Dark carbon fixation contributes to sedimentary organic carbon in the Arabian Sea oxygen minimum zone

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Key Points:

- One fifth of organic matter on Arabian Sea seafloor could stem from carbon fixation by bacteria in the oxygen minimum zone
- Evaluation of past anoxic events needs to take chemoautotrophic contribution into account in isotope balances
- Biogeochemical models ignoring dark carbon fixation could highly underestimate oxygen demand and thus expansion of oxygen minimum zones
Abstract
In response to rising CO$_2$ concentrations and increasing global sea surface temperatures, oxygen minimum zones (OMZ), or “dead zones”, are expected to expand. OMZs are fueled by high primary productivity, resulting in enhanced biological oxygen demand at depth, subsequent oxygen depletion, and attenuation of remineralization. This results in the deposition of organic carbon-rich sediments. Carbon drawdown is estimated by biogeochemical models; however, a major process is ignored: carbon fixation in the mid- and lower water column. Here, we show that chemoautotrophic carbon fixation is important in the Arabian Sea OMZ; and manifests in a $^{13}$C-depleted signature of sedimentary organic carbon. We determined the $\delta^{13}$C values of sedimentary organic carbon deposited in close spatial proximity but over a steep bottom-water oxygen gradient, and the $\delta^{13}$C composition of biomarkers of chemoautotrophic bacteria capable of anaerobic ammonia oxidation (anammox). Isotope mixing models show that detritus from anammox bacteria or other chemoautotrophs likely forms a substantial part of the organic matter deposited within the Arabian Sea OMZ (~17%), implying that the contribution of chemoautotrophs to settling organic matter is exported to the sediment. This has implications for the evaluation of past, and future, OMZs: Biogeochemical models that operate on the assumption that all sinking organic matter is photosynthetically derived, without new addition of carbon, could significantly underestimate the extent of remineralization. Oxygen demand in oxygen minimum zones could thus be higher than projections suggest, leading to a more intense expansion of OMZs than expected.

Plain Language Summary
Oxygen minimum zones are areas in the ocean in which algae produce so much organic material that sinks towards the seafloor, that all oxygen at depth gets used up. This results in vast “dead zones” where almost no oxygen is available to sustain life. With global warming, and increased nutrients from rivers, dead zones are forecast to expand. Computer models can calculate this, by considering algal production, and the amount of material delivered to the seafloor. However, these models often ignore a major process: anaerobic bacteria in the deeper water column, that can live at the edge or in the middle of these dead zones, which can also produce organic material from the dissolved CO$_2$. In this study, we used the fact that these bacteria add a distinct signature to the organic material, to show that around one fifth of the organic matter on the seafloor could stem from bacteria living in these dead zones. Thus, models who missed out on considering this contribution could have significantly underestimated the extent of oxygen depletion we are to expect in a future, warming world. A more intense expansion of dead zones than expected could have severe ecological, economical (fisheries), and climatic consequences.
1 Introduction

1.1. Organic carbon in oxygen minimum zones (OMZ)

Marine primary production fixes 50 Pg carbon per year, of which only about 1% is buried in sediments (Dunne et al., 2007; Middelburg, 2011). The majority of organic carbon derived from the photic zone is remineralised during sedimentation, fuelling heterotrophic bacterial activity in the water column (Keil et al., 2016). In marginal settings and OMZs, marine primary production in the photic zone can be significantly higher than in other settings. Organic carbon (OC) sedimentary accumulation rates within an OMZ can be in the range of tens to hundreds of mg C cm$^{-2}$ y$^{-1}$ (Hartnett et al., 1998; Hedges and Keil, 1995) higher than observed in other parts of the ocean. These high accumulation rates are most commonly attributed to attenuation in remineralization rates within the OMZ, and low bottom water oxygenation which results in decreased biodegradability of polymeric and matrix-protected substances (Burdige, 2007).

As a consequence of increasing CO$_2$ and temperature, oceanic OMZs are forecast to expand in a fashion similar to the past (Breitburg et al., 2018; Queste et al., 2018; Schmidtko et al., 2017; Shaffer et al., 2009; Stramma et al., 2010). The expansion of OMZs will result in widespread habitat loss of marine life, and could cause an increase in emissions of greenhouse gases such as N$_2$O and CH$_4$, but could also act as a long-term negative feedback on global warming, via the enhanced drawdown and storage of organic carbon in sediments.

1.2. Dark carbon fixation: chemoautotrophy in the mid- and deep water column

The biogeochemical system in subsurface waters, where light does not penetrate, has recently emerged to be substantially more complex – and possibly more important – than previously assumed. In particular, dark water column microbial activity is higher than what can be accounted for by heterotrophs (Herndl and Reinthaler, 2013), suggesting an important role for chemoautotrophy, i.e. fixation of dissolved inorganic carbon (DIC). It has been suggested to contribute substantially to the global carbon budget, with estimates ranging from 0.11 to 1.1 Pg C y$^{-1}$, equating to ca. 2% of total estimated yearly marine primary production (Middelburg, 2011; Reinthaler et al., 2010). The predominant chemoautotrophic process in the oxic, dark, pelagic ocean is thought to be nitrification (Middelburg, 2011; Pachiadaki et al., 2017). When oxygen is limited, nitrification still occurs, but other chemoautotrophic processes dominate, such as anaerobic oxidation of ammonia and sulfide oxidation (Ulloa et al., 2012; Wright et al., 2012).

Under hypoxic conditions (< 50 nM O$_2$), such as in the water column of OMZs, both archael (aerobic) and anaerobic oxidation of ammonia are thought to dominate dark inorganic carbon fixation processes (Lam and Kuypers, 2010; Pitcher et al., 2011). Here, nitrite accumulates, and other anaerobic autotrophic processes such as sulfide oxidation and methanogenesis are suppressed, most likely due to the abundance of nitrate and ammonia (Canfield, 2006; Ulloa et al., 2012).

1.3. Contribution of dark carbon fixation to particulate organic carbon flux

Of the inorganic carbon converted to organic matter within the OMZ, only a negligible fraction is presumably transported to the sediments and preserved, as this newly produced material is regarded as more labile than the sinking OC derived from the photic zone (Cowie and Hedges, 1992; Keil et al., 1994; Middelburg, 1989). Dark carbon fixation rates are challenging to quantify: they have been determined experimentally (Reinthaler et al., 2010; Taylor et al., 2001),
or have been estimated from the reaction stoichiometry of respiration based on Redfield organic matter and growth yields of nitrifiers (Middelburg, 2011; Wuchter et al., 2006). In oxygen minimum zones, such as the Peruvian margin (Lam et al., 2009), the Arabian Sea (Jensen et al., 2011), or the sulfidic Black Sea (Lam et al., 2007), the activity of some chemoautotrophs was determined via $^{15}$N- labelling, and formation of the products of their biogeochemical reactions. However, incubation methods may suffer from bias, because in situ conditions such as pressure are difficult to maintain.

However, carbon from within anoxic waters has been observed in some settings to contribute to the particulate OC flux: for example, in eutrophic lakes (Hollander and Smith, 2001) and anoxic fjords (van Breugel et al., 2005). Furthermore, discrepancies between modelled and observed organic carbon fluxes suggest that dark carbon fixation in anoxic marine settings significantly contributes to sinking material (Keil et al., 2016; Taylor et al., 2001).

One way to constrain this input into sedimentary organic matter is to use isotope mixing models. Photosynthetically fixed carbon generally has isotopic compositions of ca -19 to -21 ‰ due to Rubisco fixation. However, chemoautotrophs dwelling in OMZs typically have a more depleted $^{13}$C content as they either use $^{13}$C-depleted CO$_2$ generated by remineralization, have larger fractionation factors due to the higher abundance of CO$_2$ at depth (Freeman et al., 1994) or use carbon fixation pathways such as the acetyl coenzyme A pathway, which results in $^{13}$C-depleted biomass (Hayes, 2001). This characteristic depleted chemoautotrophic isotopic signature in the organic carbon could allow us to quantify the contribution of dark carbon fixation to sedimentary organic matter.

Here, we investigated the $\delta^{13}$C value of sedimentary organic matter of surface sediments deposited in the OMZ of the Arabian Sea and employed a simple isotope mixing model to investigate the extent of input from OMZ carbon fixation into sedimentary organic matter. As the major process in the Arabian Sea OMZ known to produce isotopically light biomass is anaerobic oxidation of ammonia (anammox; Ulloa et al., 2012; Villanueva et al., 2014), in order to determine the isotopic signature of this pathway, we developed and applied a method to determine the $\delta^{13}$C values of a novel biomarker, bacteriohopanetetrol stereoisomer (BHT'), which has been found to be unique to anammox bacteria in the marine environment in culture and environmental studies (Rush et al., 2019, 2014; Rush and Sinninghe Damsté, 2017). It has been found in the Arabian Sea and other marine anoxic settings (Matys et al., 2017; Sáenz et al., 2011). We also used stable isotope probing experiments to exclude sedimentary anammox as an important contributor to this process. This allowed us to investigate the contribution of these dark carbon fixers to sedimentary organic carbon.

2 Materials and Methods

2.1 Sediment sampling and stable isotope probing incubations

Sediments were collected with multicore devices on the R/V Pelagia in the Northern Arabian Sea in January 2009 during the PASOM cruise 64PE301 along the Murray Ridge (Fig. 1f), which protrudes into the core of the OMZ. Two cores, one each from P900 (885 m water depth) and here and P1800 (1786 m water depth), hereinafter referred to as anoxic and oxic, respectively, were incubated on board as described by Pozzato et al. (2013 a, b). In brief, particulate or dissolved organic matter from the diatom Thalassiosira pseudonana containing 20 and 18 % $^{13}$C, respectively, were added to the tops of core tubes of 10 cm internal diameter.
Figure 1. Arabian Sea depth gradients. Shown are $\delta^{13}$C$_{org}$ and % TOC values of core top sediments, and bottom water oxygenation plotted with depth (a-d), all but $\delta^{13}$C$_{org}$ replotted from Lengger et al. (2014), a scatter plot of oxygen exposure time versus $\delta^{13}$C$_{org}$ in Arabian Sea core tops along Murray Ridge (e), and a map of the sampling stations with the two main stations used for BHT analysis used here indicated with a star (f).
Eight cores are discussed here; these were incubated under oxic or suboxic conditions for 7 days (125 µM, 6 µM O₂, respectively; Table 1). At the end of incubation, cores were sliced in the intervals 0 – 2, 2 – 4, and 4 – 10 cm depth. They were then frozen and freeze dried for the isotopic analysis of the bacteriohopanepolyol lipids (BHPs), including bacteriohopanetetrol (BHT) and its stereoisomer and biomarker for anammox bacteria, BHT’. Both biomarkers have been studied previously in the Arabian Sea water column and sediments (Jaeschke et al., 2009; Sáenz et al., 2011) Furthermore, cores from 8 stations between 900 and 3000 m water depth were also collected, as described by Lengger et al. (2014, 2012b). The top 0 – 0.5 cm were used for total organic carbon (TOC) and ¹³C of organic carbon analysis. For the core from P900 (32 cm length), all depths were analysed in 0.5 cm – 4 cm resolution (Lengger et al., 2012b).

2.2 Anammox enrichment cultures

To determine the δ¹³C values for the two bacteriohopanetetrols in anammox bacteria (BHT and BHT’, the latter being unique to anammox in the marine environment), an enrichment culture of ‘Ca. Scalindua profunda’ was analysed. It was grown in a sequencing batch reactor as described by van de Vossenberg et al. (2008). Analysis of this enrichment culture that showed S. profunda comprised about 80% of the cells, while other bacteria belonging to the phyla Bacteriodetes (including Flavobacteriaceae) and Proteobacteria (including Alphaproteobacteria) accounted for the majority of the remaining populations (van de Vossenberg et al., 2008, 2013).

2.3. Extraction and purification

The freeze-dried subsamples of the unamended and incubated cores were ground, and the homogenised sediments and the culture were extracted by a modified Bligh-Dyer extraction method (Lengger et al., 2012a). Briefly, they were extracted ultrasonically three times in a mixture of methanol/dichloromethane (DCM)/phosphate buffer (2:1:0.8, v:v:v) and centrifuged, and the solvent phases were combined. The solvent ratio was then adjusted to 1:1:0.9, v:v:v to separate the DCM phase. Liquid extraction was repeated two more times, the DCM fractions were combined, the solvent was evaporated and the larger particles were filtered out over glass wool. The extraction procedure was performed on the enrichment culture material and repeated on the sediment for analysis of BHPs. An aliquot of the extract was subjected to column chromatography using 5% aminopropyl solid phase extraction (SPE), eluting with hexane, DCM, and MeOH, which contained BHPs. For analysis by chromatographic techniques, the extract was derivatised in 0.5 mL of a 1:1 (v:v) mixture of acetic anhydride and pyridine at 50 °C for 1 h, then at room temperature overnight in the case of HPLC-MS analysis. Solvent was dried under a stream of N₂ on a 50°C heating block.

2.4. Instrumental techniques

2.4.1. High temperature gas chromatography coupled to flame ionization detection (HTGC-FID)

GC analysis of acetylated BHPs was done using a HP-5890 Series II GC equipped with a flame ionization detector was fitted with a 0.25 mm x 0.1 µm VF5-ht capillary column (CP9045, CP9046, Agilent Technologies UK Ltd., Stockport, UK) of 30 m length (Lengger et al., 2018). An on-column injector was used. To the 30 m column, 1m of a 0.25 mm HT-deactivated silica tubing was attached as a guard column (Zebron Z-Guard, 7CG-G000-00GH0, Phenomenex, Macclesfield, UK). Analysis of bacteriohopanepolyols employed a constant flow of 2 ml/min He and a temperature ramp from 70°C (1 min hold) to 400°C at 7°C min⁻¹ (1 min hold).
2.4.2. High temperature gas chromatography coupled to mass spectrometric detection (HTGC-MS)

Analysis of acetylated BHPs, using HTGC-MS was performed using a Thermo Scientific Trace 1300 gas chromatograph coupled with an ISQ single quadrupole mass spectrometer. Diluted samples were introduced using a PTV injector in split mode (split flow of 30 ml min\(^{-1}\), split ratio of 6.0) onto a 0.53 mm fused silica pre-column connected to a 15 m × 0.32 mm i.d. fused-silica capillary column coated with dimethyl polysiloxane stationary phase (Rxi-1HT; film thickness, 0.1 μm; Restek). The initial injection port temperature was 50 °C with an evaporation phase of 0.05 min, followed by a transfer phase from 50 °C to 380 °C at 20 °C min\(^{-1}\). The oven temperature was held isothermally for 2 min at 50 °C, increased at a rate of 7 °C min\(^{-1}\) to 380 °C and held at 380 °C for 10 min. Helium was used as a carrier gas and maintained at a constant flow 5 ml min\(^{-1}\). The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) with a GC interface temperature of 380 °C and a source temperature of 340 °C. The emission current was 50 μA and the mass spectrometry set to acquire in the range of m/z 50–950 Daltons at two scans per second. Data acquisition and processing were carried out using the Thermo XCalibur software (version 3.0.63). Due to the lack of authentic standards for BHT and BHT’, only relative and not absolute values are reported, assuming similar ionization energies.

2.4.3. High temperature gas chromatography coupled to isotope ratio mass spectrometry (HTGC-IRMS)

The stable carbon isotopic composition (δ\(^{13}\)C) of BHPs were determined using HTGC-isotope ratio mass spectrometry. To this end, an Elementar visION IRMS with GC5 interface (Elementar UK Ltd., Cheadle, UK), and an Agilent 7890B GC were modified in-house and allowed us to achieve column temperatures of up to 400 °C, which resulted in baseline resolution of BHT and BHT’ (Fig. 2). 1 μl of the derivatized samples dissolved in ethyl acetate were injected on a cool-on-column injector, into a Zebron Z-Guard Hi-Temp Guard Column (1 m x 0.25 mm) and separated on a Zebron ZB-5HT analytical column (30 m x 0.25 mm x 0.1 μm). He was used as a carrier gas at a flow rate of 1.5 ml min\(^{-1}\) and the oven was programmed as follows: 1 min hold at 70 °C, increase by 7 °C min\(^{-1}\) to 350 °C (10 min hold). Organic compounds were combusted to CO\(_2\) in a 0.7 mm ID quartz tube with CuO pellets at 850°C. Instrumentation performance was monitored using an n-alkane standard (B3, A. Schimmelmann, Indiana University, Bloomington, IN, USA; RMS 0.4 ‰), and results were calibrated using an in-house mixture of five fatty acid methyl esters, which was injected between every six sample analyses and analyzed using a He flow of 1 ml min\(^{-1}\), with a slightly different temperature program (injection at 50 °C held for 1 min followed by an increase of 10°C min\(^{-1}\) to 300 °C and a 10 min hold). This is the first time baseline resolution between BHT and BHT’ has been achieved on a GC-IRMS, which allows the direct determination of the isotopic composition of both BHT and BHT’ in sediment samples (Fig. 2). The isotopic composition of the acetyl group used to derivatise the BHT and BHT’ was determined by acetylation of myo-inositol, and then subtracted from the values of BHT and BHT’ in a mass balance correction (Angelis et al., 2012), as authentic standards for BHT or BHT’ were not available.
2.4.4. High performance liquid chromatography coupled to positive ion atmospheric pressure chemical ionization mass spectrometry (HPLC/APCI-MS)

To verify the GC-derived assignments, an aliquot of the acetylated BHP samples was dissolved in MeOH:propan-2-ol (3:2; v:v) and filtered on 0.2 µm PTFE filters. BHPs were analysed by HPLC/APCI-MS, using a data-dependent scan mode (3 events) on an HPLC system equipped with an ion trap MS, as described in Talbot et al. (2007) and van Winden et al. (2012). Relative BHP concentrations were semi-quantitatively estimated based on the response factor of authentic standards (M. Rohmer; Strasbourg, France; Cooke et al., 2008), with a typical reproducibility of ± 20%, according to Cooke et al. (2009).

2.4.5. Bulk sedimentary organic matter and suspended particulate matter

Freeze-dried core tops from 8 stations between 900 and 3000 m for sedimentary organic matter, and punches from 0.7 µm GFF filters for suspended particulate organic matter, were decalcified with 2N HCl, washed, freeze-dried, and subjected to analysis via a Flash EA 1112 Series (Thermo Scientific) analyser, coupled via a Conflo II interface to a Finnigan Delta plus mass spectrometer as described by Lengger et al. (2014; sediment) and Pitcher et al. (2011; filters). Standards for δ13C analysis were acetanilide and benzoic acid and samples were analysed in duplicate.
3. Results

To quantify the provenance of sedimentary organic matter and the contribution of chemoautotrophs (anammox) from the OMZ, we analysed the isotopic composition of sedimentary organic matter deposited in close spatial proximity over a large depth gradient in the Arabian Sea, as well as the isotopic composition of biomarker lipids derived from anammox bacteria, chemoautotrophic microbes living in the OMZ. In order to determine whether these were water-column derived or sedimentary, we used stable isotope probing experiments on sediments retrieved from within and below the OMZ.

3.1. $\delta^{13}$C values of C$_{org}$

$\delta^{13}$C values of C$_{org}$ in surface sediments were low (-21.5 ‰) at P900 and increased with water depth to -20.2 ‰ at P2500 (Fig. 1d). $\delta^{13}$C values correlated positively and linearly (Slope 0.0057, $R^2 = 0.90$, Figure 2e) with oxygen exposure times as calculated by Koho et al., 2013 and Lengger et al., 2014). Similarly, organic carbon content in the core tops was negatively correlated with oxygen exposure times ($R^2 =0.93$, from Lengger et al., 2014). The increase mirrors the decrease in % TOC – and thus progressing degradation – with increasing oxygen exposure time (Fig. 1bc, Lengger et al., 2014). At P900, where the whole depth of the core was analysed, values increased slightly with depth, from -21.5 ‰ at the surface, to -20.9 ‰ (Fig. 3). $\delta^{13}$C values of particulate organic carbon (suspended particulate organic matter) decreased throughout the water column from –19 to –21.8 ‰, though with a very depleted, yet unexplained, value at the very surface (20 m depth) of –22.9 ‰ (Fig. S1).

![Figure 3. Depth profile of $\delta^{13}$C$_{org}$ and % TOC in an unamended core at P900.](image)

3.2. Biomarkers

3.2.1. Bacteriohopanepolyols (BHPs)

Analysis of BHPs in the Arabian Sea cores using HPLC-APCI-MS and HTGC-MS, all showed that BHT$, specific for Scalindua, was abundant. Other, relatively non-source-specific,
BHPs were also present: BHT, 35-aminobacteriohopane-32,33,34-triol (aminotriol), bacteriohopane-31,32,33,34,35-pentol (BHpentol), bacteriohopanetetrol cyclitol ether (BHT-CE) and anhydro-BHT (Fig. S3). The relative concentrations of bacteriohopanetetrols were 75 to 96 % of total bacteriohopanopolyls, an order of magnitude higher than other BHPs (Fig. S3). HTGC-MS was able to detect BHT, BHT' and BHpentol (Fig. S2), as well as small amounts of anhydro-BHT, a BHT degradation product, and BHP-570, which has been tentatively identified by Sessions et al. (2013) as acetylated bacteriohopanediol, possibly also a degradation product of bacteriohopanetetrol. In the core from P900, the ratio of BHT' over BHT increased with depth (Fig. S4).

We also analysed the BHP content of sediment cores incubated with \(^{13}\text{C}\)-labelled organic matter at both sites under the different conditions detailed by Pozzato et al. (2013 a, b). No changes were noted, except for in P900; in those, BHT abundance increased (i.e. BHT'/BHT decreased), indicating that some of the sedimentary BHT was produced in situ, likely through heterotrophy of the added labelled OM (Fig. S4).

3.2.2. BHT and BHT' \(^{\delta^{13}}\text{C}\) values

To establish the isotopic difference of BHT and BHT' derived from anammox we analysed biomass obtained from a batch reactor. BHT and BHT' were the main biohopanoids in the biomass from Ca. Scalindua profunda detected by HTGC-MS (Fig. S1b). In addition to being present in Scalindua sp. anammox bacteria, BHT is a ubiquitous lipid common to many bacteria, BHT', however, is specific to Scalindua sp. in marine environments. The \(^{\delta^{13}}\text{C}\) values of BHT and BHT' were identical within the error of analysis (-49 and -48 ‰, respectively; Table 1), indicating identical fractionation and thus biosynthetic pathways for both lipids.

In the Arabian Sea sediments (all unamended cores), BHT was markedly enriched in \(^{13}\text{C}\) relative to BHT', with values ranging from -24.7 to -28.8 ‰ and -39.1 to -48.1 ‰, respectively (Figs. 4b and 4c). At P1800 (below the OMZ), BHT and BHT' were slightly more enriched in \(^{13}\text{C}\), with BHT at -27 ± 3 ‰ and BHT' at -47 ± 4 ‰, as compared to -26 ± 1 ‰ and -43 ± 5 ‰ for BHT and BHT' at P900 (in the OMZ). However, this difference was not statistically significant. Moreover, even though the proportion of BHT' increased with depth in the anoxic core, the \(^{\delta^{13}}\text{C}\) values did not change. We also analysed BHT and BHT' in the cores that had been incubated with \(^{13}\text{C}\)-labeled POM and DOM, and these showed no indication of \(^{13}\text{C}\)-enrichment in BHT'. Values, excluding outliers (defined by a Grubbs test at 99% confidence level and indicated in Table 1), were on average -48 ± 4 ‰ and -46 ± 2 ‰ at P1800, and P900, respectively. BHT was slightly enriched compared to the unamended incubations at 3 cm depth (\(\Delta^{\delta^{13}}\text{C} = 4.6 ± 0.7 ‰\)), but not at 1 cm depth. BHpentol concentrations were too low to allow reliable isotopic determination, and anhydroBHT and BHP-570 co-eluted with other compounds, also precluding their isotopic characterization.

4. Discussion

4.1. Origin of sedimentary organic matter

The Murray Ridge represents an open ocean setting, and the selected coring sites were in close proximity to each other, with no substantial terrigenous contribution (Koho et al., 2013; Lengger et al., 2014, 2012a; Nierop et al., 2017). Despite this, \(^{\delta^{13}}\text{C}\) values of sedimentary organic matter of a purely marine origin varied systematically by 1.3 ‰ across the core top
sediments of the Murray Ridge (Fig. 1d). The value at the shallowest location (~21.5 °‰; P900), within the OMZ, was 2.5 °‰ more depleted than the estimated value for surface water-derived OM -19.8 °‰ (Fontugne and Duplessy, 1986). Moreover, the δ13C values of sedimentary Corg increased with an increasing amount of oxygen bottom water concentration / oxygen exposure time (Fig. 1e). These observations are in agreement with earlier studies from this setting. Cowie et al. (2009, 1999) detected similar trends in surficial sediments across different settings in the Arabian Sea, with values of -21 °‰ within and -19 °‰ above and below the OMZ. Organic matter δ13C values in sediment traps collected in the north western Arabian Sea (i.e. sinking POC) had a value of -22.4 °‰ (composite of the OMZ between 500 and 900 m depth). The corresponding sedimentary δ13Corg value, from oxygenated bottom waters at 1445 m depth, was, however, enriched (-20.8°‰; Wakeham and McNichol, 2014). Fernandes et al. (2018) detected similar, though less pronounced, trends in sediments collected from the Pakistan margin. An increase in δ13C with enhanced degradation, i.e. 13C-enriched sediments vs. depleted POM, was also observed in the South China Sea (Liu et al., 2007), and in the Eastern Tropical North Pacific (Jeffrey et al., 1983).

Despite its common occurrence in OMZ settings, this trend is unusual and, at present, not explained: degradation of organic carbon in marine environments usually preferentially removes isotopically heavy carbon (Hatch and Leventhal, 1997), causing a depletion in δ13C with increased degradation of the sediment. This can be due to the removal of the more labile marine carbon, and subsequent relative enrichment of terrigenous organic material of a lower initial reactivity and lower δ13C values (Huguet et al., 2008; Middelburg et al., 1993). However, progressive depletion also occurs in areas with purely marine input; this is due to preferential loss of 13C-enriched carbohydrates over the more 13C-depleted lipids, and preferential degradation of easily accessible material over biopolymers (Spiker and Hatcher, 1987), and polymerization and elimination of functional groups (Galimov, 1988; Balabane et al., 1987). Conversely, sulfurization, a process observed in euxinic settings appears to preferentially preserve 13C-enriched material such as carbohydrates (Van Kaam-Peters et al., 1998); however, this process is not expected to occur here - the Arabian Sea is anoxic but not sulfidic (Ulloa et al., 2012) and has not experienced euxinia for the past 120ka (Schenau et al., 2002). Nonetheless, in Arabian Sea sediment, δ13Corg values increased by 1.8 °‰ with increasing oxygen exposure time and thus increasing degradation; at the same time, organic carbon contents decreased from 60 to 10 mg g dw⁻¹, indicating progressing remineralization (Fig. 1c, Lengger et al., 2014). Cowie (2005) attributed this to the contribution of – potentially – organic matter from the facultatively autotrophic, chemosynthetic sulfur-bacterium Thioploca sp., which has been observed in the Arabian Sea (Schmaljohann et al., 2001). However, Thioploca sp. has only been reported for shelf and upper slope sediments in the Arabian Sea (above and upper part of OMZ), and it is unlikely that the Sulfur-dependent Thioploca sp. could have caused this significant depletion by chemoaototrophy, as sulfide concentrations are negligible within the OMZ (Kraal et al., 2012), and there is no evidence for the production of severely 13C-depleted biomass by filamentous sulfur bacteria (Zhang et al., 2005). Further, the depletion is not only observed in sediments, but also in particle fluxes (Wakeham and McNichol, 2014), which allows the exclusion of sedimentary sources for 13C-depleted organic matter. This is also supported by the δ13Corg values of the suspended particulate matter (Fig. S1), which becomes gradually more depleted with depth.

On the other hand, an active nitrogen-cycle in the water column within the Arabian Sea oxygen minimum zone is undisputedly present, with heterotrophic denitrifying bacteria,
nitrifying archaea, nitrite-oxidising and anammox bacteria present in high abundances (Lüke et al., 2016; Villanueva et al., 2014). The $^{13}$C fractionation for the carbon fixation pathways employed by nitrifying archaea (3-HP/4-HB) is similar to that of phytoplankton using Rubisco (Könneke et al., 2012). The biomass of heterotrophic denitrifiers is close to the value of the source organic matter (1‰ more enriched; Hayes, 2001), and the biochemical pathway that nitrite oxidisers employ (reverse TCA cycle) produces $^{13}$C-enriched biomass (Pearson, 2010). Anammox bacteria, on the other hand, are abundant and active in the Arabian Sea (Jensen et al., 2011) and known to produce severely $^{13}$C-depleted biomass by inorganic carbon fixation (Schouten et al., 2004). Anammox bacteria, and other, yet undescribed chemoautotrophs, could thus present a potential pathway for addition of $^{13}$C-depleted organic matter to sinking organic matter, something which we explore below.

4.2. OMZ-derived anammox biomass

A first indication for the significant contribution of anammox biomass to sedimentary organic matter is the observation that BHT’, a biomarker likely unique for anammox in marine environments (Rush et al., 2014), is highly abundant in the sediment and occurred throughout the depth profiles of both sediment cores (Fig. 4a). In the anoxic core (P900), BHT’ was the most abundant of the two stereoisomers (Fig. 4a). Most importantly, BHT and BHT’ were present in concentrations at least an order of magnitude higher than other BHPs (Fig. S2). The relative proportions of BHT’ compared to the BHT (0.4 – 0.6) we report are higher than those reported previously in nearby core tops (0.22-0.30) by Saenz et al. (2011). This could be caused by a difference in settings, or in BHP extraction protocol. BHT and BHT’ in enrichment cultures also have higher ratios of BHT’ to BHT than reported by Saenz et al. (2011). The high proportion of BHT’ is contrary to our expectations, as BHT is a ubiquitous lipid and presumed to derive from both anammox and non-anammox sources such as cyanobacteria or other bacteria (Pearson and Rusch, 2009); it is, therefore, expected to be abundant in most depositional contexts. However, the high proportions of BHT’ observed in the Arabian Sea are not unprecedented: they are lower than BHT’ proportions in sediments underlying the Humboldt Current System OMZ offshore Peru (0.45 – 0.69 in surface sediments, Matys et al., 2017).
Further evidence for the unique source of BHT' comes from its $^{13}$C value. Anammox bacteria are known to fractionate strongly against $^{13}$C and produce severely depleted biomass in cultures (Brocadia sp., $-47\%$) and sediment (Scalindua sp., $-49\%$; Schouten et al., 2004). This is decidedly more depleted than other biomarkers in the Arabian Sea (Wakeham and McNichol, 2014), though distinctly depleted highly branched isoprenoids (HBIs, $-37\%$) have been found in Arabian Sea cores from the Holocene (Schouten et al., 2000). BHT, however, which in cultures is produced in similar proportions and with similar isotope values as BHT' (Rush et al., 2014; Fig. S4, Table 1), is much more enriched in $^{13}$C in the Arabian Sea sediments, with values of $-29$ to $-30\%$ (Fig. 4bc). This supports the idea that BHT is a common lipid and can also be derived from cyanobacteria or heterotrophic bacteria thriving in the OMZ or the sediment (Pearson and Rusch, 2009), possibly including nitrite-oxidising bacteria (Kharbush et al., 2018). The sources for BHT could be varied, and the $^{13}$C values of BHT derived from these alternative sources are poorly constrained (Hayes, 2001; Kharbush et al., 2018; Pearson, 2010; Sakata et al., 1997; Schouten et al., 1998). However, they would be expected to be substantially less depleted than anammox BHPs. Methane cycling could also lead to $^{13}$C depleted OM, but is not important in the Arabian Sea (Lüke et al., 2016), and no biomarker evidence for it, such as $^{13}$C depleted archaeal lipids, or methylated BHPs, were detected. Thus, a BHT $^{13}$C value of $-30\%$ could suggest a mixture of heterotrophic (and other) bacterial sources with an anammox source.
Anammox activity has been shown to occur both within the OMZ water column (Jensen et al., 2011; Lüke et al., 2016; Pitcher et al., 2011; Villanueva et al., 2014) as well as in sediments (Sokoll et al., 2012). To test whether the anammox-derived biomarkers are formed in the sediment or are derived from the OMZ, the labelled cores were incubated for 7 days with particulate and dissolved organic matter (Table 1), which resulted in the substantial incorporation of $^{13}$C into bacterial fatty acids, as well as in $^{13}$C-enriched CO$_2$ due to heterotrophic respiration (Pozzato et al., 2013a, b). Anammox bacteria are autotrophic, and it is therefore likely that an active sedimentary community could result in some uptake of this $^{13}$C labelled CO$_2$ formed by respiration. However, no labelling was observed in BHT$'$ (Table 1), despite some minimal incorporation into BHT, suggesting that most of the BHT$'$ pool is water-column derived. BHT, on the other hand, could thus also be sourced partially from sedimentary heterotrophic bacteria. Further, BHT and BHT$'$ are also present in the surface of the oxic sediments (P1800), in proportions similar to the anoxic sediments at P900. Even if anammox growth was too slow for labelling, substantial sedimentary production would have resulted in a decreasing $\delta^{13}$C value of BHT$'$ in the unamended cores, as DIC gradually becomes more $^{13}$C depleted with sediment depth (Fernandes et al., 2018), but this is not observed (Fig. 4bc). This supports the idea that the vast majority of BHT$'$ is derived from anammox bacteria living in the oxygen minimum zone of the water column, and that BHT also has a mostly pelagic origin with limited sedimentary production.

4.3. Proportion of chemoautotrophy-derived organic carbon

The water-column derived $^{13}$C-depleted BHT$'$ suggests that there may be a substantial contribution of $^{13}$C depleted organic carbon, produced by anammox bacteria, to the sedimentary organic matter. Based on BHT$'$ $\delta^{13}$C values determined in this study, and the fractionation factor associated with anammox lipid biosynthesis of 16 ‰ (diplolene versus total biomass; Schouten et al., 2004), we can estimate a $\delta^{13}$C value for anammox biomass: of ca.-28.6 ± 6 ‰, which is very similar to the expected value calculated from $\epsilon_{\text{biomass-DIC}}$ (22-26 ‰; Schouten et al., 2004) and the generally observed DIC value in the Arabian Sea OMZ, 0 ‰ (Moos, 2000). This is significantly depleted compared to phytoplankton biomass in the Arabian Sea (-19.8 ‰, Fontugne and Duplessy, 1978), and would add depleted organic carbon to the sinking POM. Additionally, the increased availability of CO$_2$ derived from heterotrophic respiration of the POM pool would also result in greater discrimination against $^{13}$C (Freeman et al., 1994). This is also apparent in the lipids of ammonia-oxidising archaea, which have previously been shown to be produced in the OMZ and transported to the sediment (Lengger et al., 2014, 2012b; Schouten et al., 2012). Here, IPL-derived GDGTs were also more depleted when deposited in the OMZ than below the OMZ (Table S1), further supporting the hypothesis that some deep water column production is exported to, and preserved in, the anoxically deposited sediments. While the high abundance of surface lipids are the result of a mixed signal of pelagic and sedimentary for archaeal lipids (contrary to anammox, which are presumed from the OMZ), and thus precludes the incorporation of these ammonia oxidising, autotrophic archaea in this mixing model, it is further evidence that other chemoautotrophs might also contribute to this depleted signature.

Hence, chemoautotrophic carbon fixation could explain the low $\delta^{13}$C values of sedimentary OM observed within the OMZ. As the newly produced organic carbon is more labile than surface-produced organic matter, it degrades more quickly upon exposure to oxic bottom waters. This poorer preservation of anammox biomass results in a shift back towards the $^{13}$C signature of the primary photosynthetic production, which is observed as the enrichment in...
sedimentary d\textsuperscript{13}C\textsubscript{org} with increasing oxygen exposure times (Fig. 1c). A likely explanation for this lability of anammox biomass is that, due to the lack of zooplankton in the OMZ, this organic matter is not fecal-pellet packaged or adsorbed to inorganic particles and thus not matrix protected (cf. Burdige, 2007). Wakeham et al. (2002) analysed the biomarker fluxes in sinking particles in the Arabian Sea, and they also found that surface-produced lipids such as alkenones (which would be fecal pellet packaged) were exceptionally well-preserved compared to the total organic carbon of the SPM. Gong and Hollander (1997) also noted an enhanced contribution of bacterial biomass to sediment deposited under anoxic conditions in the Santa Monica Basin, when compared to sediment located in nearby oxygenated bottom waters. Studies examining carbon fluxes in the Arabian Sea (Keil et al., 2016) and the Cariaco basin (Taylor et al., 2001) invoked the addition of chemoautotrophically derived carbon from a midwater source in order to explain the enhanced carbon fluxes observed, even if oxygen depletion and other factors such as the lack of zooplankton were taken into account.

We modelled the contribution of anammox biomass ($\chi_{\text{anammox}}$) to sedimentary organic carbon using an isotopic mass balance approach (using IsoError; Phillips et al., 2005), which employs error estimates and allows to determine contributions of likely sources of organic matter to the sediment. The end member value of $-19.8 \pm 0.5$ ‰ was used for phytoplankton-derived sedimentary organic carbon (Fontugne and Duplessy, 1986; Ziegler et al., 2008). This value is slightly lower than the $-19$ ‰ observed from the surface particulate carbon, but is representative of sedimentary organic matter, as it accounts for the decreased $\delta^{13}$C values of POM caused by degradation of $^{13}$C enriched compounds such as carbohydrates (Close, 2019; Ziegler et al., 2008). More recent values are, to the best of our knowledge, not available. Planktonic, sinking organic matter, and organic material produced by heterotrophs also contribute to sedimentary OM. However, this OM value would likely be similar to the organic matter assimilated (Blair et al., 1985; 2001). $-28.6 \pm 6$ ‰ was used for anammox-derived organic carbon, as derived from the $\delta^{13}$C value of BHT' and $\epsilon_{\text{bm/lipid}}$ of $16 \pm 4$ ‰ (Schouten et al., 2004; Table 1; uncertainty represents combined standard deviations of $\delta^{13}$C\textsubscript{BHT'} and $\epsilon_{\text{lipid/bm}}$). The $\delta^{13}$C value $-21.5$ ‰ of surface sediment C\textsubscript{org} in the oxygen minimum zone was used as the value of the mixture (Table 1):

$$\chi_{\text{anammox}} \cdot \delta^{13}C_{\text{anammox}} + \chi_{\text{pp}} \cdot \delta^{13}C_{\text{pp}} = -21.5 \text{ ‰}$$

\text{Eqn 1}

The modelling, assuming the above end member contributions and their statistical uncertainties, yields a proportion of anammox with a mean of approximately 17% (Fig. 5), and confirms that, with 95% confidence, anammox contribution to the sedimentary organic matter is between 3 and 30% between the different cores. This means that it is likely that some of the sedimentary organic carbon present at P900 is anammox-derived. However, other chemoautotrophic bacteria also present, or suspected to be present, in the Arabian Sea OMZ (e.g. ammonia-oxidizing archaea, Pitcher et al., 2011) possess metabolisms which would lead to different $\delta^{13}$C values, and could therefore be diluting this signal (Hayes, 2001; Pearson, 2010). These results suggest that the contribution of organic material produced in the oxygen minimum zone to sedimentary organic carbon is larger than estimated in this mass balance.
Results from the isotope mass balance model, showing the calculated means, standard error, and confidence intervals for contribution from anammox, and planktonic-derived OM (POM) to the surface sediment at P900. This estimate, at a first glance, appears large. However, we can convert experimental rates of anammox-mediated ammonia oxidation of 27 – 38 nmol L\(^{-1}\) d\(^{-1}\) as determined in the Arabian Sea (Jensen et al., 2011) and alternative rates of 0.24 -4.32 nmol L\(^{-1}\) d\(^{-1}\) estimated by Ward et al. (2009), to carbon fixed using stoichiometric rates determined for anammox bacteria of 0.07 mol C mol N\(^{-1}\) (Jetten et al., 2001; Strous et al., 1999; van de Vossenberg et al., 2008). Assuming a production depth of 300 to 900 m water depth, with a maximum at 600 m (Pitcher et al., 2011), anammox production over the whole depth could be estimated using Equation 2,

\[
P = 2 \cdot \int_{300}^{600} k \cdot x \cdot dx = 2 \cdot [k \cdot x^2]_{300}^{600} = 2 \cdot (k \cdot 600^2 - k \cdot 300^2)
\]

in which \(x\) corresponds to depth in m, and \(k\) corresponds to the linear constant describing the increase in production from 300 m (0) to 600 m / mirrored decrease from 600 to 900 m, with maximum production calculated from aforementioned published anammox rates (138.7 – 9855 µmol m\(^{-3}\) yr\(^{-1}\), Jensen et al. 2011; 87.6 – 1576.8 µmol m\(^{-3}\) yr\(^{-1}\), Ward et al. 2009).

Using this equation, we calculated that water-column anammox produce 2 to 7 g organic C m\(^{-2}\) yr\(^{-1}\). These estimates dwarf sedimentary anammox rates, with C-fixation determined to be at 64 pg organic C m\(^{-2}\) yr\(^{-1}\) in the Arabian Sea (Sokoll et al., 2012), indication that most of the anammox from the sediment is water-column derived. When compared to
organic carbon accumulation rates (Lengger et al., 2012a) of 3 to 5 g C m$^{-2}$ yr$^{-1}$ at P900 and assuming a 17 % anammox contribution to sedimentary organic matter at this site, this would mean that 6 to 10 % of annually produced, water-column produced anammox biomass would be preserved in this anoxic setting. Within the sediment from the OMZ, as seen from a sedimentary depth profile at P900 in the Oxygen Minimum Zone (Fig. 3), $\delta^{13}C_{org}$ also shifts to higher values with depth, to a maximum of -20.8 ‰ at 8 cmbsf. However, contrary to oxic degradation, no decrease in organic matter contents occurs over the first 14 cm (Fig. 3). In this case, the increase in $\delta^{13}C_{org}$ most likely reflects input by other sedimentary autotrophs, producing enriched organic carbon. This suggests that the depleted signal from the OMZ is not completely preserved.

In summary, biomarker and isotope evidence strongly suggest that at least some of the organic carbon deposited in the oxygen minimum zone in the Arabian Sea is derived from anammox bacteria. This carbon is labile and appears not to be well preserved when bottom waters are oxygenated. However, should the depositional setting remain anoxic, the depleted anammox signal could potentially be preserved in the $\delta^{13}C$ of the C$_{org}$.

4.4. Implications for past, present, and future global biogeochemical cycles

4.4.1 Interpretation regarding ancient sedimentary OM $\delta^{13}C$ values

The $\delta^{13}C$ values of total organic carbon are commonly used in combination with organic carbon contents in sediment cores to interpret changes in sea surface biogeochemistry and to discern the causes for ocean deoxygenation (Jenkyns, 2010; Meyers, 2014). Negative $\delta^{13}C$ excursions are associated with either atmospheric injection of depleted CO$_2$ and/or increased CO$_2$ availability (Pagani, 2005; Pancost and Pagani, 2005), or a higher input of terrigenous organic matter. Positive excursions are inferred to reflect increased burial rates of organic carbon, i.e. the removal of ‘light’ carbon from the ocean-atmosphere reservoir (e.g. Kuypers, Marcel M. M. et al., 2002). An increase in TOC coinciding with a negative carbon isotope excursion has been observed in the sedimentary record numerous times. They are a prominent feature in OAE 1a (Leckie et al., 2002), during the PETM (Zachos et al., 2005), and in the Eastern Mediterranean during Sapropel deposition (Meyers and Arnaboldi, 2008). Our results show that if non-sulfidic oxygen minimum zones expand to impinge on the sediment, a significant amount of $^{13}C$-depleted organic carbon can be preserved, changing the carbon isotope composition of organic matter by up to – 1.6 ‰ in the case of Arabian Sea surface sediments, and by -0.5 to -1 ‰ in deeper sediments. When evaluating past events based on bulk $\delta^{13}C$ values, the incorporation of chemooautotrophically produced carbon must thus be considered. Notably, even in settings dominated by a euxinic water column, e.g. the Black Sea, anammox has been shown to be an important process in the anoxic water column overlying euxinia (Kuypers et al., 2005). Anammox also plays an important role in the nitrogen cycle of Mediterranean sapropel events, even in sapropels where euxinia appears to have occurred (Rush et al., 2019). Therefore, the significant contribution of anammox to the depleted $\delta^{13}C_{org}$ values of these sapropels should be considered. However, anammox can be inhibited by sulfide and alternative chemooautotrophic metabolism could produce a different isotopic signature in other euxinic water columns. This conceptual model has also been invoked to explain e.g. the End-Permian (Luo et al., 2014) and the Lomagundi isotope excursion (Bekker et al., 2008).

4.4.2 Paleo-CO$_2$ reconstructions
The offset between organic and inorganic δ\textsuperscript{13}C values is often used to reconstruct paleo-CO\textsubscript{2} (Kump and Arthur, 1999) by assuming that the organic material reflects algal input. Here, we demonstrate that the δ\textsuperscript{13}C\textsubscript{org} in anoxic settings can contain a substantial amount of chemoautotrophically derived carbon, which could be more depleted or enriched than phytoplanktonic, surface-derived carbon. This could explain changes in offsets between bulk and compound-specific δ\textsuperscript{13}C values observed in some black shales. The δ\textsuperscript{13}C value of algal biomass is approximately 4 ‰ higher than the δ\textsuperscript{13}C value of an algal biomarker, phytane (Schouten et al., 1998), however, in many ancient settings, deviations are observed (Meyers, 2014; Witkowski et al., 2018). At some sites, such as the Cretaceous OAE 2 interval at Tarfaya (Tsikos et al., 2004), ODP 1260 (van Bentum et al., 2012) or ODP site 367 (Kuypers et al., 2002), phytane is only 1.4 – 2 ‰ more depleted than bulk organic matter. Similarly, the offset is 1.3 ‰ for the Toarcian at Whitby, which has previously been explained by a release of isotopically light CO\textsubscript{2} from the underlying sediment (Sælen et al., 1998). Some records of the Ordovician (Early Katian) Guttenberg Carbon Isotope Excursion show large changes in δ\textsuperscript{13}C\textsubscript{org} (+ 8 ‰), but not in phytoplanктон biomarkers (+ 4 ‰) or carbonates (+ 2 ‰), potentially caused by local effects via the incorporation of enriched, chemoautotrophic signals (Pancost et al., 2013, 1999). Our results show that calculations of pCO\textsubscript{2} based on differences between δ\textsuperscript{13}C of organic and inorganic carbon should not rely on bulk organic, but instead use compound specific δ\textsuperscript{13}C values derived from algae, consistent with previous investigations (cf. Witkowski et al., 2018). A deviation from the relationship between δ\textsuperscript{13}C bulk and δ\textsuperscript{13}C phytane could thus be used as an indicator for chemoautotrophic contribution to sedimentary OM.

4.4.3 Sources of 13C-depleted geohopanoids

The δ\textsuperscript{13}C records for geohopanoids (the geological degradation product of biohopanoids such as BHT and BHT\textsuperscript{+}) can exhibit dramatic variability, and often pronounced depletion in terrestrial (Pancost et al., 2007) and marine settings (Köster et al., 1998). These are commonly attributed to aerobic methane oxidising bacteria, and thus regarded as evidence for a significant contribution of methane oxidisers to sedimentary organic matter. However, our data show that hopanes of -35 to -50 ‰ could also indicate the presence of a significant amount of anammox bacteria in an anoxic water column. Several factors could attenuate the anammox signal. The decrease of biohopanoids in structural complexity upon degradation means that the anammox signal would be diluted by mixing with aerobically and anaerobically produced hopanoids such as those of Geobacter (Fischer et al., 2005, Härtner et al., 2005). Chemoautotrophs operating in euxinic settings employ biochemical pathways resulting in enriched rather than depleted biomass and lipids, which could also dilute this signal. This may explain why, in some marine anoxic settings where we might expect to see an anammox signature, the δ\textsuperscript{13}C depletion of hopanes parallels that of algal biomarkers (Sinninghe Damsté et al., 2008; van Breugel et al., 2005). Nonetheless, we suggest that potential anammox contributions to the sedimentary hopanoid pool should be considered when interpreting their abundances, distributions and isotopic compositions.

4.4.4. Quantifying the biological pump and remineralization rates

In models of the biological pump, sedimentary organic matter is traditionally regarded as a reflection of exported organic material from the photic zone, and export models consider only remineralization and C-loss, rather than C-production, in the deep ocean (Cabré et al., 2015; Schlitzer, 2002). Particle flux data from sediment traps suggest decreasing carbon
remineralization at greater depths, in particular in the oxygen minimum zone (Marsay et al., 2015; Roullier et al., 2014). However, if a significant proportion of carbon incorporated into sinking particles is derived from bacterial activity in the deep ocean, remineralization rates would be higher than previously estimated. This could explain why remineralization estimates can be too low to sustain oxygen deficiency in the Arabian Sea (Andrews Oliver et al., 2017; Rixen and Ittekkot, 2005). This also affects estimates of denitrification as the major heterotrophic pathway in the anoxic environment of the Arabian Sea OMZ, and thus nitrogen losses. We thus propose a revised conceptual model for the carbon cycle in the Arabian Sea oxygen minimum zone (and other anoxic environments) and suggest that chemooautoautotrophy, i.e. inorganic carbon fixation in the OMZ, needs to be included in models assessing organic matter remineralization rates, oxygen demand, and the nitrogen cycle. This has direct implications for the forecasting of OMZs in a warming world. If anammox and other autotrophic carbon fixation processes increase due to a higher inorganic nitrogen supply by nitrogen fixers, this will constitute an additional pool of labile organic carbon exported from the OMZ that will result in increased oxygen demand and result in expansion of OMZs.

5 Conclusions

Combining the δ¹³C values of BHT', a biomarker from anammox bacteria, and the δ¹³C values of sedimentary organic matter allowed us to estimate the contribution of anammox to organic matter in the Arabian Sea. This can be as much as 30 % of organic matter deposited within an oxygen minimum zone. In the sediments underlying the Arabian Sea, δ¹³C values of total organic matter can shift by 1 to 2 ‰ due to these bacterial contributions, but it remains unclear how well and under what conditions this signature is preserved. This implies that, when past occurrences of OMZs are evaluated based on δ¹³C values of C₉ org, a ¹³C-depleted (anoxic) or possibly ¹³C-enriched (euxinic) contribution of bacteria needs to be considered. Our results also suggest that chemooautoautotrophs (e.g. anammox bacteria) contribute more than previously believed to the burial of carbon in oxygen deficient zones, and remineralization rates are potentially higher than inferred from organic matter decreases. Chemoautoautotrophic carbon fixation thus represents a mechanism of CO₂ removal from the pelagic water column; it should therefore be included in biogeochemical models predicting feedbacks to a warming world.

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**Table 1.** δ¹³C values of analysed samples. s.d. = standard deviation of three repeat analyses, n.d. = not determined (single analysis). The values shown are natural abundance from unamended cores, and from incubations with ^¹³C labelled DOM and POM, under oxic and suboxic conditions. * indicates outliers.

| Type                     | Sample          | BHT | s.d. | BHT’ | s.d. | C<sub>org</sub> |
|--------------------------|-----------------|-----|------|------|------|-----------------|
| **Biomass**              |                 |     |      |      |      |                 |
|                          | Scalindua sp.   | -47.8 | 1 | -47.6 | 1 | - |
|                          | P900 - 1 cm     | -24.7 | 1.3 | -39.1 | 1.3 | -21.5 |
|                          | P900 - 3 cm     | -27.0 | 1.4 | -48.1 | 1.1 | - |
|                          | P900 - 7 cm     | -26.1 | 0.4 | -41.4 | 0.5 | - |
|                          | P1800 - 1 cm    | -25.1 | 1.7 | -48.1 | 1.6 | -20.3 |
|                          | P1800 - 3 cm    | -28.8 | 2.1 | -46.6 | 4.1 | - |
|                          | P1800 - 7 cm    | -27.1 | 0.6 | -45.5 | 1.0 | - |
| Arabian Sea Natural abundance | P900, suboxic, DOM - 1 cm | -27.9 | n.d. | -45.9 | n.d. | - |
|                          | P900, suboxic, DOM - 3 cm | -26.6 | n.d. | -42.0 | n.d. | - |
|                          | P900, suboxic, POM - 1 cm | -41.6* | n.d. | -75.2* | n.d. | - |
|                          | P900, suboxic, POM - 3 cm | -26.4 | n.d. | -49.8 | n.d. | - |
|                          | P900, oxic, POM - 1 cm | -27.1 | n.d. | -51.5 | n.d. | - |
|                          | P900, oxic, POM - 3 cm | -25.2 | n.d. | -47.8 | n.d. | - |
|                          | P1800, oxic, DOM - 1 cm | -21.3 | n.d. | -42.0 | n.d. | - |
|                          | P1800, oxic, DOM - 3 cm | -28.3 | n.d. | -45.6 | n.d. | - |
| Arabian Sea Incubations  | P1800, suboxic, POM - 1 cm | -52.2* | n.d. | -101.9* | n.d. | - |
|                          | P1800, suboxic, POM - 3 cm | -28.8 | n.d. | -47.1 | n.d. | - |
|                          | P1800, oxic, POM - 1 cm | -30.2 | n.d. | -51.3 | n.d. | - |
|                          | P1800, oxic, POM - 3 cm | -28.0 | n.d. | -46.1 | n.d. | - |
Supporting Information for

Dark carbon fixation contributes to sedimentary organic carbon in the Arabian Sea oxygen minimum zone

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Introduction

This supporting information includes additional figures to those presented in the main text. A table showing the δ¹³C values of archaeal lipids is added (Table S1), including information on how this data was acquired (Text S1).
Figure S1. $\delta^{13}C_{\text{org}}$ values of organic matter in the Arabian Sea. Sedimentary values (white circles), and suspended particulate matter (black circles) in the water column as sampled by McLane in situ filter pumps are shown.
Figure S2. HTGC-MS chromatograms and mass spectra of bacteriohopanepolyols. a Arabian Sea sediment and b ‘Ca. Scalindua profunda’ biomass, and mass spectra of identified BHPs: c bacteriohopanetetrol (BHT), d bacteriohopanetetrol stereoisomer (BHT’), and e bacteriohopanepentol (BHpentol), with BHT as the only compound with known stereochemistry.
Figure S3. BHP composition in core tops at P1800 and P900 as detected by HPLC-APCI-Iontrap MS.
Figure S4. BHT'/BHT ratios compared between incubated cores and unamended. UA – unamended, OP – oxic incubation, particulate $^{13}$C-labelled OM, OD – oxic incubation, dissolved $^{13}$C-labelled OM, SP – suboxic incubation, particulate $^{13}$C-labelled OM, OD – oxic incubation, dissolved $^{13}$C-labelled OM.
Table S1. $\delta^{13}C$ values of analysed samples. bp = biphytane with 0, 2, or 3 cyclopentane moieties, all from intact polar lipids, and single analysis due to low amounts. The values shown are natural abundance from background cores, and from incubations with $^{13}C$ labelled DOM and POM, under oxic and suboxic conditions.

Text S1. Analysis of archaeal lipids

The values presented in Table S1 were determined as follows:

The freeze-dried subsamples of the background and incubated cores were ground and extracted by a modified Bligh-Dyer extraction method (Lenger et al., 2012b). Briefly, they were extracted ultrasonically three times in a mixture of methanol/dichloromethane (DCM)/phosphate buffer (2:1:0.8, v:v:v) and centrifuged, and the solvent phases were combined. The solvent ratio was then adjusted to 1:1:0.9, v:v:v to separate the DCM phase. Liquid extraction was repeated two more times, the DCM fractions were combined, the solvent was evaporated and the larger particles were filtered out over glass wool. An aliquot was separated into CL and IPL-GDGTs by silica column separation with hexane/ethyl acetate (1:2, v:v) for the CL-fraction and MeOH to elute the IPL-fraction.

The IPL fraction was then subjected to ether cleavage in order to release biphytanyl chains from GDGTs (Fig. 1c), following procedures described by Schouten et al. (1998a). To this end, the IPL fraction was refluxed in 57% HI for 1 h to cleave the ether bonds and produce alkyl iodides and subsequently extracted 3 times with hexane. The hexane phase was washed with 5% Na$_2$S$_2$O$_7$ and twice with water. The alkyl iodides were purified over Al$_2$O$_3$ with hexane/DCM 9:1, reduced
with LiAlH$_4$ in 1,4-dioxane for 1 h under reflux, the remaining LiAlH$_4$ was reacted with ethyl acetate, bidistilled H$_2$O was added and the biphytanes were extracted with DCM from the dioxane/H$_2$O mixture. Additional purification was achieved by elution over an Al$_2$O$_3$ column using hexane.

GC-MS was used to identify the biphytanes formed upon ether cleavage of GDGTs and PLFAs using a TRACE GC with a DSQ mass spectrometer. The gas chromatograph was equipped with a fused silica capillary column (25 m, 0.32 mm internal diameter) coated with CP Sil-5 (film thickness 0.12 µm). The carrier gas was helium.

The compound specific carbon isotopic composition of the biphytanes was measured with an Agilent 6800 GC, using the same GC column conditions, coupled to a ThermoFisher Delta V isotope ratio monitoring mass spectrometer. The isotopic values were calculated by integrating the 44, 45 and 46 ion currents of the peaks and that of CO$_2$-spikes produced by admitting CO$_2$ with a known $^{13}$C-content into the mass spectrometer at regular intervals. The performance of the instrument was checked by daily injections of a standard mixture of a C$_{20}$ and a C$_{24}$ perdeuterated n-alkane of known isotopic composition. Material from the Arabian Sea, outside the OMZ and incubated suboxically with POM yielded insufficient material for $\delta^{13}$C determination. The stable carbon isotope compositions are reported in the delta notation against the V-PDB standard.