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Microbial bioavailability regulates organic matter preservation in marine sediments

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Abstract. Burial of organic matter (OM) plays an important role in marine sediments, linking the short-term, biological carbon cycle with the long-term, geological subsurface cycle. It is well established that low-oxygen conditions promote organic carbon burial in marine sediments. However, the mechanism remains enigmatic. Here we report biochemical quality, microbial degradability, OM preservation and accumulation along an oxygen gradient in the Indian Ocean. Our results show that more OM, with biochemically higher quality, accumulates under low oxygen conditions. Nevertheless, microbial degradability does not correlate with the biochemical quality of OM. This decoupling of OM biochemical quality and microbial degradability, or bioavailability, violates the ruling paradigm that higher quality implies higher microbial processing. The inhibition of bacterial OM remineralisation may play an important role in the burial of organic matter in marine sediments and formation of oil source rocks.

1 Introduction

Degradation of marine organic matter (OM) begins in the water column, immediately upon the death of marine organisms, and continues at and below the sediment–water interface (Hedges et al., 2000). Oxygen deficiency of the depositional setting has been shown to favour the formation of organic-rich deposits (Hartnett et al., 1998) and oil source rocks (Demaison and Moore, 1980; Gélinas et al., 2001), implying lower degradation rates of organic matter under low oxygen conditions. However, this paradigm has been challenged (e.g. Canfield, 1994), and many other parameters including enhanced primary productivity (Pedersen and Calvert, 1990), sorption to surfaces (Keil et al., 1994a) and high sediment-accumulation rates (Hedges and Keil, 1995) may be associated with the formation of OM-rich sediments.

OM remineralisation in marine sediments is mainly attributed to bacteria (Turley et al., 2000). A wide array of bacteria are required to carry out successful OM degradation, of which hydrolytic and fermentative bacteria play a key role, being able to break down (hydrolyse) complex polymeric compounds into smaller, more soluble and digestible products. Therefore, the activity of these bacteria is often proposed to limit OM degradation rates (Tyson, 1995; Arnosti, 2004). However, OM remineralisation is also influenced by macrofauna. For example, experimental work has shown that under oxic bottom-water conditions, the redistribution and transport of OM from surface sediments to deeper units makes OM more available to a wider bacterial community, and thus substantially stimulates bacterial OM remineralisation (Kristensen and Mikkelsen, 2003; Van Nugteren et al., 2009). Furthermore, macrofaunal bioirrigation will bring oxygen deeper into the sediment and increase solute transport, stimulating microbial activity and net remineralisation (e.g. Aller, 1982, 1994; Aller and Aller, 1998).

Macrofauna also degrade OM directly by ingestion and subsequent mineralisation. Macrofaunal deposit feeders employ a high intensity digestive system, whereas bacteria use low intensity hydrolysis based on extracellular enzymes (Mayer et al., 2001). These different degradation pathways may lead to variations in the biochemical composition of remaining sedimentary OM (Woulds et al., 2012). Specifically, macrofaunal digestion (manipulation) has been suggested to enhance the degradability of OM by microbes (Van Nugteren et al., 2009). Similarly, in soils, particle manipulation by animals is known to promote microbial OM degradation (Brussaard et al., 1997).

From a geological and oil-source rock perspective, it is important to understand which fraction of the OM survives the early degradation stages and is left behind in the rock record, potentially becoming a hydrocarbon source. Traditionally, OM degradation (or OM bioavailability) has been observed to co-vary with OM biochemical quality and quantity, with higher biochemical quality and quantity typically leading to higher remineralisation rates (Henrichs, 1992; Cowie et al., 1995). Robust and commonly applied indirect indicators of biochemical quality of sedimentary OM are the concentrations of chlorophyll a (Chl a) and other intact (or non-altered) pigments. The degradation products of Chl a, phaeopigments (phaeo), in turn serve as indicators of more degraded OM, and the ratio of the two is a commonly applied to examine the quality of sedimentary OM (e.g. Jeffrey and Vesk, 1997; Woulds and Cowie, 2009). Amino acid composition provides another powerful indirect tool for examination of OM biochemical composition, with certain amino acids becoming preferentially enriched (e.g. β-alanine and γ-aminobutyric acid) during degradation while others (e.g. aspartic acid and glutamic acid) are lost (e.g. Cowie and Hedges, 1992, 1994; Dauwe and Middelburg, 1998; Dauwe et al., 1999). Moreover, a quantitative degradation index (DI), based on a range of amino acids and reflecting the progressive compositional change during OM remineralisation, provides another tool to assess the biochemical composition of OM (Dauwe et al., 1999; Vandewiele et al., 2009).

The aim of this study was to investigate two independent parameters – microbial mineralisation and biochemical quality – in order to study the drivers and constraints of OM degradation in marine sediments. Previous work has investigated the biochemical quality of OM in Arabian Sea sediments (e.g. Cowie and Levin, 2009; Vandewiele et al., 2009; Woulds and Cowie, 2009), and, through an experimental approach, microbial degradation in these sediments (Moodley et al., 2011). However, a combined study of both parameters is lacking. Here we report the biochemical quality of OM, including amino acid and pigment analyses, and the potential (oxic) microbial remineralisation rates of OM along a bottom-water oxygen gradient. In addition, biological mixing, the result of metazoan activity, was assessed by means of downcore ^{14}C, phaeopigment and ^{210}Pb profiles. OM accumulation rates were estimated using ^{14}C-dating.

2 Materials and methods

The Arabian Sea is characterised by a pronounced mid-water column oxygen minimum zone (OMZ), which is sustained through monsoon-driven high surface water primary productivity and relatively weak bottom-water ventilation via Antarctic Intermediate Water (Wyrtki, 1973). The modern-day OMZ ($O_2 < 22\,\mu M$ after Helly and Levin, 2004) extends from ± 100 to ± 1400 m water depth with some spatial and seasonal variability. However, the core of the OMZ is relatively stable, with bottom-water oxygen (BWO) values falling to $2\,\mu M$ (Cowie and Levin, 2009). The intensity of Arabian Sea OMZ appears to fluctuate on orbital and suborbital time scales, with minimum OMZ intensity coinciding with low productivity and high winter mixing during the climatic cooling in the North Atlantic (Reichart et al., 1998).

In January 2009, during the PASOM (process study on the Arabian Sea oxygen minimum zone) cruise in the northeastern Arabian Sea, undisturbed surface sediments were collected with a multiple corer, along a BWO gradient ranging from $2\,\mu M$ to $80\,\mu M$, on the Murray Ridge. The studied sites also lie along a depth transect ranging from 900 m to 3000 m water depth (Fig. 1, Table 1). In addition to coring, a CTD profile, including an attached oxygen sensor (Sea-Bird SBE43, accuracy 2 %), was determined at each station to monitor the water-column properties.

2.1 Degradability potential of organic matter

To assess the potential microbial decomposition of OM under oxic conditions, we performed a series of oxic sediment incubations where CO_2 production per unit of OM was quantified (Dauwe et al., 2001). We decided that an oxic approach was the most suitable for indicating the potential net rates of OM remineralisation as oxic conditions have been shown to produce higher, or similar, remineralisation rates than anoxic ones (e.g. Hulthe et al., 1998; Moodley et al., 2011). Nevertheless, some bias in the potential availability rates may result from the adaptation of different bacterial communities. However, we believe this is unlikely to be of a major concern as bacteria are ubiquitous (Fenchel and Finlay, 2004; De Wit and Bouvier, 2006). Furthermore, very similar carbon remineralisation rates were observed for surface sediments of the eastern Arabian Sea OMZ sediment, where both anoxic and oxic incubations were performed (Moodley et al., 2011).

The incubations to assess the in situ OM microbial bioavailability and to quantify the organic carbon (C_{org}) remineralisation through CO_2 production were performed in duplicate on homogenised surface sediments (top 3 cm), following the main protocol outlined in Moodley et al. (2011). Here in short: following core recovery, samples were stored in darkness, in sealed plastic bags at 4 °C. This was chosen over freezing of the sediment, which could have been detrimental to the bacterial community. Storage in sealed plastic bags most likely also resulted in anoxic conditions, although
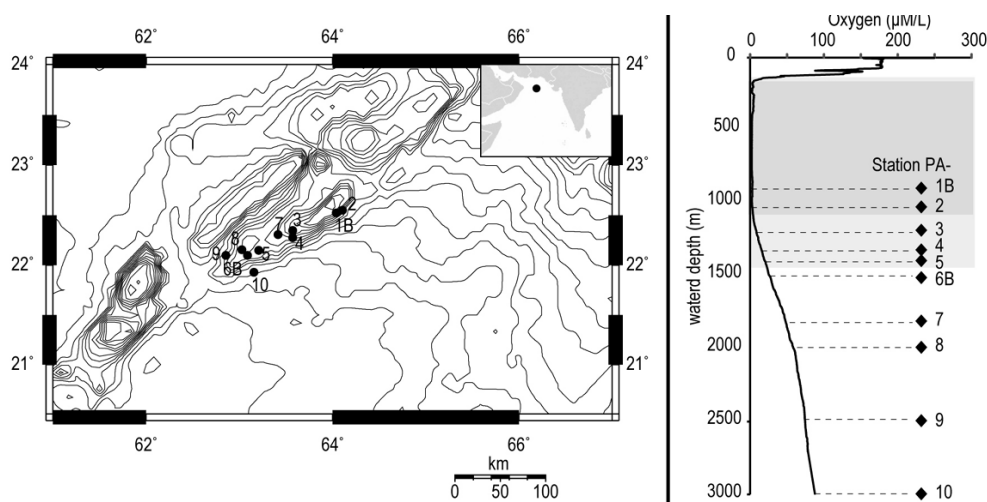
Fig. 1. Left: map of Murray Ridge, Arabian Sea, showing the station locations with an annex of the Indian Ocean with the Murray Ridge indicated with a solid symbol. Right: water-column profile of dissolved oxygen in the study area. Water depth and BWO content of each station shown. Dark grey shaded area = core of the OMZ, BWO content $< 5\,\mu$M; light grey shaded area = OMZ, BWO content $< 22\,\mu$M (or $0.5\,\text{mL}\,\text{L}^{-1}$).

this was not monitored. The incubations were initiated after two months and performed in darkness at $10\,°C$. The incubations were carried out in 80 mL bottles. Into each bottle 10 mL of homogenised sediment was inserted. The bottles were then filled with well-aerated $0.2\,\mu$m-filtered seawater (low-nutrient deep Atlantic water). Total water content, as well as accurate conversion of wet and dry weight sediment, was obtained by direct weighing. Throughout the experiment, the bottles were periodically shaken to mix the slurry. At the end of the incubations the oxygen content was measured with an oxygen optode (Presens, Germany). The oxygen content in each bottle at the end of the incubation was always $> 20\,\mu$M.

At the start of the experiment, sediment was sub-sampled for background analysis (C_{org}, total hydrolysable amino acids, grain size, Sect. 2.2). At the end of the incubations (18 days) the sediment from duplicate bottles was combined (due to expected low concentration of polar lipid-derived fatty acids, PLFAs) for PLFA extraction used to estimate bacterial biomass (Sect. 2.2).

The OM reactivity, expressed as a half-life, was calculated as $-\ln(0.5)/k$ with k (decay constant) based on the quotient of CO_2 production and C_{org} content per mL wet sediment (Hargrave and Phillips, 1981).

2.2 Analytical measurements

Organic carbon and nitrogen contents of ground freeze-dried sediments were measured using an elemental analyzer following acidification to remove any carbonate (Nieuwenhuize et al., 1994).

The total hydrolysable amino acids (THAA) were measured after Vandewiele et al. (2009). Here in short: samples

of 0.1 g freeze-dried sediment were hydrolysed (6 N HCl, $110\,°C$, 20 h, under N_2 atmosphere). To measure total concentrations, 0.1 mL of the hydrolysate was added to 2 mL potassium borate buffer (pH 10) and neutralised with 0.1 mL of 6 N NaOH. The solutions were vortexed and left standing at room temperature for 1 h, after which they were vortexed again to eliminate any ammonium present in the mixture. Fluorescent derivatives were obtained by adding 0.2–0.4 mL of the solutions and an equal amount of ortho-phthaldialdehyde reagents to 2 mL phosphate buffer (pH 8) in a cuvette and vortexing the solution. After 5 min, total concentrations were determined by measuring the fluorescence in a spectrofluorometer (excitation wavelength: 340 nm; emission wavelength: 455 nm). By comparison with a standard amino acid mixture (Sigma), these measurements were then converted to concentrations. Individual amino acids were determined by reverse-phase HPLC after Fitznar et al. (1999). The obtained THAA distribution was used to calculate the DI index, which translates subtle differences in the amino acid composition into one number indicative of the degradation state of particulate OM: from -2 for extensively degraded to $+1$ for fresh algae (Dauwe et al., 1999).

At the end of the slurry incubations, the sediment was freeze-dried and subsequently analyzed for polar lipid-derived fatty acid (PLFA) content in order to estimate the bacterial biomass, which was based on concentration of bacteria-specific PLFAs (i14C:0, i15C:0, a15C:0 and i16C:0) after Middelburg et al. (2000). Here in short: lipids were extracted from 3 g of sediment (wet weight) with a modified Bligh and Dyer extraction (Boschker et al., 1999). The lipid extract was then further fractionated on silicic acid (60, Merck) into different polarity classes by sequential eluting with chloroform, acetone and methanol. The

methanol fraction, which contained the PLFAs, was derivatized using mild alkaline methanolysis to yield fatty acid methyl esters (FAMEs). Internal FAME standards of 12 : 0 and 19 : 0 were used. The analyses were carried out using a gas chromatography combustion isotope ratio mass spectrometry (GC-c-IRMS).

The grain size measurements of the surface sediment (top 3 cm) were performed using a Malvern Particle Analyzer. The sediment was not acidified prior to analyses.

Sedimentary pigments were analyzed for all ten stations. Onboard, the top 10 cm of a multicore subcore (6 cm diameter) was subsampled into 10 slices: the top 2 cm every 0.5 cm, from 2–6 cm every 1 cm and from 6–10 cm at every 2 cm. The samples were stored at $-80\,°C$, and freeze-dried prior to pigment extraction in 10 mL of acetone : water (90 : 10). The full pigment composition was gained through application of high-performance liquid chromatography (HPLC) equipped with a C_{18} reverse phase column. See Barranguet et al. (1998) for the full methodological description. The calibration was based on working standards prepared from commercially available compounds (DHI, Denmark). The pigment concentrations are reported per $\mu g\,g^{-1}$ of sediment. The pigment inventories were calculated as a depth-integrated sum of pigments in the top 10 cm of sediment.

2.3 Bioturbation and sediment mixing: ^{210}Pb and ^{14}C profiles

Downcore ^{210}Pb profiles were measured for four stations (water depths: 1013 m, 1172 m, 1306 m and 1495 m). Samples were taken from the top 6 cm of sediment. The top 2 cm was sampled at every 0.5 cm and thereafter at 1 cm intervals. The ^{210}Pb activity in 100 mg dry weight of sample was measured at Royal NIOZ by α-spectrometry of its granddaughter ^{210}Po, which was precipitated on silver after digestion of sample in an acid solution (Boer et al., 2006). It should be noted that in open marine sediments, like the Murray Ridge, with relatively low sediment accumulation rates (typically a few cm kyr^{-1}), the down-core changes in ^{210}Pb (and phaeopigment) content are due to particle mixing (bioturbation) rather than accumulation. If the ^{210}Pb profile would represent isotope decay, and thus reflect the sedimentation rate, it should not penetrate the surficial sediments but complete decay within the first cm of the sediment. The same principle applies to phaeopigments, however, at stations where the surficial pigment concentrations are very low, microbial degradation may play a role.

The ^{14}C-AMS dating was performed on carbonate from handpicked planktonic foraminiferal tests from three depth intervals (top, middle, bottom) of the multicores. The ^{14}C dating was carried out at each station. The ^{14}C ages were corrected using a marine reservoir age of 400 yr and calibrated using the Int09 calibration curve with CALIB software package version 6.0.1 (Stuiver and Reimer, 1993).

2.4 Carbon accumulation rates

Organic carbon accumulation, or burial, rates were based on the C_{org} content of the top 3 cm of sediment. The surface C_{org} values in the OMZ sediments can be taken to represent the burial values, as the downcore profiles are relatively constant (Vandewiele et al., 2009; Kraal et al., 2012). Furthermore, the typical decrease in C_{org} content, which may be anticipated at the oxic sites occurred within the top 3 cm of the sediment (data not shown). In fact, if using deeper sedimentary C_{org} content (e.g 18–20 cm depth) to calculate carbon burial rates a slight increase rather than decrease is observed, although the pattern remains the same. As the trend in the burial calculations remains the same if using the top 3 cm or a deeper horizon, we believe that our burial estimates are valid in their current form.

For the 0–3 cm sediment interval, the dry bulk density was determined for each station by measuring the weight of a known volume of freeze dried sediment (data not shown). The accumulation rates were inferred from ^{14}C ages. If a clear linear average age vs. depth correlation was not possible, maximum and minimum accumulation rates (and average) were calculated. No C_{org} accumulation rate was calculated for the station from 1495 m water depth as no clear relationship between ^{14}C-data and depth was observed.

3 Results and discussion

3.1 Sediment characteristics: OM quantity and biochemical quality

None of our stations contained clearly laminated sediments, although the four deepest sites outside the OMZ (water depth 1791–3010 m) could be argued to show some subsurface very fine scale lamina (Fig. 2). However, distinct changes in the sediment characteristics were observed along the oxygen gradient, with respect to colour (Fig. 2), C_{org} content, C_{org} accumulation rates and biochemical composition (Fig. 3). The OMZ stations at 885 m and 1013 m water depth, with BWO contents of 2–3 μM, were distinctly darker (dark-olive brown vs. olive brown) than sediments from somewhat deeper sites (depth: 1172 m to 1379 m; with BWO ranging 5–17 μM). With BWO increasing to 27 μM at 1495 m water depth, a colour change from light-olive-brown to more greyish sediment underneath was seen in the top 1 cm, indicating a shallow oxidation front. However, clearly bioturbated sediments with red-brownish surfaces overlying more grey sediments were not seen until 1791 m water depth with a BWO content of 45 μM.

All measured parameters indicative of OM quantity in recent sediments, C_{org} (Fig. 3a), C_{org} accumulation (Fig. 3b), total pigment inventory (Fig. 3c) and total hydrolysable amino acids (Fig. 3d), showed a clear exponential decline with increasing BWO content. The

Table 1. Original station name, station positions, water depth, bottom-water oxygen (BWO) content, median grain size and silt content. In the text and figures stations are referred to according to their water depth.

Station PASOM-	Lat (N)	Lat (E)	Depth (m)	BWO (μM)	Median (μm)	Silt (%)
1B	22°32.9′	64°02.4′	885	2.1	35.4	70.4
2	22°33.9′	64°03.8′	1013	2.6	41.2	63.3
3	22°19.9′	63°36.0′	1172	5.1	38.6	65.2
4	22°18.0′	63°36.0′	1306	13.8	29.6	71.4
5	22°09.3′	63°12.8′	1379	16.8	74.7	46.5
6B	22°04.7′	63°04.5′	1495	26.8	27.4	73.8
7	22°18.5′	63°24.5′	1791	45.2	16.6	92.2
8	22°08.7′	63°01.1′	1970	56.9	15.2	91.8
9	22°06.3'	62°53.7'	2470	66.3	No data	No data
10	21°55.6′	63°10.6′	3010	76.9	14.8	94.7

exponentialrelationship between C_{org} content and BWO in the Arabian Sea OMZ closely corresponds to data from Slater and Kroopnick (1984).

The biochemical composition of OM changes during remineralisation due to preferential loss of reactive compounds and accumulation of other, more refractory compounds (Cowie and Hedges, 1992; Dauwe et al., 1999). Therefore, biochemical OM quality indicators, like amino acids and photosynthetic pigments, provide powerful tools to assess the extent of the OM degradation. Our biochemical OM quality indicators showed clear linear trends with the BWO content, with the highest quality coinciding with the lowest oxygen concentrations in the OMZ (Fig. 3e, f). The quantitative amino-acid degradation index (DI), which is based on subtle changes in amino acids composition reflecting the progressive compositional change during OM remineralisation (Dauwe et al., 1999), ranged from −0.45 to −1.4 and related strongly with the BWO content ($R^2 = 0.95$, $p = < 0.001$). As the DI was negative at all sites (+1 represents freshly produced algal matter and −2 corresponds to extensively degraded deep-sea sediments; Dauwe et al., 1999; Vandewiele et al., 2009), the OM in the core of the OMZ was moderately degraded. However, extensive degradation (DI = −1.4) was seen at the deepest better-oxygenated site, consistently with observations on the Pakistan margin (Vandewiele et al., 2009). Similarly, the phytopigment OM quality indicator (the content of Chl a and other intact phytopigments over total pigments) showed a strong relation with the BWO content ($R^2 = 0.73$, $p = 0.02$), implying enhanced degradation with elevated bottom-water oxygenation as previously reported by Woulds and Cowie (2009). The pigment index also showed a relation to the DI ($R^2 = 0.65$, $p = 0.009$). Clearly, biochemical indices for OM quality consistently show preservation of high quality OM under low oxygen conditions.
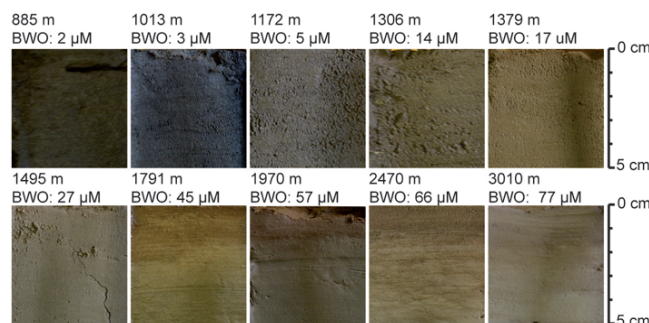

Fig. 2. Images of surface sediments from the studied oxygen transect. First row: stations from 885 m water depth down to 1379 m depth. These are located in the OMZ where bottom-water oxygen (BWO) is less than 22 μM. Second row: stations from 1495 m water depth down to 3010 m depth. These sites are located below the OMZ where BWO ranges from 27 μM to 77 μM.

3.2 Bacterial biomass and OM (microbial) degradability

The bacterial biomass was relatively constant averaging $248 \pm 67 \, \mathrm{mmol\,C\,m^{-2}}$ and did not show a clear trend along the study transect (Fig. 3g). The only exception was the station located at 2470 m water depth, where a bacterial biomass minimum ($127 \, \mathrm{mmol\,C\,m^{-2}}$) was recorded. Nevertheless, our biomass data fit with the general observation that bacterial biomass is rather constant in oceanic sediments, regardless of the depositional setting (Wei et al., 2010). Furthermore, despite the high quantity and high biochemical quality of OM in the OMZ sites, the potential remineralisation rates under oxic conditions were remarkably constant along our study transect, averaging $2.01 \pm 0.33 \, \mathrm{mmol\,C\,m^{-2}\,d^{-1}}$ (Fig. 3g). Our remineralisation rates are similar to those measured for OMZ sediments along the eastern Arabian Sea and one order of magnitude lower than those for continental shelf sediments (Moodley et al., 2011). Hence, the abundant OM of moderately high quality in OMZ sediments exhibits surprisingly poor microbial degradability (bioavailability). The examination of the OM decay constants (k) derived from the incubation experiments, supports this observation, showing that the OM accumulating in the OMZ is significantly less biodegradable than the OM deposited in the oxygenated zone below the OMZ (Mann–Whitney test, 1-tailed, $p < 0.005$, $n = 18$; Fig. 3h). The resulting average OM half-life for OMZ sediments is 35 ± 14 yr while the corresponding numbers for the zone below the OMZ is only a half of this (15 ± 2 yr; not shown). As the incubations were carried under oxic conditions, some bias may result from the adaptation of different bacterial communities. However, we believe this is unlikely to be a major concern as very similar carbon remineralisation rates were observed for surface sediments of the eastern Arabian Sea OMZ sediment, where both anoxic and oxic incubations were performed (Moodley et al., 2011).
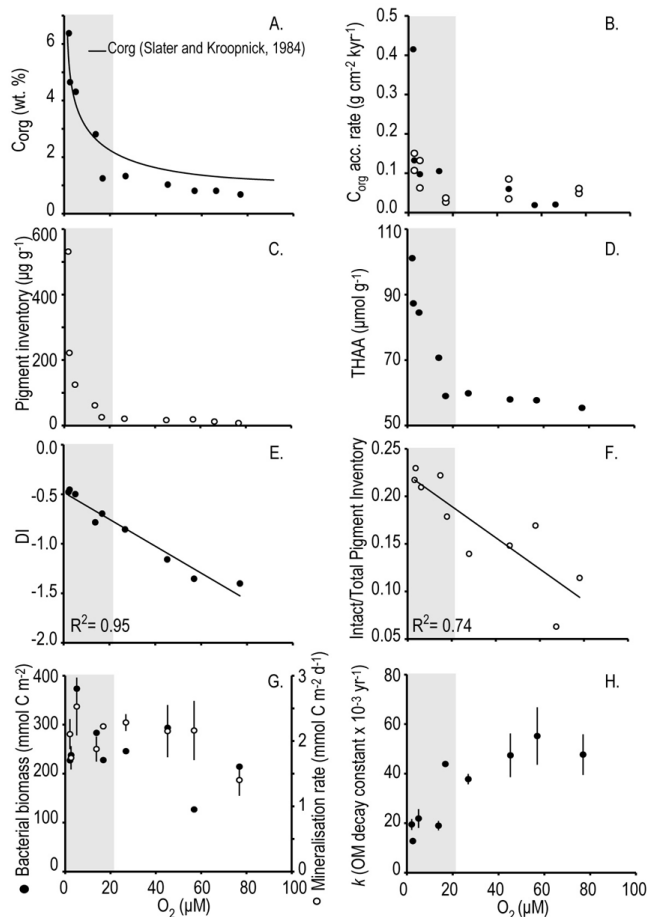
Fig. 3. Organic matter quantity, organic carbon accumulation and biochemical quality indices versus bottom-water oxygen content. **(A)** Organic carbon with a trend line of Slater and Kroopnick (1984); **(B)** organic carbon accumulation rates. The solid circles indicate the values based on average sedimentation rates where as the open circles indicate the maximum and minimum accumulation rates. **(C)** Total pigment inventory; **(D)** total hydrolysable amino acid content (THAA); **(E)** DI, the amino acid degradation index; **(F)** intact/total pigment inventory; **(G)** bacterial biomass (closed symbol) and mineralisation rate (open symbol); **(H)** OM decay constant versus BWO content along the studies transect. The area shaded in grey represents the zone where BWO content is $< 22 \, \mu M$ and invertebrate fauna are accordingly affected by the low O_2 concentrations (Levin, 2003). The error bars in plots G and H represent the standard deviation of two replicate incubations. No error bars are available for bacterial biomass as the sediment was pooled for bacterial PLFA analyses **(G)**.

Furthermore, it should be noted that our remineralisation rates reflect the potential degradation rates under oxic conditions. Thus, the in situ rates in the OMZ of moderately degraded OM may be expected to be lower due to the near absence of oxygen (Hulthe et al., 1998; Dauwe et al., 2001).

3.3 OM bioavailability versus biochemical OM quality

The apparent paradox that biochemical quality and direct microbial bioavailability of OM are not coupled is intriguing. This is in contrast with the ruling paradigm that less degraded OM, or OM of higher quality as inferred from biochemical composition, is typically more readily degradable (Henrichs, 1992; Cowie et al., 1995; Hedges and Keil, 1995). We suggest that this retardation of OM remineralisation in OMZ sediments may be the controlling parameter for the accumulation of OM in the OMZ sediments.

Several mechanisms have been linked to the inhibition of OM remineralisation. For example, physical protection through encapsulation of reactive OM with algaenans, compounds present in algal cell walls, or other hydrolysis-resistant matrices may inhibit the remineralisation of OM of high biochemical quality (Knicker, 2004). However, we do not believe that algaenans play a major role in the protection of OM in the OMZ sediments. The relative abundance of amino acids typically found in the algal cell wall, such as of glycine and threonine, were equally abundant in the OMZ sediments ($27.2 \pm 0.4 \%$) and in sediments outside the OMZ ($27.8 \pm 0.7 \%$; data not shown). Sedimentary OM has also been shown to be commonly enriched in finely grained sediments (e.g. Bordovskiy, 1965; Premuzic et al., 1982; Keil et al., 1994b) and it has been suggested that this may be due to OM association with, or sorption to, mineral surfaces (e.g. Keil et al., 1994a; Mayer, 1994; Hedges and Keil, 1995). In this study, no data for mineral surface area is available. Nevertheless, no correlation was observed between the median grain size or clay content (Table 1) and sedimentary C_{org} content (Pearson correlation: $p = 0.513$, $n = 9$; and $p = 0.247$, $n = 9$, respectively). Moreover, Vandewiele et al. (2009) showed that sediments below the Pakistan OMZ are also enriched in organic carbon when normalised to specific mineral surface area. Therefore, we believe that mineral surface sorption is not the primary preservation mechanism of OM in the OMZ sediments.

OM depolymerisation via extracellular enzymatic hydrolysis has been shown to be the rate-limiting step in OM remineralisation (e.g. Hoppe, 1991; Arnosti, 2004). Microbial remineralisation of OM of high molecular weight substrate typically begins with extracellular enzymatic hydrolysis, which produces compounds small enough to be taken up by a bacterial cell. The typical molecular size limit for microbial uptake is around 600 Da (Weiss et al., 1991). Thus, the inhibition of OM remineralisation may be due to the presence of higher molecular weight compounds, which are not bioavailable to microbes despite their higher biochemical quality (Arnosti and Holmer, 2003). This may also be the case in our OMZ transect, as pyrolysis results indicate relatively higher concentrations of pigment-derived, macromolecular-bound tetrapyrrole compounds in the OMZ sediments and the absence of these pigment related macromolecules outside the OMZ (K. Nierop, unpublished data).
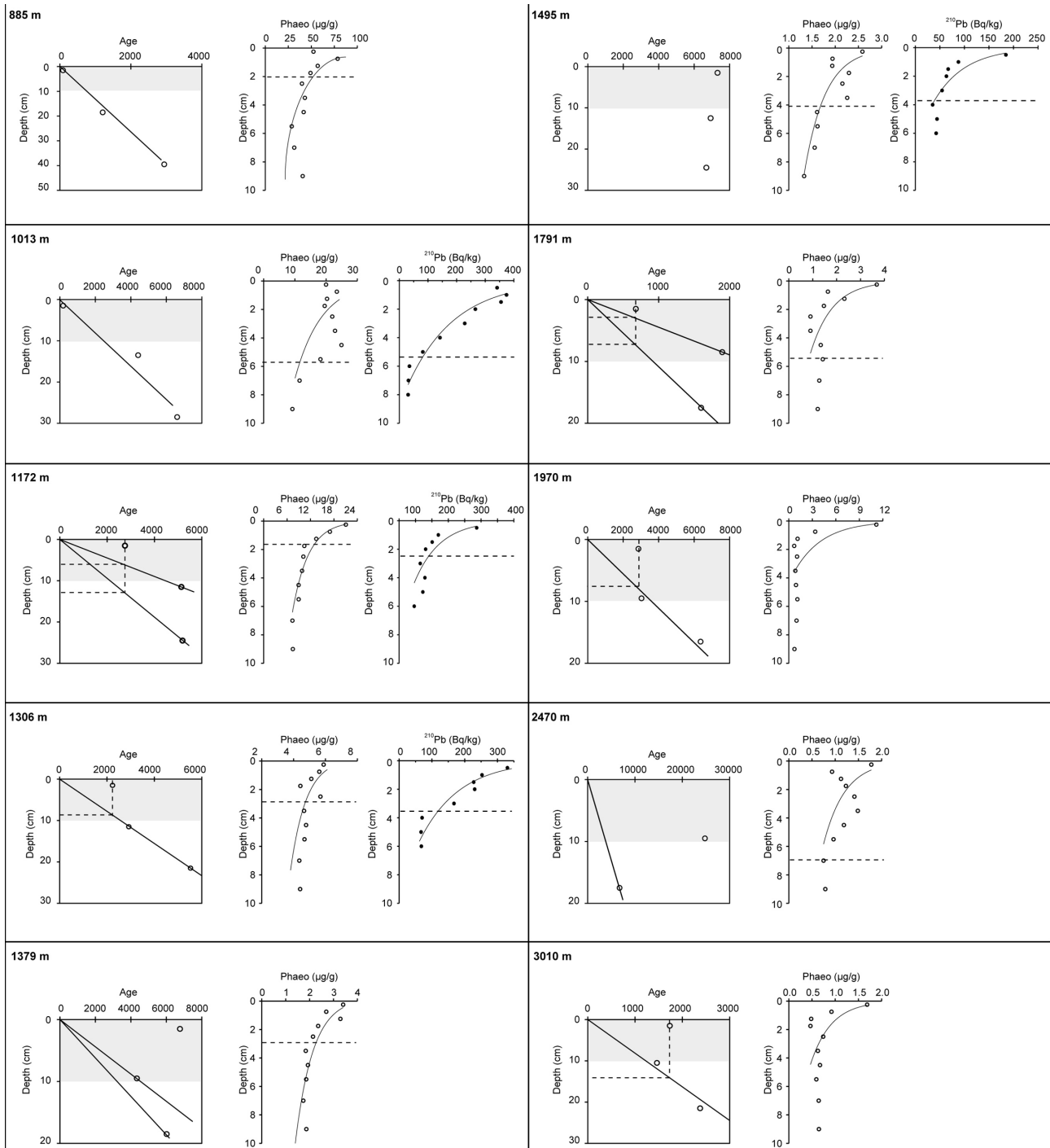
Fig. 4. Sediment mixing or bioturbation indicators used in this study. Stations from 885 m depth down to 1379 m water depth are located in the OMZ and stations from 1495 m depth to 3010 m depth are located outside the OMZ. Three mixing indicators used: [14]C data to give age of sediment in absolute years, and down core phaeopigment and ^{210}Pb data. Due to various half-lives of the mixing indicators, long-term and short-term mixing can be examined independently, see main body of text for more detail. Light grey shading in the ^{14}C age plots indicates the top 10 cm of sediment, which is also shown in phaeopigment and ^{210}Pb profiles. Horizontal, dashed lines indicate the inferred mixing/bioturbation zone.

Particle manipulation by macrofauna, which have relatively complex digestion pathways and involve many enzymes, makes OM more accessible to microbes by changing the surface area of particles, simultaneously making nutrient-rich molecules more easily obtainable (Mayer et al., 2001). Through this mechanism, macrofauna may catalyse microbial degradation, aiding the breakdown of macromolecular compounds and providing bacteria with bioavailable OM. Such particle manipulation by fauna and associated bioturbation would also enhance the diffusion of enzymes, thus accelerating microbial degradation in agreement with the OM degradation model of Rothman and Forney (2007). A recent in situ labelling study by Hunter et al. (2012) also points out the importance of macrofauna in regulating bacterial carbon and nitrogen uptake in OMZ sediments. In OMZ sediments in the presence of macrofauna (as in this study), OM processing by bacteria was observed to be retarded, occurring only after OM was first processed by macrofauna. The study of Hunter et al. (2012) thus supports the idea that OM particle manipulation by macrofauna mediates heterotrophic bacterial utilisation. In addition, the study of Hunter et al. (2012) is in agreement with the abyssal uptake experiment of Witte et al. (2003) where faunal activity appeared to control bacterial OM uptake. The work of van Nugteren et al. (2009) has also demonstrated enhanced degradation of OM in the presence of macrofauna.

We suggest that poorly developed macrofaunal communities in the OMZ are responsible for the enhanced preservation of OM in these sediments. Direct macrofaunal data for the Murray Ridge suggest relatively high macrofaunal abundance within the OMZ (± 1400 mg C m^{-2}; Pozzato, 2012), despite the BWO concentrations (2–3 μM) being substantially lower than the accepted lower limit of long-term oxygen tolerance of macrofauna (22 μM; Levin, 2003). However, the diversity of the macrofaunal community is very low; 65 % of the total biomass (including bacteria) is attributed to the polycheate *Linopherus* sp. This species is known to live close to the sediment–water interface, where it makes shallow, vertical burrows (Gooday et al., 2009; Levin et al., 2009). Similarly low macrofaunal diversity inside the OMZ has also been highlighted in studies of the adjacent Pakistan margin (Gooday et al., 2009; Hughes et al., 2009). Apparently, these shallow, low-diversity assemblages are less efficient than high-diversity assemblages at manipulating sedimentary OM into particles which are readily available for bacterial remineralisation.

In the absence of more complete macrofaunal data, we use bioturbation estimates (Fig. 4) as a proxy for macrofaunal activity along the complete transect. Comparing organic carbon accumulation to bioturbation depth Van Der Weijden et al. (1999) showed an inverse relation. The linear ^{14}C age vs. depth relationships at the 885 m and 1013 m sites suggest that bioturbation is limited to the very surficial sediments in the OMZ itself, supporting our theory of inefficient sedimentary recycling by the low-diversity macrofaunal assemblage

(Fig. 4). Generally, bioturbation then increases with increasing bottom-water oxygenation. Between 1172 m and 1379 m water depth, the bioturbation horizon appears to reach ± 4 cm sediment depth, based on pigment and ^{210}Pb profiles (Fig. 4). The ^{14}C-profiles suggest somewhat deeper mixing for the stations located at 1306 m and 1379 m water depth, implying that the long-term mean mixing depth at these stations may be greater (up to 10 cm). Such deep mixing may be related to the burrowing activity of *Zoophycos* (Leuschner et al., 2002). At 1791 m water depth the bioturbation zone reaches down to ± 6–7 cm depth as indicated by the phaeopigment and deeper sediment ^{14}C-data. The offset of the intermediate and deep ^{14}C-data may be due to *Zoophycos* or other macrofaunal activity. Below 1970 m water depth, the bioturbation horizon reaches beyond 7 cm depth and at 3010 m water depth it reaches below 10 cm depth. We suspect that the anomalously shallow mixing, according to pigment profile at the depths at 1970 m and 3010 m water depth may be due to the very low surficial OM concentrations at these depths. The constant age vs. depth relationship in the ^{14}C data at 1495 m is interpreted as a mass deposit such as a slump or turbidite, however, this event does not seem to be of very recent age as both the ^{210}Pb and phaeopigment profiles imply that hemipelagic sedimentation has continued since than.

More targeted experiments are required to further examine our hypothesis of macrofaunal control on organic matter bioavailability to bacteria.

4   Implications and conclusions

Our data support the view that the enhanced preservation of OM occurs in the OMZ sediments where the BWO content is < 22 μM. The biochemical quality of the OM (phytopigments and amino acids) also shows a negative linear relationship with BWO content, implying that the OM in the OMZ sediments is indeed of high quality. In addition, the biochemical quality indicators correlate well with each other, thus providing a robust and consistent impression of the composition of OM in Arabian Sea sediments. However, in contradiction to the ruling paradigm of OM preservation in marine sediments, microbial bioavailability does not reflect the biochemical quality of OM in the OMZ. In light of recent literature (van Nugteren et al., 2009; Hunter et al., 2012), we propose that the enhanced preservation of OM in the Arabian Sea OMZ is controlled by the poorly developed, low-diversity macrofaunal assemblage in this location. The lack of a well-developed macrofauna leads to inefficient breakdown of OM into smaller substrates more readily ingestible to bacteria. The proposed mechanisms would lead to preservation and burial of OM with high biochemical composition, thus providing an analogue for oil source rocks.

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