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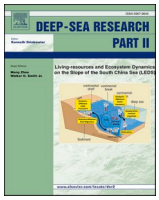

Suspended particles are hotspots of microbial remineralization in the ocean's twilight zone

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ABSTRACT

The sinking of photosynthetically produced organic carbon from the ocean surface to its interior is a significant term in the global carbon cycle. Most sinking organic carbon is, however, remineralized in the mesopelagic zone (~100 m–1000 m), thereby exerting control over ocean-atmosphere carbon dioxide (CO_2) partitioning and hence global climate. Sinking particles are considered hotspots of microbial respiration in the dark ocean. However, our observations in the contrasting Scotia Sea and the Benguela Current show that >90% of microbial remineralisation is associated with suspended, rather than sinking, organic matter, resulting in rapid turnover of the suspended carbon pool and demonstrating its central role in mesopelagic carbon cycling. A non-steady-state model indicates that temporally variable particle fluxes, particle injection pumps and local chemoautotrophy are necessary to help balance the observed mesopelagic respiration. Temperature and oxygen exert control over microbial respiration, particularly for the suspended fraction, further demonstrating the susceptibility of microbial remineralisation to the ongoing decline in oxygen at mid-ocean depths. These observations suggest a partial decoupling of carbon cycling between non-sinking and fast-sinking organic matter, challenging our understanding of how oceanic biological processes regulate climate.

1. Introduction

Export of organic matter produced in the surface ocean to its interior by the biological carbon pump (BCP) (Volk and Hoffert, 1985; Fowler and Knauer, 1986; Boyd and Trull, 2007; Steinberg et al., 2008) annually removes about one third of anthropogenically produced CO_2 from the atmosphere (Siegenthaler and Sarmiento, 1993; Gruber et al., 2019). Attenuation of the gravitational draw-down of particulate carbon in the upper 1000 m of the ocean is a key determinant of the size of carbon storage mediated by the BCP (Martin et al., 1987; Boyd and Trull, 2007), with microorganisms and zooplankton playing an important role in this process (Ploug et al., 1999; Arístegui, 2005; Steinberg et al., 2008; Jackson and Checkley, 2011; Marsay et al., 2015; McDonnell and Boyd, 2015). Nonetheless, estimates of microbial respiration and production suggest that the sinking particulate organic carbon (POC) flux into the mesopelagic ocean is often insufficient to meet total microbial carbon demand (respiration + biomass production) within these depths (Steinberg et al., 2008; Baltar et al., 2009; Burd and Jackson, 2009; Herndl and Reinthaler, 2013). This gap may be partially met by the only recently fully recognized gravity-independent particle injection pumps, which can act on sinking and suspended particles, as well as dissolved organic carbon (DOC), to transport additional carbon into the ocean's interior in likely similar magnitude as the gravitational POC flux (Hansell et al., 2009; Lévy et al., 2013; Boyd et al., 2019).

Suspended particles that are either directly injected (Boyd et al., 2019), result from the fragmentation of larger particles (Christina and

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Passow, 2007; Steinberg et al., 2008; Briggs et al., 2020), are produced locally by coagulation of dissolved organic matter (DOM) (Chin et al., 1998; Passow, 2000) or chemoautotrophic dark carbon fixation (Baltar et al., 2010; Reinthaler et al., 2010; Herndl and Reinthaler, 2013; Guerrero-Feijóo et al., 2018), interlink the different carbon pools in the mesopelagic zone, where they account for the majority (>80%) of POC by concentration. Given its large size, the suspended POC pool could potentially sustain a substantial fraction of microbial respiration in the mesopelagic (Karl et al., 1988; Baltar et al., 2010; Giering et al., 2014), though to date it is the larger, sinking particles that have been a primary focus for respiration (Ploug and Grossart, 2000; Ploug et al., 2008; Bianchi et al., 2018). However, not only are direct measurements of microbial respiration in the dark ocean scarce (Robinson and Williams, 2005), but they also rarely differentiate between different particle pools (Cavan et al., 2017).

As the Ocean continues to warm and deoxygenate further (Keeling et al., 2010), the cycling of organic matter is bound to change. On one hand, rising temperatures likely accelerate microbial production and consumption processes (Iversen and Ploug, 2013) while, on the other hand, lower oxygen concentrations can promote the preservation of POC (Devol and Hartnett, 2001), potentially as a result of reduced heterotrophic microbial activity (Van Mooy et al., 2002), though a recent study (Maβmig et al., 2020) observed no major influence of low oxygen concentrations on bacterial degradation of organic matter. An alternative likely explanation is the reduced fragmentation of large particles owing to the absence of zooplankton (Cavan et al., 2017). Further studies are therefore needed to ascertain how the future ocean may influence the remineralisation of carbon.

Here we compare the rates of microbial remineralisation measured on fast-sinking and suspended particles and the estimated fraction sustained by DOC in the mesopelagic depths of the Scotia Sea and the Benguela Current, representing cold, well oxygenated waters and a warm rapidly expanding oxygen minimum zone (Schmidtko et al., 2017), respectively. Fast-sinking particles were separated from non-sinking organic matter in the same water sample collected by Marine Snow Catchers (MSC) (Lampitt et al., 1993). The direct measurements of microbial oxygen consumption in short-term incubations (<24 h), along with parallel measurements of net-ammonification, show substantially faster turnover of suspended than fast-sinking organic matter. These striking differences between fast-sinking and suspended particles demonstrate a minor direct contribution of microbial respiration to POC flux attenuation and highlight the suspended organic matter pool as the "centre stage" for microbial activity in the ocean's twilight zone. We further demonstrate that microbial respiration is influenced by both the concentration and quality of particulate organic matter (POM) (inferred from C:N), as well as the temperature and oxygen concentration of the water column. The several-fold difference in turnover times of suspended POC between the contrasting ocean biomes, represented by the Scotia Sea and the Benguela Current, indicate the susceptibility of microbially mediated mineralisation of suspended organic matter to CO_2 to a changing ocean.

2. Material and Methods

2.1. Sampling of fast-sinking and suspended particles

Samples from the surface mixed layer (<100 m) and the mesopelagic zone (100 m–750 m) were obtained during two research expeditions on board the *RRS Discovery* to the Scotia Sea in the Southern Ocean (52 41° S 40° W) on 12th November to December 16, 2017 (DY086), and to the Benguela Current (18° S 11° E) on 25th May to June 21, 2018 (DY090), respectively. We used 100 L capacity Marine Snow Catchers (MSC) to collect and separate suspended and fast-sinking particles (Lampitt et al., 1993; Riley et al., 2012; Lampitt et al., 1993, 1993). On any given sampling occasion we would use two consecutive MSCs to each collect 100 L of seawater from any one depth between 45 m and 750 m, overall. A total of 11 separate MSCs deployments were conducted on both cruises. On recovery, an initial 4 L was removed for POM analyses from each MSC ($n = 2$) for a time zero sample (see section on "POM analysis" below). The remaining 96 L of seawater were allowed to settle for approximately 2 h at ambient air temperature (Riley et al., 2012) to separate fast-sinking (>25 m d^{-1}) from suspended organic matter including DOM and suspended particles (<10 m d^{-1}). Seawater containing suspended organic matter was obtained from the top section of the MSC for respiration and net-ammonification rate measurements as well as POM elemental analysis. The remaining water in the upper section of the MSC was gently released and the section removed. The base of the MSC was immediately transferred to a 4 °C or 12 °C (for the Scotia Sea and Benguela Current, respectively) cold room, where the fast-sinking particles were collected from one MSC base with a glass Pasteur pipette and immediately transferred to micro-respiration chambers (see "Rates of Oxygen Consumption" section). Bulk particles and water from the other MSC base were collected with a silicon syphon pump and transferred to amber high-density polyethylene bottles for net-ammonification rate measurements (See "Ammonia measurements and calculation of net-ammonification rates"). The number of particles varied, with the particle mass in each chamber measuring between 0.22 μg to 2.57 μg and 0.15 μg–2.07 μg in the Scotia Sea and Benguela Current, respectively, with a mean weight of 1.2 μg for both regions.

2.2. In situ sinking velocity of fast-settling particles

A neutrally buoyant platform (PELAGRA (Saw et al., 2004)) equipped with a camera system was used to obtain *in situ* image sequences of settling particles (Iversen and Lampitt, 2020). The sinking particles were illuminated with a flash gun (Canon Speedlite 600EX-RT) that was mounted perpendicular to the camera (Canon EOS 6D DSLR) to create collimated light to illuminate a water volume of 3.32 L. Particle sizes and settling velocities were analysed from image sequences where the neutrally buoyant platform was stable in the water column (following the description in Iversen and Lampitt, 2020). This was done by converting the images into a grey scale and identifying each individual particle by applying a threshold value. Article size was determined from their pixel area at 40.8 μm per pixel for conversion. Sinking velocities were measured by following individual particles throughout the image sequences and determining the length of the vertical trajectory particles settled per unit time. These sinking velocities were then used in the non-steady state model (see "Modelling the suspended POC").

2.3. Hydrochemical data

Profiles of temperature, salinity, chlorophyll fluorescence and oxygen concentration were taken using a Conductivity-Temperature Depth (CTD, SeaBird SBE911plus/917 plus) unit during the two research cruises. Dissolved oxygen concentrations were calibrated using a semi-automated whole-bottle Winkler titration unit.

2.4. POM analyses

Samples for POC and PON concentration analyses were obtained directly after retrieval of the MSC (4 L) and vacuum filtered at < 200 mbar onto pre-combusted 25 mm glass fibre filters (GF/F) (Whatman) and dried in an oven at 50 °C for ≥12 h. Further samples were obtained after 2 h of settling time for suspended POM analysis (4 L). To estimate the concentration of POM associated with fast-sinking particles in the water column, representative samples (100 ml–500 ml) were taken from the base of the snow catcher using a silicon syphon pump as described below for "ammonia measurements and calculations of net-ammonification rates''. The POM concentrations were corrected for the collected water volume, assuming that all fast-sinking particles had accumulated in the bottom section following a 2 h settling time. To arrive at the POM concentration associated with fast-sinking particles,

the fraction of POM associated with suspended particles was subtracted. To determine carbon specific respiration rates, POC concentrations were measured directly for the fast-sinking particles in the micro-respiration chambers following the oxygen consumption experiments and treated as described above. In the home laboratory, samples were acid-fumed overnight with hydrochloric acid, dried to a constant weight and packed into tin cups (Elemental Microanalysis, 5 × 8 mm). Concentrations of PON and POC were measured with an elemental analyser (Sercon Integra 2, 1000 °C combustion temperature) along with respective standards (urea and casin) and blanks.

2.5. Rates of oxygen consumption

Oxygen consumption was measured using a micro-respiration set-up (MicroRespiration System, Unisense, Denmark). The different fractions from the MSC were placed into eight 750 μl and eight 2 ml glass chambers and oxygen consumption over time was measured using an oxygen microelectrode (Oxygen MicroRespiration sensor, Unisense, Denmark). The chambers were held in a dedicated rack with integrated stirrers and kept at a stable temperature (variation ± <0.01 °C) in a water bath connected to a chiller (Grant, RC350G). The fast-sinking particles were placed in seawater that had previously been filtered through polycarbonate filters (0.22 μm pore size, Sterivex, Millipore). The chambers were then sealed and placed in the water bath at 2 °C and 12 °C, for the Scotia Sea and Benguela Current, respectively, for 30 min to equilibrate, before the first measurements were made. The chambers were stirred with glass coated stirrers set underneath a mesh to avoid formation of oxygen gradients without breaking up particles. Triplicate blank measurements were run in parallel with 0.22 μm-filtered seawater. Rates of oxygen consumption were calculated using linear regression. We measured oxygen consumption in 149 incubations in the Scotia Sea and in 87 in the Benguela Current. Results were discarded if: the blank oxygen concentrations did not remain stable over time; the r^2 obtained from linear regression of the incubation experiments i.e., rate estimate was below 0.6; or if the rate of oxygen consumption was plus or minus the standard deviation from the mean (52 and 19, from the Scotia Sea and Benguela Current respectively). A linear mixed effects model showed that the blanks had a significantly different slope from the other particle fractions for both regions ($P < 0.01$).

2.6. Ammonia measurements and calculation of net-ammonification rates

Samples of suspended organic matter and fast-sinking particles were obtained as described above and initial ammonia concentrations were measured on board fluorometrically on a Turner Trilogy Fluorometer (detection limit 0.01 μM) (Holmes et al., 1999). To measure net ammonification, triplicate samples of both particle fractions were distributed into 30 ml acid-washed (10% HCl for >24 h) and pre-combusted glass vials without headspace, closed with butyl septa and incubated for 7–21 h in the dark on roller tables to avoid settling of aggregates and formation of chemical gradients. Incubations were conducted at 1 °C and 12 °C in the Scotia Sea and the Benguela Current, respectively. Ammonia concentrations were measured as described above. Net ammonification rates were calculated from the difference in ammonia between the initial and final concentrations over the incubation period, and negative rates (=net uptake) were discounted (45 out of 114 samples). To obtain rates of net-ammonification in fast-sinking particles, rates measured in the corresponding suspended fractions were subtracted. Nitrogen-specific turnover k (d^{-1}) was calculated by dividing the net rate of ammonification by the concentration of particulate organic nitrogen measured in each particle fraction:

$k(d^{-1})$ = microbial ammonification (mol NH_3 $chamber^{-1}$ d^{-1})/ mol N $chamber^{-1}$ Equation 1

The mean values of the triplicates were used for this analysis and in cases where the standard deviation was more than plus or minus the mean, the results were discarded ($n = 14$ out of 114).

Expected rates of ammonification were calculated from the rates of oxygen consumption assuming complete aerobic remineralisation and release of ammonia (NH_3):

$$C_{106}H_{175}O_{42}N_{16}P + 118O_2 \rightarrow 106CO_2 + 16NH_3 + H_3PO_4 + 62H_2O \quad \text{Equation 2}$$

However, rather than use Redfield ratios for the calculation, the C:N ratio measured in our samples was used instead (see POM analyses). The mean of the samples run for each deployment and fraction was taken to allow for a comparison between the calculated and measured ammonification rates; a paired t-test was used for this analysis.

2.7. Microbial respiration and carbon turnover

We estimated the rates of respiration fueled by suspended POC using a linear regression model describing the relationship between oxygen consumption and POC concentrations. The intercept of the model represents the rate of oxygen consumption at zero POC, which we considered to account for microbial respiration directly fueled by DOM and other oxygen consuming processes, such as nitrification. The mean percentage difference between the two slopes, at the measured intercept and the intercept set at zero POC, was calculated to estimate the amount not accounted for by the suspended POC concentration. This fraction accounted for 45% in the Scotia Sea and 13% in the Benguela Current and was subtracted from microbial respiration rates associated with suspended particles.

The rate of respiration directly attributable to DOM within the samples containing suspended particles was further estimated using the relationship between DOC concentrations and apparent oxygen utilisation for samples from the Benguela Current. The proportion of respiration fueled directly by DOM was then estimated using the measured C:N ratio and the expected $-\Delta C/\Delta O2$ ratio of 0.72 (Anderson, 1995). Apparent oxygen utilisation was calculated from the measured oxygen concentration and the expected saturated concentration at 1 atm, using the Matlab Gibbs-Seawater toolbox (McDougall and Barker, 2011).

Carbon specific turnover k (d^{-1}) was calculated (equation (3)) using the POC concentrations measured in parallel samples for the suspended fraction and subsequent direct POC measurements of the particles used in the respiration incubations for the fast-sinking fraction (see "POM measurements" below):

$k(d^{-1})$ = microbial respiration (mol C $chamber^{-1}$ d^{-1})/ mol C $chamber^{-1}$ Equation 3

Rates of respiration and turnover times were corrected for *in situ* temperature using measured water column temperatures and an experimentally derived activation energy of 0.6 eV (see below). The samples in the Benguela Current were corrected further to *in situ* oxygen concentrations (see below). To calculate the rate of microbial remineralisation in the water column for fast-sinking particles, we first calculated the concentration of POC in the water column by scaling the combined fast-sinking POC measurements in all the parallel incubations for one deployment, to the volume of the MSC. This assumes that all fast-sinking particles were collected for the analysis. To then calculate the rate in the water column the carbon specific turnover was multiplied by the calculated water column POC.

2.8. Temperature and oxygen corrections

Temperature-manipulation experiments were conducted in the Benguela Current to determine the temperature effect on rates of respiration. Suspended and fast-sinking fractions from 100 m depth were placed into respiration chambers as described above. The rate of

oxygen consumption was determined for approximately 10 h. For each batch of samples two different temperatures were tested, after the 10 h the temperature was lowered 2–3 °C (The temperatures used were: 18, 15, 13, 12, 11, 10, 7, and 5 °C). Once the temperature had stabilised (approximately 2 h), the next measurement was taken. The decrease in oxygen was checked for linearity. The apparent activation energy (eV) was calculated by regressing natural log transformed, C specific respiration rates (ln (d^{-1}) against centred, absolute temperature ($1/K_bT_c$ - $1/K_bT$), where K_b is the Boltzmann constant, T_c is the mean temperature in Kelvin (285 K for the incubations and for the mesopelagic depths investigated i.e., 12 °C) and T is the temperature of each individual incubation, also in Kelvin. *In situ* temperatures were taken from the CTD profiles and *in situ* respiration estimated using the characterised temperature sensitivity i.e., activation energy (eV).

Oxygen manipulation experiments were conducted to determine how changes in oxygen concentration would affect respiration rates for the two particle fractions. Suspended particles were collected from 100 m depth using the marine snow catchers as described above. The water was bubbled gently with helium until the oxygen concentration was near zero. The deoxygenated water was added to oxygen saturated samples of suspended particles from the same snow catcher sample to adjust the oxygen concentration to 3, 6, 12.5, 25, 50, 75 and 100% saturation. These oxygen adjusted samples were placed into 2 ml chambers for respiration measurements. The material in the base section of the MSC from these stations was insufficient to set up oxygen manipulation experiments with fast-sinking particles. Instead, material was collected with Pelagra traps at 100 m (Salter et al., 2007). The material was gently stirred to homogenise the sample and a set volume (250 µl) added to the respiration chambers and serum bottles. To achieve the desired oxygen concentrations, sterile filtered seawater at different oxygen concentrations was added to each respiration chamber rather than directly deoxygenating the Pelagra material to prevent break-up of the fast-sinking particles. The high oxygen levels in the fast-sinking particles samples resulted in initial oxygen concentrations ranging between 45 µM and 235 µM O_2 and only a few incubations started below 50 µM O_2. Quantile regression was used to estimate the median rate of change in turnover in relation to the starting oxygen concentration within the incubations, with the quantile set to the conditional median = 0.5 ($p < 0.001$). The relationship was used to estimate *in situ* rates of oxygen consumption using the CTD profile (Fig. 1).

2.9. Total microbial cell counts

Water samples (50 ml–100 ml) were collected from different particle fractions and immediately preserved in paraformaldehyde (2% final concentration) at 4 °C. After 8 h–12 h, water samples were filtered onto polycarbonate membrane filters. The Scotia Sea samples were sequentially filtered first through a 3 µm-pore-size filter to collect for particle-associated microbes, then through a 0.2 µm-pore-size filter to collect for free-living microbes. For the Benguela Current samples, the whole water samples were filtered through 0.2 µm-pore-size membranes. These filters were then air-dried and stored at −80 °C. In a shore-based laboratory, a filter section was cut out and stained with nucleic-acid-binding dye 4′6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980), and mounted on a microscope slide. Cell counting was conducted with a Zeiss Axioplan 2 Fluorescence Microscope at the Imaging and Microscopy Centre (University of Southampton). At least 20 fields were counted for each filter sample, and cell counts were converted to cell abundance by normalising to the volume of water filtered.

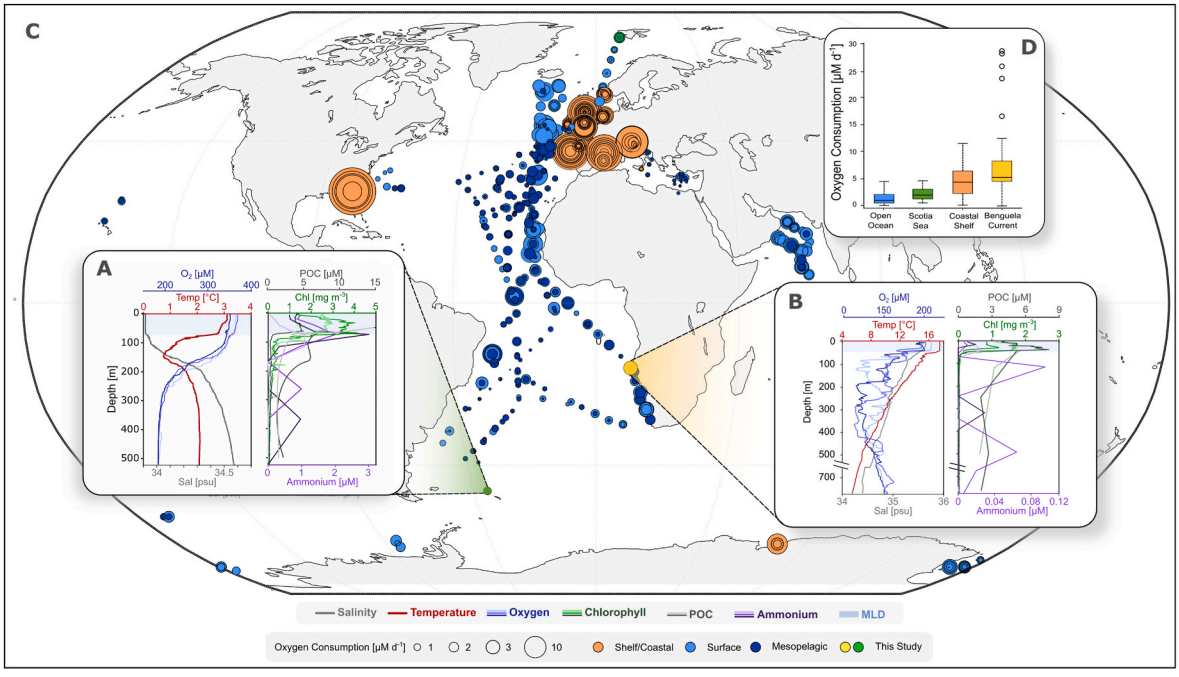

Fig. 1. Global observations of rates of oxygen consumption (µM O_2 d^{-1}) in contrasting regions of the ocean. Scotia Sea (A): Temperature (red), salinity (grey) and oxygen (light to dark blue over time) remained temporally stable, whereas the chlorophyll *a* peak gradually declined and deepened from the surface to the base of the mixed layer (light to dark green) during the 1-month period of our expedition (November to December 2017). While total POC concentrations remained comparatively stable below the mixed layer (light to dark grey), ammonia, a product of remineralisation of the decaying bloom, showed gradually deepening subsurface peaks over time (light to dark purple). **Benguela Current (B):** Temperature and salinity remained stable throughout the expedition (May to June 2018), while oxygen, chlorophyll *a* and ammonia varied temporally. These variations were, however, not apparent in the vertical POC profiles. Oxygen consumption at both sites is comparable to previous observations in contrasting ocean waters (database maintained by Carol Robinson) (Robinson and Williams, 2005) **(C** - size and colour of circle on bottom scale-bar**)**, with the rates observed in the Scotia Sea being within the range of previous open ocean measurements, and the rates in the Benguela Current comparable to previous measurements in coastal and shelf waters **(C & D).** Upper and lower boundaries for the boxplots in **D** give the 75th and 25th percentiles, respectively, bars are the medians and whiskers the minima and maxima. No data in C have been obscured by subpanels A, B and D.

2.10. Modelling suspended POC inventory

To determine if the mesopelagic suspended POC pool could be maintained by the flux of POC from the surface, while being turned over as per our measurements, we used a non-steady state model with suspended POC as the output to compare to our observations.

The fast-sinking particle export was calculated over depth and time to estimate an input term for the suspended POC from fragmentation. First, daily chlorophyll *a*, temperature, and photosynthetically available radiation (PAR) data were extracted from MODIS aqua for our regions of interest for three months prior to and during each expedition. The data were run through a Vertically Generalised Production Model (VGPM) to calculate primary production (mg C m^{-2} d^{-1}) (Behrenfeld and Falkowski, 1997).

We used export efficiencies from the literature to calculate the flux from the euphotic to the mesopelagic zone and compared them to the observed POC below the mixed layer depth to check for consistency (Student t-test, scaled mean modelled POC over the cruise time period to mean POC from the MSC mmol m^{-3}). We used efficiencies of 0.3 and 0.2 for the export efficiencies for the Scotia Sea and Benguela Current, respectively, which are within literature values and match well with the measured POC concentrations below the mixed layer (SI Fig. 6C and D) (Buesseler and Boyd, 2009; Henson et al., 2012; Siegel et al., 2014). These efficiencies were then used for the whole three-month period to estimate the flux below 100 m from the calculated rates of primary production. Mean particle sinking speeds of 50 ± 4.3 m d^{-1} derived from observations were used as described in "*In situ* sinking velocity of fast-settling particles".

We used equations from Kriest and Oschilies (Kriest and Oschlies, 2008), which were based on the equations from Martin (Martin et al., 1987), assuming a constant sinking speed (*w,* 50 m d^{-1}) with depth (*z,* m) and using the estimated export flux, the fast-sinking POC concentration (M_f, mmol m^{-3} d^{-1}) was calculated over time (*t,* d)

$$\frac{\delta M_f}{\delta t} = w\frac{M_f}{\delta z} - rM_f - fragM_f \quad \text{Equation 4}$$

where *w* is the observed mean sinking velocity (50 m d^{-1}). As the data for carbon specific turnover (*r)* were not normally distributed, the median value was used from our observations (0.03 d^{-1} and 0.02 d^{-1}, for the Scotia Sea and Benguela Current, respectively. See SI Table 4). We included fragmentation from the fast-sinking fraction (*frag*) as a term at 0.1% per metre, based on the change in the observed fast-sinking volume with depth (Model 1). The fast-sinking volume was calculated from photos taken under a microscope and measured for length and width with the ImageJ software and using the equation of a cylinder for faecal pellets (Supplementary Fig. 1).

Suspended POC (M_s) was calculated over time as above, without the sinking rate and with inputs from fragmentation of fast-sinking particles, measured carbon turnover by respiration on suspended particles (*rMs*, See Supplementary Table 4) and estimates of carbon fixation ($C_{fix,}$ d^{-1}) with equations (6) and (7), below.

$$\frac{\delta M_s}{\delta t} = \frac{M_s}{\delta z} - rM_s + fragM_f + C_{fix}M_s \quad \text{Equation 5}$$

Where equation (6) recycles 1.5% of mineralised CO_2 back into the suspended carbon pool as CH_2O i.e.,1.5% fixed as new nitrifier biomass (Zhang et al., 2020) or, equation (7), with 9% of mineralised CO_2 fixed into new nitrifier biomass (Meador et al., 2020). In equation (6), the O_2: C ratio is 1:1.4 and in equation (7) and (1):1.3:

$$C_{106}H_{175}O_{42}N_{16}P + 148.4O_2 \rightarrow 104.4CO_2 + 1.6CH_2O + 16HNO_3 + H_3PO_4 + 76.4H_2O \quad \text{Equation 6}$$

$$C_{106}H_{175}O_{42}N_{16}P + 140.5O_2 \rightarrow 96.5CO_2 + 9.5CH_2O + 16HNO_3 + H_3PO_4 + 68.5H_2O \quad \text{Equation 7}$$

Additionally, for the oxygen-depleted Benguela Current waters, a profile for suspended carbon turnover was calculated over depth using a mean oxygen concentration profile over the cruise period and calculating the turnover using the relationship determined in the oxygen manipulation experiments (Fig. 4). The model was first run to steady state using a constant input flux and was then run with a variable temporal flux based on satellite observations. Results from the model were compared to the observed suspended POC profile during the time of sampling (Normalised Root Mean Square Error) and see Supplementary Table 4 for model run scenarios.

3. Results and discussion

3.1. Hydrochemical characteristics

Although the cold (1–3 °C), oxygen-rich (60–110% O_2 saturation) Scotia Sea represents a distinctly different ocean biome compared to the warm (6–16 °C), oxygen-poor (20–86% O_2 saturation) Benguela Current (Fig. 1A and B, respectively), internal POC inventories were comparable and temporally stable over our approximately 1-month-long expeditions to each of the two sites (Fig. 1A and B), despite the higher levels of chlorophyll *a* and POC in the surface waters of the Scotia Sea (Fig. 1A). Suspended POC (here defined as particles that did not sink to the base of the MSC within 2 h, often considered to have sinking velocities <10 m d^{-1}) (Riley et al., 2012) accounted for the majority of POC (98%, 1 ± 0.02 mmol m^{-2} integrated to 500 m), while fast-sinking particles (sinking velocities of >25 m d^{-1}) only accounted for 2% of the total POC (0.15 ± 0.03 μmol m^{-2}, integrated to 500 m) on average (Fig. 2A and B). The ammonia profile varied substantially with depth as well as with time at both sites (Fig. 1A and B), often accumulating at or just below the mixed layer depth (up to 3 μM and 0.04 μM in the Scotia Sea and Benguela Current, respectively) and additional maxima within the mesopelagic zone (up to 1 μM and 0.1 μM), indicating temporal decoupling between ammonia production and consumption.

3.2. Suspended organic matter sustains high oxygen consumption rates

In comparison to a global ocean database containing >3000 measurements of 'overall' microbial community oxygen consumption rates (Robinson and Williams, 2005) (Fig. 1C, SI Table 1A,B), the mean overall microbial oxygen consumption in the Epi- and Mesopelagic (45 m–700 m) of the Scotia Sea (2 ± 0.2 μM O_2 d^{-1})is similar to previous observations from open ocean waters (1.4 ± 0.04 μM O_2 d^{-1}) (Fig. 1D and SI Tables 1A and 2). The mean oxygen consumption rate in the Benguela Current (45 m–750 m) (7.3 ± 0.8 μM O_2 d^{-1}), in contrast, is comparable to shelf and coastal waters (4.5 ± 0.22 μM O_2 d^{-1}) (Fig. 1D and SI Tables 1B and 2) and the high average respiration rates at both sites are strongly driven by the epipelagic (<100 m). Nevertheless, the measured cell-specific respiration rates associated with non-sinking OM (mean 9.0 ± 1.2 and 4.3 ± 1.7 fmol O_2 $cell^{-1}$ d^{-1} for the Scotia Sea and Benguela Current, respectively) (SI Table 3) are comparable to previous reports (1–5 fmol O_2 $cell^{-1}$ d^{-1}) on mesopelagic community respiration (Reinthaler et al., 2010; Baltar et al., 2009). The geographical distribution of publicly available measurements of community oxygen consumption in the global ocean database is biased toward the Atlantic Ocean and a considerable fraction of observations are located within the highly oligotrophic South and North Atlantic Ocean Gyres (~20% of all open ocean measurements) (Fig. 1C), which represent the lower end of primary productivity and heterotrophic microbial activity (Serret et al., 2015). The Benguela Current, however, drives one of the most productive marine ecosystems (Shannon and Nelson, 1996; Carr, 2001) and previous measurements of oxygen consumption in surface waters and the mesopelagic ocean from such productive waters often exceed 3 μM O_2 d^{-1} (Fig. 1C).

To estimate *in situ* respiration rates (e.g. nM C d^{-1}) associated with the different organic matter pools, i. e. fast-sinking and suspended

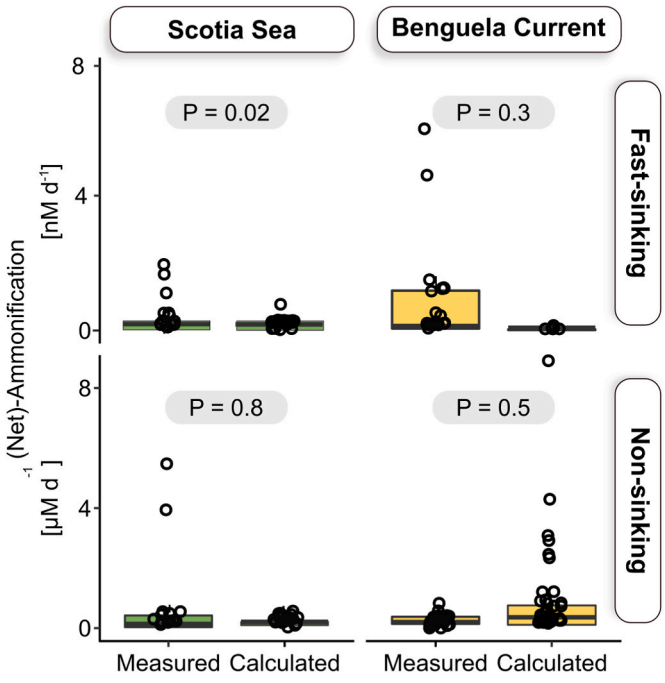

Fig. 2. Comparison of measured against stoichiometrically calculated net-ammonification rates. Measured net-ammonification rates in fast-sinking particles were highly variable but only differed significantly from those calculated based on measured rates of respiration and concurrent C:N ratios in the fast-sinking pool of the Scotia Sea (Equation (1), Paired *t*-test, $P = 0.02$, $n = 7$). In the Benguela Current, the difference between calculated and measured net-ammonification rates was not significant in the fast-sinking pool (Paired *t*-test, $P = 0.3$, $n = 7$). The rates measured in non-sinking organic matter were less variable and not significantly different to the stoichiometrically calculated rates neither in the Scotia Sea (Paired *t*-test, $P = 0.8$, $n = 8$) nor the Benguela Current (Paired *t*-test, $P = 0.5$, $n = 8$). The net rates of ammonification in both regions were significantly higher in the non-sinking fraction (note the different nM and µM units on the respective y-axes). The black central line indicates the median, and the bottom and top edges of each box indicate the 25th and 75th percentiles, respectively.

particles and DOM, we applied several corrections and conversions to our oxygen-consumption data: 1) To calculate microbial respiration in fast-sinking particles, we subtracted the fraction associated with the dissolved/suspended pool and corrected for the enrichment of fast-sinking POC within each incubation chamber relative to their ambient concentration in the ocean (see Material and Methods and Supplementary Discussion). (2) We corrected all rates of microbial oxygen consumption to *in situ* temperature and oxygen concentration (see Material and Methods); (3) We used a novel linear regression approach to estimate oxygen consumption driven by POC in contrast to the fraction attributed to dissolved organic carbon (DOC) (see below). We validated this strategy by modelling the changes in apparent oxygen utilisation (AOU) driven by DOC in the Benguela Current (see Supplementary Discussion). Although each of these steps has its own short-comings, they allow us to discern the relative contribution of the different organic matter pools to microbial 'carbon' respiration through independent measures of net-ammonification and oxygen consumption.

3.3. Net-ammonification serves as an additional proxy for microbial respiration

Net ammonification rates (taken as net production of ammonia) were used as an additional proxy for organic matter remineralisation and showed similar patterns to the oxygen-based estimates of remineralisation (Supplementary Figs. 2A and B). The use of 'net' (as opposed to 'gross') ammonification rate is confounded by oxidation of ammonia via nitrification and its assimilation, and so only positive rates (i.e., net production of ammonia) are used here to estimate remineralisation (61% of all measurements). Nevertheless, apart from fast-sinking particles in the Scotia Sea, the measured net-ammonification rates did not differ significantly from those calculated stoichiometrically from microbial respiration and the carbon to nitrogen ratio of POM (Fig. 2 and Equation (1)). Similar to the oxygen-based estimates, net ammonification was two orders of magnitude lower in fast-sinking particles at 6.4 ± 1.5 nM d^{-1} and 2.2 ± 0.6 nM d^{-1}, in the Scotia Sea and the Benguela Current, respectively, compared to suspended organic matter at 743 ± 296 nM d^{-1} in the Scotia Sea and 270 ± 33 nM d^{-1} in the Benguela Current, on average (Supplementary Fig. 2B and Supplementary Table 2). While net-ammonification can only serve as an indirect proxy for microbial respiration, the technical requirements for its determination are minimal and the measurement is rapid and accurate even at low ammonia concentrations (~2 nM) (Holmes et al., 1999). The comparison with direct measurements of oxygen consumption demonstrates the validity of this proxy and provides an additional line of evidence for substantial contribution of suspended particles in fuelling heterotrophic microbial activity.

3.4. Oxygen and temperature represent major controls over microbial respiration

The temperature and oxygen concentrations differed dramatically between the Scotia Sea and Benguela Current (Fig. 1). To determine the effects of these parameters on respiration, we undertook separate temperature (5 °C–18 °C, Fig. 3) and oxygen (9 µM O_2 to 240 µM O_2, Fig. 4) manipulation experiments with particles from 100 m depth in the Benguela Current. Rates of oxygen consumption were measured over time by varying the temperature and, in separate incubation experiments, varying the initial oxygen concentration (see Materials and Methods). Plotting the natural log of the turnover rates (ln d^{-1}) for both fast-sinking and suspended particles against absolute incubation temperature revealed an apparent activation energy for microbial respiration of 0.6 eV, which is in line with a comprehensive meta-analysis of respiration in relation to temperature in many biomes, including the ocean (Yvon-Durocher et al., 2012) (Fig. 3B). Further, by extending the regression analysis to include the C to N ratio of both particle fractions we could see how lower-quality organic matter (i.e., high C to N) acted counter to the stimulatory effect of the highest temperatures (Fig. 3C).

Manipulating the initial oxygen concentration revealed a significant linear decrease in turnover rate for the suspended particles (Quantile Regression, p = 0.01) (Fig. 4B) but not for the fast-sinking (Fig. 4A). Though the fast-sinking suggested some influence by oxygen i.e., a positive slope (0.00010 d^{-1} µM O_2^{-1}, 95% CI: −0.00011 to 0.00129) we had difficulty manipulating oxygen below 50 µM without breaking up the particles. While we encountered oxygen concentrations as low as 20 µM at mid-water depths in the Benguela Current, likely the signature of horizontal advection of oxygen depleted water from the coastal OMZ (Hutchings et al., 2009), on average, oxygen declined from 146 µM at 100 m to 85 µM at 400 m. Assuming a constant microbial sensitivity to oxygen at these depths, such a decline in oxygen could decrease turnover by 1.7-fold. In comparison, water temperatures declined from 16 °C to 11 °C over the same depth interval, on average, which could further decrease turnover by a comparable 1.5-fold. In contrast, mineralisation of POC would be strongly governed by concentration and organic matter quality in the mesopelagic of the Scotia Sea owing to the relatively constant oxygen and temperature regimes. As the lowest oxygen concentration of 20 µM in the Benguela Current could potentially decrease mineralisation by 7-fold, the net effect of the projected ongoing deoxygenation of the ocean through global warming, and expansion of oxygen minimum zones (Stramma et al., 2008; Schmidtko et al., 2017), will likely increase the efficiency of the biological carbon pump in low oxygen waters.

Visible fast-sinking particles (FS) were scarce at both sites and were

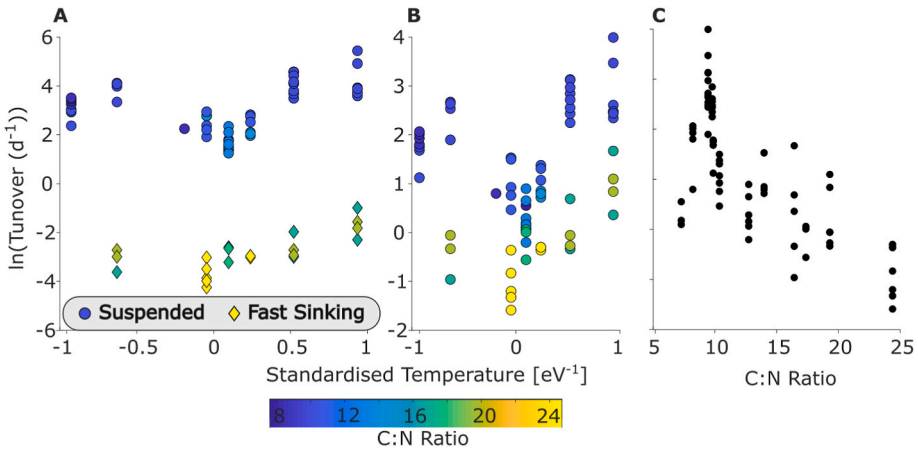

Fig. 3. Analysis of the temperature manipulation experiments conducted in the Benguela Current. A linear mixed effects model was applied to the natural log of turnover (d^{-1}) against standardised and centred temperature [$(1/K_bT_c - 1/K_bT)$ eV^{-1}] **(A)**, where K_b is the Boltzmann constant and T_c is the mean temperature in Kelvin (285 K) and T is the temperature of each individual incubation, in Kelvin. The influence of fraction (suspended or fast-sinking) on the intercept was removed from the data **(B)** and linear regression applied to the natural log of carbon turnover against absolute temperature to estimate the apparent activation energy of 0.6 eV. Once the effects of particle fraction and temperature were removed, a significant inverse relationship between the C:N ratio and turnover of carbon was revealed **(C)**.

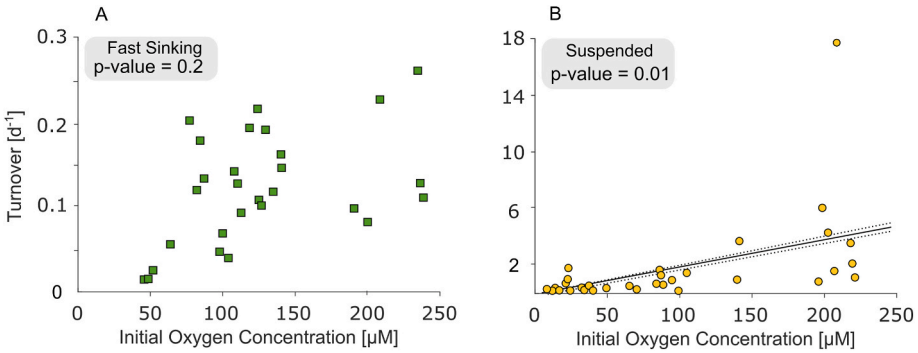

Fig. 4. Carbon specific respiration as a function of manipulated oxygen for fast-sinking and suspended particles taken from 100 m depth in the Benguela Current. Quantile regression was used to determine the median rate of decline in turnover with the starting oxygen concentration within the fast-sinking (A) and suspended (B) particle incubations. Each point shows a single chamber measurement. The relationship for the fast-sinking particles was not significant ($p = 0.2$, Panel A), but there was a significant effect of oxygen for turnover of the suspended particles ($p = 0.01$, Panel B), $y = 0.008x$. The intercept for the suspended particles was not significantly different to zero. The black line is the median regression fit to the data with the quantile set to the conditional median = 0.5.

dominated by small faecal pellets (0.3–1.2 mm) (Supplementary Fig. 1). Microbial respiration associated with this organic matter pool was 0.9 ± 0.12 nM C d^{-1} in the Scotia Sea and 0.8 ± 0.09 nM C d^{-1} in the Benguela Current (Supplementary Fig. 2A and SI Table 2). Microorganisms associated with suspended organic matter consumed 1.12 ± 0.1 µM C d^{-1} and 6.4 ± 1.3 µM C d^{-1}, on average, in the Scotia Sea and Benguela Current, respectively, exceeding respiration in fast-sinking particles by 4 orders of magnitude (Supplementary Fig. 2A and Supplementary Table 2). Despite the regional variability in microbial respiration rates associated with suspended particles, our oxygen-based measurements of microbial respiration, and the ammonification proxy of organic matter remineralisation, demonstrate that heterotrophic microbial activity on fast-sinking particles accounts for a negligible fraction of mesopelagic microbial respiration. In both contrasting parts of the ocean, suspended particles fuel the majority of microbial respiration.

3.5. The relative contribution of DOM in sustaining microbial oxygen consumption is higher in Scotia Sea compared to the Benguela Current

There are currently no methods available to directly measure respiration rates of DOM and suspended particulate organic matter (POM) separately, as separation of these two organic matter pools is typically achieved by filtration through 0.2 to 0.7 µm-pore-size filters that would also remove most free-living microorganisms. Ultimately, microbial communities rely on the uptake of DOM as their source of energy and nutrients. However, labile DOM released by phytoplankton in the surface ocean, as a by-product of feeding by larger organisms and the activity of microbial communities is rapidly consumed and largely escapes our measurements. Instead, the high concentrations of DOC observed throughout the water column (~40 µM–80 µM) represent biological recalcitrant DOM with lifetimes of months to millennia (Hansell et al., 2009; Hansell, 2013). To indirectly estimate the fraction of microbial respiration supported by DOM we used a linear regression model to characterise the fraction of oxygen consumption within the incubations that could be explained by POC concentration (see Supplementary Information for more details). The relationship for fast-sinking particles showed an increase with POC concentration to a plateau, with no offset from the zero intercept, indicating that the signal of oxygen consumption for fast-sinking particles swamped any other particle fraction due to the high concentration of fast-sinking particles in the incubation chambers (Fig. 5A). On the other hand, there was a significant offset from the zero intercept for suspended particles (Fig. 5B). We therefore calculated the mean difference in the rate of oxygen consumption between the measured slope and the slope where the intercept was set to

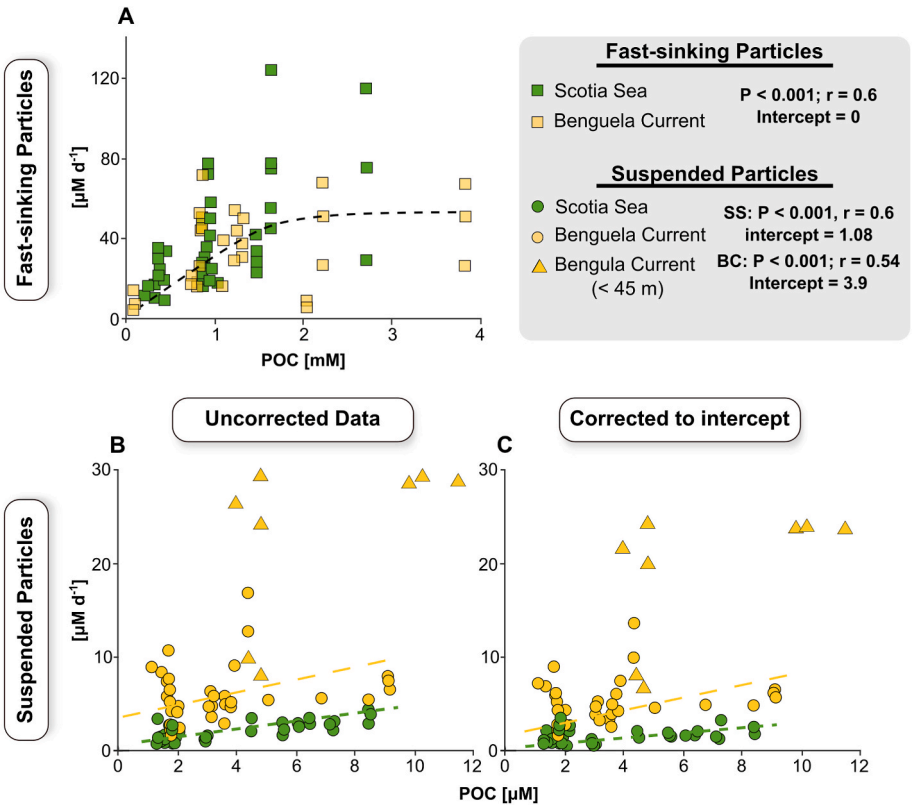

Fig. 5. Correlation of microbial respiration with POC concentration in the incubation chambers: Rates of microbial respiration, for each separate chamber for all deployments, in fast-sinking and suspended particles are correlated with concentrations of POC in each respective particle fraction in both the Scotia Sea (SS) and Benguela Current (BC) (**A** & **B**, $n = 18$ & 20, respectively; Spearman Correlation). The intercept in **B** was used as an estimate for oxygen consumption due to other processes e.g., respiration of DOC within the incubation and not associated directly with POC. This effect was removed from the data to give an estimate of oxygen consumption only associated with the suspended particle fraction and which is shown in panel **C**. Note, the very high rates at 45 m in the Benguela Current (triangles) were omitted from this analysis.

zero suspended POC. We inferred that the offset represented other oxygen sinks, such as microbial respiration driven by DOC and nitrification. Consequently, subtracting this offset resulted in a reduction in oxygen consumption attributed to suspended particles, on average, by 45% in the Scotia Sea and 13% in the Benguela Current, giving an average signal of DOC related respiration of 0.88 μM C d^{-1} and 0.95 μM C d^{-1} for the Scotia Sea and Benguela Current, respectively. This assumes that the relationship is constant with depth and time, although samples from 45 m in the surface mixed layer in the Benguela Current were clearly outside of the relationship (Fig. 5). We validated this approach in the Benguela Current, where parallel measurements of DOC concentrations were available, with a commonly used linear regression model describing changes in measured DOC concentration by apparent oxygen utilisation (AOU) to estimate the relative contribution of DOC to community respiration (Aristegui, 2002) (Supplementary Fig. 3). This method estimated that DOC fueled 8% ± 3% of oxygen consumption in the upper 1500 m in the Benguela Current (Supplementary Fig. 3), comparable to 13%, estimated by the "POC intercept" method. Our estimates are within the range of previous reports on the relative contribution of DOC to microbial oxygen consumption, that range between ~10 and >50% (Aristegui, 2002; Santana-Falcón et al., 2017; Calleja et al., 2019; Pan et al., 2014). The dark ocean holds a DOC pool approximating the size of the atmospheric carbon pool (Hansell, 2013) and the mechanisms behind the production and utilisation of this apparently stable organic carbon pool are still debated (Shen and Benner, 2020, 2022; Lennartz and Dittmar, 2022). The relatively high contribution of DOC to microbial respiration in the Scotia Sea could be fueled by the inflow of Weddell Sea Water (Locarnini et al., 1993; Meredith et al., 2008) and a potential priming effect of DOM degradation by labile organic matter released from sinking particles in mesopelagic depths (Farjalla et al., 2009; Guenet et al., 2010; Shen and Benner, 2018). In contrast, in the Benguela Current lateral advection of particles may have fueled the high particle associated respiration rates we observed (Bauer and Druffel, 1998; Shen et al., 2020).

3.6. *The turnover of suspended particles exceeds that of fast-sinking particles several fold*

We used our estimates of carbon respiration (Supplementary Fig. 2A) and net ammonification (Supplementary Fig. 2B) to calculate carbon- and nitrogen-specific turnover times for POC (Supplementary Fig. 2C) and PON (Supplementary Fig. 2D), respectively, in the suspended and fast-sinking particle fractions. Based on microbial respiration alone, mean turnover times for carbon in fast-sinking particles would be 33 days in both the Scotia Sea and Benguela Current (0.03 ± 0.003 d^{-1} and 0.03 ± 0.004 d^{-1}). Nitrogen specific turnover times in fast-sinking particles were substantially shorter at 10 and 13 days (0.1 ± 0.04 d^{-1} and 0.08 ± 0.02 d^{-1}) for the Scotia Sea and the Benguela Current, respectively, indicating preferential remineralisation of nitrogen-rich organic matter in these particles (Thomas et al., 1999; Schneider et al., 2003) (Supplementary Figs. 2C and D and SI Table 2). This is consistent with the significant relationship between lower C:N, thus higher nitrogen content, and faster turnover of fast-sinking particles (Fig. 6C). Our estimates of microbial carbon specific turnover are within the range of previous measurements in marine snow (0.08 d^{-1} to 0.2 d^{-1}) (Ploug et al., 1999; Iversen and Ploug, 2010; Belcher et al., 2016a; Cavan et al., 2017), and consistent with the microbial carbon turnover measured previously in faecal pellets from the Scotia Sea (0.01 d^{-1} to

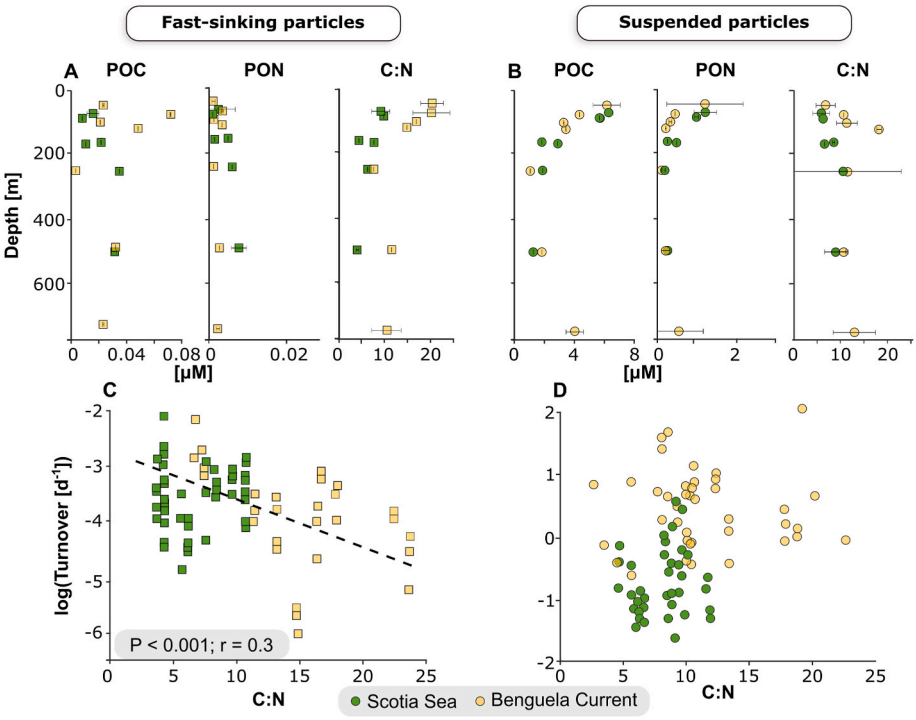

Fig. 6. Correlation of carbon specific turnover with the C:N of POM. POC and PON concentrations were an order of magnitude lower in the fast-sinking particle fraction (**A,** squares) compared with the suspended organic matter pool (**B,** circles). The range in POC at specirfic depths was generally quite low, where the error bars show the standard deviation of POC measurements taken at that specific depth (n was variable but a minimum of 3). The C:N ratio, which can serve as an indicator of the lability of organic matter, decreased with depth in fast-sinking particles but remained stable or increased slightly within the suspended organic matter fraction (**A** and **B**), thus indicating that the two types of POM are compositionally different. Significant correlation between rates of carbon specific turnover (natural log) and the C:N ratio of fast-sinking particles (**C**) indicated preferential remineralisation of nitrogen rich organic matter. We could find no significant correlation between turnover and C:N in the suspended particles, but, for the same C:N ratio, turnover in the Scotia Sea (SS) was lower compared to the Benguela Current (**D**).

0.06 d^{-1}) (Belcher et al., 2016b; Iversen et al., 2017).

Despite the suspended particle pool containing an order of magnitude more carbon and nitrogen than the fast-sinking fraction (Fig. 6A and B), turnover times were at least an order of magnitude more rapid in both regions. Carbon specific turnover times were 2.3 and 0.6 days (i.e., turnover rates of 0.43 ± 0.05 d^{-1} and 1.7 ± 0.3 d^{-1}) for the Scotia Sea and Benguela Current, respectively, while those for nitrogen were comparable at 1.6 and 0.6 days (i.e., turnover rates of 0.6 ± 0.1 d^{-1} and 1.8 ± 0.4 d^{-1}, respectively (Supplementary Fig. 2C). This observation is in line with a previous report from the Eastern Tropical North Pacific, where carbon turnover of slow-sinking particles exceeded that of fast-sinking particles by 50-fold (Cavan et al., 2017), and confirms the previously anticipated key role of suspended organic matter in sustaining microbial activity in mesopelagic waters (Karl et al., 1988; Baltar et al., 2009; Baltar et al., 2010 Giering et al., 2014). The high turnover rates of suspended organic matter in the Benguela Current are likely in part sustained through lateral transport, as observed widely elsewhere (Bauer and Druffel, 1998; Alonso-González et al., 2009; Shen et al., 2020) of organic matter from the highly productive eastern boundary upwelling system over the Southern Atlantic shelf (Kämpf and Chapman, 2016). Similar to fast-sinking particles, high organic turnover within suspended particles was linked to the lability of organic matter or higher nitrogen content in the Benguela Current at 100 m, as indicated by the significant negative relationship between turnover and C:N ratio (Fig. 3C), however this relationship was only apparent when turnover was analysed together for both fractions with temperature and C:N ratio (Fig. 3C vs Fig. 6D). Unlike the fast-sinking particles, for which POC turnover followed the same log-linear relationship with C:N at both sites, turnover times of suspended POC in the Benguela Current were higher compared to the Scotia Sea for the same C:N ratio (Fig. 6D). This is despite the comparable sizes of the suspended carbon pool in both regions (3.3 ± 2.2 μM and 3.6 ± 2.6 μM, SS and BC respectively), yet generally lower C:N ratios, thus indicating higher-quality organic matter in the Scotia Sea.

Correcting all the rates to the same temperature showed that the higher water temperatures in the Benguela Current can explain 53% of the higher microbial respiration rates of suspended organic matter compared to the colder Scotia Sea (Brown et al., 2004) (Supplementary Fig. 4). The higher microbial abundances in the Benguela Current relative to the Scotia Sea provides an additional explanation for the faster turnover of suspended organic matter in the Benguela Current (1.9×10^6 and 2.0×10^5 cells ml^{-1}, respectively, SI Table 3). However, these differences are not reflected in the pool of fast-sinking particles. Instead, rates in the concentrated fast-sinking particles within the incubations showed a nearly universal relationship with POC concentration that is consistent with previous studies (Iversen and Ploug, 2010; Belcher et al., 2016a,b; Cavan et al., 2017); although our data showed a plateau at 2 mM POC concentration (Fig. 5A) i.e., many-fold higher than the *in situ* POC concentration for either particle fraction.

Based on a mean sinking velocity of 50 ± 4.3 m d^{-1}, measured with an *in situ* imaging system at both sites (see Materials and Methods) (Iversen and Lampitt, 2020), and the median turnover of organic carbon and nitrogen, the fast-sinking particles appeared to largely escape microbial remineralisation to CO_2 in both parts of the ocean, with only 25% and 28% of carbon being respired by microorganisms in the upper 500 m of the Scotia Sea and the Benguela Current, respectively. In contrast, fitting a traditional power function to POC and depth (Martin et al., 1987) would predict an ~75% decrease in POC by 500 m. Our findings demonstrate that, among the various processes that impact particle flux attenuation: zooplankton consumption and fragmentation and microbial enzymatic dissolution to DOM (Lampitt et al., 1993; Smith et al., 1992; Steinberg et al., 2008), microbial respiration on

fast-sinking particles only plays a minor role in their attenuation in the mesopelagic zone. Instead, suspended particles are the hotspots of microbial activity.

3.7. The suspended organic matter pool plays a central role in the mesopelagic carbon cycle

Despite the rapid microbial turnover of suspended organic matter, its concentration remained temporally stable in the mesopelagic zone of both the Scotia Sea and the Benguela Current and was similar between the two sites (overall mean of both regions: 1.8 ± 0.23 μM between 50 m and 500 m), suggesting constant replenishment of this carbon pool (Fig. 6B). This has been described previously by Baltar et al. (2010), who found that 4–12% of sinking POC could support prokarotic demand. We used a simple one-dimensional model to explore whether the fragmentation of sinking particles (Dilling and Alldredge, 2000; Goldthwait et al., 2004) nitrification, and other chemolithoautotrophic processes that replenish the POM pool *in situ* (Reinthaler et al., 2010; Herndl and Reinthaler, 2013; Baltar and Herndl, 2019; Swan et al., 2011) could sustain the rapid turnover by modelling the integrated suspended POC concentration (50 m–500 m in the Scotia Sea, 50 m–750 m in the Benguela Current) (Kriest and Oschlies, 2008). To account for the variable particle flux over time we estimated export production using satellite observations of primary production over a period of 90 days both prior to and during our expeditions (For more details see Supplementary Information). The non-steady state model accounted for 62% and 15% of the observed integrated suspended carbon pool in the Scotia Sea and Benguela Current, respectively (Supplementary Table 4, Model 6). The model uses turnover compared to a fixed rate of mineralisation and, therefore, as the POC pool decreases the rate of microbial respiration also decreases.

Our model failed to fully reproduce the mesopelagic concentration of suspended POC in the Benguela Current, irrespective of the contribution of variable fluxes and dark carbon fixation. Although our expedition focused on an area 89 km–444 km offshore and did not include the highly productive upwelling region over the Namibian shelf (Carr, 2001), upwelling filaments may stretch out as far as 250 km into the open ocean (Shannon and Nelson, 1996) and horizontal advection of low-oxygen, POM-rich water may partly explain the comparably large suspended POC inventory (Supplementary Table 4) and its replenishment. The effect of low oxygen acting to preserve POC can be seen as peaks in POC in the Benguela Current (Fig. 7B).

Despite the limitations of our simple one-dimensional model, it could account for a good portion of the suspended POC in the Scotia Sea, and thus indicates that the carbon turnover rates, estimated here from direct measurements of microbial oxygen consumption and particulate organic carbon and nitrogen, provide valid insights into the relative importance of carbon cycling dynamics within the mesopelagic ocean. Our simulations also demonstrate the importance of POC sources other than vertical particle flux contributing to the suspended POC pool. Especially in dynamic and highly productive regions such as the Benguela Current, these likely include particle injection pumps (Steinberg et al., 2008; Bianchi et al., 2013; Omand et al., 2015; Waite et al., 2016; Boyd et al., 2019), *in situ* production of suspended particles from coagulation of DOM (Chin et al., 1998) and horizontal advection of oxygen depleted water originating on the shelf bringing "low-oxygen preserved" POC.

Our results are the first to directly demonstrate that the majority of organic matter mineralisation occurs on suspended, rather than fast-sinking particles such as marine snow aggregates or faecal pellets, confirming hypotheses postulated in several studies (Arístegui et al., 2020; McDonnell and Boyd, 2015; Belcher et al., 2016a; Baltar et al., 2009; Cavan et al., 2017). This is consistent with the previously observed niche differentiation of microbial communities between particle-types: fast-sinking particles are enriched in opportunistic, *r*-strategists originating from surface waters; whereas suspended particles harbour microbial taxa more versatile in exploiting diverse substrates, including complex compounds, especially in mesopelagic depths (Duret et al., 2019). It is therefore not surprising to find suspended particles supporting a larger and more active microbial community. Attenuation of fast-sinking particles should not be translated to CO_2 production directly, as it has also been reported that about half of the sinking particle attenuation is due to particle fragmentation (Briggs et al., 2020). Nor can the estimation of gravity-driven particle flux alone fully characterise the strength of the biological carbon pump. The total integrated effect of ocean biological processes on atmospheric CO_2 is affected by the fraction of upwelled nutrients used in production, which itself is driven by remineralisation depth. This study shows the importance of temperature, oxygen and organic matter quality on remineralisation rates in mesopelagic suspended carbon and moves our thinking forward from a simple consideration of fast-sinking particles as the sole primary substrate for remineralisation, which is integral to understanding biological controls over atmospheric CO_2 levels and planetary climate.

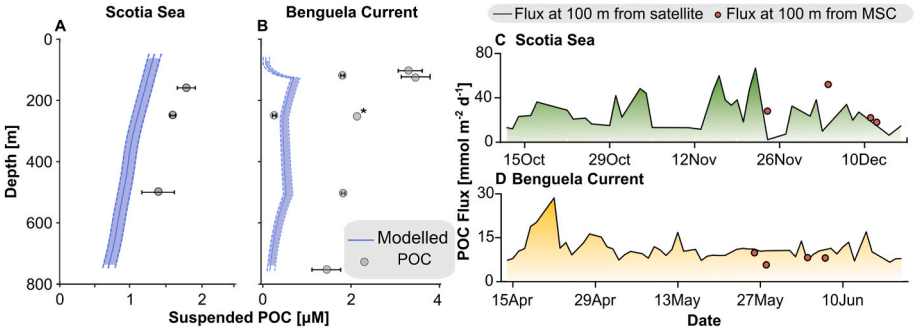

Fig. 7. Modelling POC concentration profiles: Our one-dimensional model is based on a temporally variable POC flux for 90 days both prior to and including the cruise period; a fragmentation rate of 0.1% m^{-1} of fast-sinking particles; and scenarios either with or without complete nitrification, based on aerobic remineralisation equations driving different rates of dark carbon fixation (Equations (6) and (7), Material and Methods). A summary of models 1–6 are found in SI Table 4. In Model 6 (blue), our model includes dark carbon fixation of 9%, which reduces net-carbon turnover and the modelled suspended POC increasingly reflects the observed mean profile (**A** & **B**, Model 6, blue). The solid blue lines represent the mean POC concentration and the dashed are the 95th percentiles over the period that the model is run. The modelled suspended POC concentration varies over time in accordance with the fluctuating POC flux (shaded areas coloured according to each model). The modelled POC flux at 100 m is based on satellite derived primary production estimates covering 90 days before and during our sampling periods and agrees well with the observed flux at 100 m (red dots) in the Scotia Sea (**C,** green) and the Benguela Current (**D,** yellow). The kinks in the modelled POC profile for the Benguela Current reflect the more dramatic changes in oxygen and temperature over the mesopelagic depths there relative to gentler changes in the Scotia Sea (Fig. 1).

CRediT authorship contribution statement

V. Hemsley: Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft. **J. Füssel:** Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft. **M.T. Duret:** Investigation, Writing – review & editing. **R. R. Rayne:** Investigation, Writing – review & editing. **M.H. Iversen:** Investigation, Writing – review & editing. **S.A. Henson:** Funding acquisition, Project administration, Formal analysis, Writing – review & editing. **R. Sanders:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **P. Lam:** Conceptualization, Funding acquisition, Supervision, Project administration, Data curation, Writing – review & editing. **M. Trimmer:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Funding acquisition, Supervision, Project administration, Data curation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr2.2023.105339.

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