., 2000; Doughty et al., 2009; Welander et al., 2009; van Winden, 2011).

4.4. TRANSPORT, DEPOSITION, AND AGE OF BHP RECORDS IN THE CONGO DEEP-SEA FAN

We recognise two principal challenges to constrain the sensitivity of CH₄ oxidation markers in the deep-sea fan within the context of West African climate dynamics. These are (1) the mechanisms and locations of aminopentol signal formation and its transport and burial in the deep marine sediments and (2) a robust high resolution stratigraphic framework to place possible mechanisms into the climatic and sedimentological context of the African-Congo catchment/deep-sea fan system. Here, we further discuss these control mechanisms within a conceptual framework for further research.

4.4.1. TRANSPORT AND DEPOSITION OF BHP SIGNAL IN THE CONGO CATCHMENT

Previous endmember analysis suggests that the source of Congo methane oxidation signature may indeed come from the deep interior of the Congo catchment (Spencer-Jones et al., 2015), and is potentially controlled by central African climate evolution (Schefuß et al., 2016). However, the extent to which the biomarker signal is reworked during transport from source environments to the Congo fan is yet to be elucidated. During riverine transport, a large proportion of sediment is trapped on the Cuvette Centrale with a lesser proportion of sediment subsequently trapped at Malebo pool (Laraque et al., 2009; Spencer et al., 2012). Furthermore, the fine particulate organic matter (FPOM, 0.7-63µm) fraction potentially undergoes some
form of extended degradation in the Cuvette Centrale (Laraque et al., 2009; Spencer et al., 2012). However, Spencer et al. (2012) found little evidence to suggest degradation or variation in the FPOM fraction at the mainstem sites before and after Malebo pool on the Congo River. Therefore, it remains unclear to what extent the BHP component of FPOM may be reworked in the Cuvette Centrale prior to delivery on the Congo fan. The biomarker signal may also be influenced by changes in the topography and run-off patterns as the landscape of the catchment evolved throughout the late Quaternary, possibly with more direct export during earlier interglacial phases in comparison to their more recent analogues.

4.4.2. LAGS IN BIOMARKER RESPONSE

For some but not all glacial-interglacial transitions a delay in biomarker response is noted (e.g. transition from MIS 12-11 in Fig. 4). During MIS 11 a decrease in CH₄ oxidation marker concentration is observed at 388 ka which is 15 ka prior to the beginning of MIS 10. This delay appears to be long taking other studies addressing lead-lag relationships in the Congo system into consideration (e.g. Schneider et al., 1995; Zabel et al., 2001; Holtvoeth et al., 2003) therefore we assume that this is a result of uncertainties in the age model during that specific time period (see section 2.2). Schneider et al. (1995) and Dupont et al. (1999) demonstrated that fluctuations in SST, salinity, runoff, upwelling, organic carbon burial and vegetation in the Congo catchment were in phase and highly sensitive to climate forcing at orbital 23- and 100-kyr periodicities, but did not follow the pacing of global ice-volume and glacial stages, emphasizing the relevance of monsoonal impact on tropical climate systems. The response of the terrestrial biome to climate and hydrological change may possibly have resulted in leads and lags in biomarker response on shorter timescales. Holtvoeth et al. (2003) reports a 2-4 ka time shift of bulk organic
geochemical signatures that correspond to the delayed development of vegetation and soil with respect to atmospheric circulation and insolation, however, the absolute duration of the lag remains unclear within the context of the error associated with the age model of ODP 1075 (see section 2.2). This lag is supported by Zabel et al. (2001), who observe lags between the fluctuation in the suspension load of the Niger River and insolation during the Pleistocene where oscillation of solar radiation led variations of the terrigenous composition by ~4100-5100 yr. In addition to the age model limitations between the comparison of GHG and biomarker records, lag time between vegetation build up and rapid warming, and temporary storage (pre-aging) of the terrestrial carbon within the catchment (Schefuß et al., 2016) may also need to be taken into account when interpreting the observed time differences in the GHG and aminopentol records.

5. CONCLUSIONS

35-amino BHPs including aminotriol, aminotetrol and aminopentol are found in high concentrations throughout ODP 1075 to a maximal depth of 201.25 m.b.s.f, equivalent to ~2.5 Ma. This represents the oldest record of 35-amino BHPs and suggests that these biomarkers may well be preserved in much deeper and older sedimentary archives. Low concentrations of CH$_4$ oxidation markers, identified between 1865 and 1713 ka (‘a’) and 1099 and 826 ka (‘b’), suggest a reduction in wetland extent in response to more arid tropical African environmental conditions, consistent with a reported increase in climate variability and aridity near 1.7 Ma and 1 Ma. Correlation of high resolution CH$_4$ oxidation marker signatures with global atmospheric methane concentrations during MIS 5, 11, and 13 further suggests periods of enhanced tropical methane cycling. Furthermore, we observe a decoupling between CH$_4$ oxidation marker concentrations and atmospheric GHG
concentrations potentially due to a range of physical (West African climate and hydrological cycles) and/or biological parameters impacting on the tropical C cycle. Moreover, subsequent analysis is required to extrapolate the relative importance of tropical methane sources as a driver of global methane concentrations observed in ice core records.

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**References**


Table 1. List of 35-amino containing compounds identified in samples with corresponding abbreviated names, structures, and base peak (m/z) values. [M+H]^+

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Abbreviated name</th>
<th>Structure</th>
<th>Base peak m/z</th>
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<tr>
<td>35-aminobacteriohopane-32,33,34-triol</td>
<td>aminotriol</td>
<td>I</td>
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<tr>
<td>35-aminobacteriohopane-31,32,33,34-tetrol</td>
<td>aminotetrol</td>
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<td>35-aminobacteriohopene-30,31,32,33,34-pentol</td>
<td>unsaturated aminopentol</td>
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<tr>
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<td>aminopentol</td>
<td>III</td>
<td>830</td>
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<tr>
<td>35-aminobacteriohopane-30,31,32,33,34-pentol</td>
<td>aminopentol isomer</td>
<td>III'</td>
<td>788</td>
</tr>
</tbody>
</table>

Figure 1. Map of Congo including locations of ODP 1075 and Malebo Pool.
Figure 2. Concentration (µg gTOC$^{-1}$) of aminopentol (a), unsaturated aminopentol (b), aminopentol isomer (c), and CH$_4$ oxidation marker concentration (d) in ODP 1075 from 10 ka to 2.5 Ma, +/- 20% analytical error. In graph d, the black line indicates 3 point rolling average, hatched panel ‘a’ represents an interval from 1865–1713 ka and hatched panel ‘b’ represents an interval from 1099–826 ka. Correlation between aminopentol (AP) vs. aminotriol (AT) ($R_s$ 0.891, <0.05) and aminopentol vs. aminotetrol (ATT) ($R_s$ 0.908, P<0.05) shown in e (insert). Grey bars across a, b, and c indicate selected marine isotope stages (MIS).
Figure 3. A; Global CH$_4$ (Loulergue et al., 2008; Spahni et al., 2005; black, ppbv) and CO$_2$ concentrations (Lüthi et al., 2008; grey, ppmv). B; Concentration of CH$_4$ oxidation markers (µg gTOC$^{-1}$; ODP 1075), from 350 ka to 540 ka. Grey bars indicate MIS 10 and 12.
Figure 4. A; Global CH₄ (Loulergue et al., 2008; Spahni et al., 2005; black, ppbv) and CO₂ concentrations (Lüthi et al., 2008; grey, ppmv). B; Concentration of CH₄ oxidation markers (µg gTOC⁻¹; ODP 1075), from 10 ka to 200 ka. Grey bars indicate MIS 2, 4 and 6.
Figure 5. CH₄ oxidation marker concentration (µg gTOC⁻¹) during MIS 5 (n=20), 6 (n=8), 10 (n=10), 11 (n=36), 12 (n=38), and 13 (n=21) with median (black line) and mean (grey line) shown on each box.