https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

Biogeosciences Discussions

High bacterial organic carbon uptake in the Eastern Tropical South Pacific oxygen minimum zone

Marie Maßmig, Jan Lüdke, Gerd Krahmann, Anja Engel*

GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, D-24105 Kiel, Germany

Correspondence to: Anja Engel (aengel@geomar.de)

Abstract. Oxygen minimum zones (OMZs) show distinct biogeochemical processes that relate to microorganisms being able to thrive under low or even absent oxygen. Microbial degradation of organic matter is expected to be reduced in OMZs, although quantitative evidence is low. Here, we present heterotrophic bacterial production (^{3}H leucine-incorporation), extracellular enzyme rates (leucine aminopeptidase /ß-glucosidase) and bacterial cell abundance for various *in situ* oxygen concentrations in the water column of the Eastern Tropical South Pacific off Peru. Bacterial heterotrophic activity in the suboxic core of the OMZ (at *in situ* ≤5 µmol O_2 kg^{-1}) ranged from 0.6 to 160 µmol C m^{-3} d^{-1} and was not significantly lower than in waters of 5-60 µmol O_2 kg^{-1}. Moreover, bacterial abundance in the OMZ was slightly and leucine aminopeptidase activity even significantly higher in suboxic waters compared to the upper oxycline suggesting no impairment of bacterial organic matter degradation in the core of the OMZ. Nevertheless, high cell-specific bacterial production and extracellular enzyme rates were observed in samples from the upper or lower oxyclines corroborating earlier findings of highly active and distinct micro-aerobic bacterial communities. To assess the impact of bacterial degradation of dissolved organic matter for oxygen loss in the Peruvian OMZ, we compared diapycnal fluxes of oxygen and dissolved organic carbon (DOC) and their microbial uptake within the upper 60m of the water column. Our data indicate bacterial growth efficiencies of 0.5-8.6% at the upper oxycline, resulting in a high bacterial oxygen demand that can explain up to 33% of the observed average oxygen loss over depth. Our study therewith shows that microbial degradation of DOM has a considerable share in sustaining the OMZ off Peru.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

1. Introduction

In upwelling zones at eastern continental margins, oxygen minimum zones (OMZs) with hypoxic (<60 µmol O_2 kg^{-1}), suboxic (<5 µmol O_2 kg^{-1}) or even anoxic conditions occur (Gruber, 2011; Thamdrup et al., 2012). OMZs have expanded over the past years resulting in an ~3.7 % increase of hypoxic waters at depth (200 dbar) between 1960 and 2008 (Stramma et al., 2010). One of the largest anoxic water masses in the global ocean (2.4 x 10^{13} m^3) is located in the Eastern Tropical South Pacific and includes the Peruvian upwelling system (Kämpf and Chapman, 2016; Thamdrup et al., 2012). There, nutrient-rich water is upwelled and supports high rates of primary production and organic matter accumulation. Biological degradation of organic matter subsequently reduces oxygen below the surface mixed layer (Kämpf and Chapman, 2016). As a consequence, and supported by sluggish ventilation of water masses, a permanent OMZ forms between 100 and 500 m depth, with upper and lower boundaries, i.e. oxyclines, varying within seasonal and inter-annual cycles (Czeschel et al., 2011; Graco et al., 2017; Kämpf and Chapman, 2016).

Within OMZs, microbes apply anaerobic respiratory pathways that yield less metabolic energy compared to aerobic respiration. For instance, denitrification or dissimilatory nitrate reduction to ammonia (DNRA) result only in 99 %, or 64 % of the energy (kJ) per oxidized carbon atom that is produced by aerobic respiration (Lam and Kuypers, 2011). Meanwhile, bacteria are mainly responsible for the remineralization of organic matter into nutrients and carbon dioxide (CO_2) in the ocean (Azam et al., 1983). Thus, microbial activity and consequently organic matter remineralization in anoxic waters might be reduced, possibly explaining observations of enhanced vertical carbon export in OMZ regions (Devol and Hartnett, 2001; Roullier et al., 2014). As a consequence, expanding OMZs could result in increased CO_2 storage in the ocean.

During the degradation process, low molecular weight (LMW <1 kDa) organic compounds can directly be taken up by bacteria (Azam et al., 1983; Weiss et al., 1991). However, in the ocean, bioavailable organic matter is commonly in the form of particulate organic matter or high molecular weight (HMW) dissolved organic matter (Benner and Amon, 2015). To access this organic matter pool, bacteria produce extracellular, substrate specific enzymes that hydrolyse polymers into LMW units (Hoppe et al., 2002). Taken-up, organic matter is partly incorporated into bacterial biomass, or respired to CO_2, which may evade to the atmosphere (Azam et al., 1983). Rates of enzymatic organic matter hydrolysis or bacterial production are controlled by the environment, i.e. temperature and pH, but can be actively regulated e.g. in response to changing organic matter supply and quality (Boetius and Lochte, 1996; Grossart et al., 2006; Pantoja et al., 2009; Piontek et al., 2014). However, the effect of oxygen concentration, which dictates the respiratory pathway and thus energy gain, on bacterial production and the expression of extracellular enzymes in aquatic systems, is poorly understood and previous results are ambiguous. For instance, volumetric rates of protein hydrolysing enzymes (peptidases) did not differ between oxic and anoxic incubations of Baltic Sea water (Hoppe et al., 1990), but were significantly higher in oxic compared to anoxic waters in Cariaco waters (Taylor et al., 2009). Bacterial production was higher in anoxic lake waters (Cole and Pace, 1995), whereas in the Pacific waters off Chile bacterial production and dissolved organic matter decomposition rates did not change in relation to oxygen concentrations (Lee, 1992; Pantoja et al., 2009). Further measurements of microbial activity such as extracellular

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

enzyme rates, the initial step of organic matter degradation (Hoppe et al., 2002), and bacterial production in OMZs are necessary to unravel possible adaptation strategies of bacterial communities to suboxic and anoxic conditions as for instance high extracellular enzyme rates compensating a putative lower efficiency of anaerobic respiration and the subsequent biogeochemical effects. Especially, combined investigations of extracellular enzymatic rates, bacterial production (measured by ^{3}H leucine-incorporation) and carbon fluxes sampled at various *in situ* oxygen concentrations are still missing. These data, however, are crucial to inform ocean biogeochemical models that aim at quantification of CO_2 uptake and nitrogen loss processes in oxygen depleted areas.

We studied bacterial degradation of organic matter in the OMZ off Peru during an extensive sampling campaign in the Austral winter 2017. We determined rates of total and cell-specific bacterial production (^{3}H leucine-incorporation) as well as of leucine aminopeptidase (LAPase) and ß-glucosidase (GLUCase). We estimate bacterial utilisation of dissolved organic carbon (DOC) supplied by diapycnal transport into the OMZ and discuss the contribution of bacterial degradation activity to the formation and persistence of the OMZ off Peru.

2. Methods

2.1. Study site and CTD measurements

Samples were taken during the cruises M136 and M138 on the R/V METEOR off Peru in April and June 2017, respectively (Fig. 1). Seawater was sampled with 24 Niskin bottles (10 L) on a general oceanic rosette system. At each station, 5 to 11 depths were sampled between 3 and 800 m (supplementary Table 1). Oxygen concentrations, temperature and depth were measured with a Sea-Bird SBE 9-plus CTD System (Sea-Bird Electronics, Inc., USA). Oxygen concentrations at each depth were determined with a SBE 43 oxygen sensor, calibrated with Winkler titrations (Winkler, 1888), resulting in an overall accuracy of 1.5 µmol kg^{-1} oxygen. Chlorophyll *a* fluorescence was detected with a WETStar chlorophyll *a* sensor (WET Labs, USA) and converted to µg l^{-1} using factors given by the manufacturer (Wetlabs).

2.2. Dissolved organic carbon, total dissolved nitrogen, dissolved hydrolysable amino acids and dissolved high molecular weight carbohydrates

DOC and total dissolved nitrogen (TDN) samples were taken at all stations, whereas the further analysis of DOC data was limited to stations with compatible bacterial production data and turbulence measurements (stations G-T). For DOC and TDN 20 ml of seawater was sampled in replicates, whereas both replicates were only analysed in case of conspicuous data. Samples were filtered through a syringe filter (0.45 µm glass microfiber GD/X membrane, Whatman ™), rinsed with 50 ml sample, into a combusted glass ampoule (8 h, 500 °C). Before sealing the ampules, 20 µl of 30 % ultrapure hydrochloric acid were added. Samples were stored at 4 °C in the dark for 3 months until analyses. DOC and TDN were analysed using a TOC−VCSH with a TNM-1 detector (Shimadzu), applying a high-temperature catalytic oxidation method modified from Sugimura and Suzuki (1988). The instrument was calibrated with potassium hydrogen phthalate standard solutions (0 to 416.7 µmol C l^{-1}) (Merck 109017) and a potassium nitrate standard solution (0-57.1 µmol N l^{-1}) (Merck 105065). The instrument blank was examined with reference seawater standards (Hansell laboratory RSMAS University of Miami). The relative standard deviation (RSD)

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

between repeated measurements is <1.1 % and <3.6 % and the detection limit is 1 µmol l^{-1} and 2 µmol l^{-1} for DOC and TDN, respectively.

At each station replicate 6 ml and 20 ml sample for the analysis of dissolved amino acids (DHAA) and dissolved combined carbohydrates (DCHO) were filtered through rinsed Acrodisc® 0.45µm GHP membrane (Pall) and stored in combusted vials (8 h, 500 °C) at -20 °C, respectively. Replicates were only analysed, if the first sample analyses resulted conspicuous data. The following DHAA were analysed: Alanine, Arginine, Glycine, Leucine, Phenylalanine, Serine, Threonine, Tyrosine, Valine, Aspartic acid + Asparagine (co-eluted), Glutamine + Glutamic acid (co-eluted), γ-Aminobutyric acid and Isoleucine. DHAA samples were analysed with a high performance liquid chromatograph (1260 HPLC system, Aglient Technologies) using a C_{18} column (Phenomex Kinetex) after in line ortho-phthaldialdehyde derivatization with mercaptoethanol after Lindroth and Mopper (1979) and Dittmar et al. (2009) with slight modifications after Engel and Galgani (2016). DCHO samples were desalted by membrane dialysis (1kDa, Spectra Por) and analysed with a high performance anion exchange chromatography (HPAEC) (DIONEX ICS3000DC) after Engel and Händel (2011).

2.3. Diapycnal fluxes of oxygen and dissolved organic carbon

In this study, we calculated DOC and oxygen loss rates (mmol m^{-3} d^{-1}) from the changes in diapycnal fluxes over depth. Therefore, oxygen and DOC profiles were used (stations G-T), excluding the mixed layer, defined by temperature deviating ≤0.2°C from the maximum, but excluding at least the upper 10 m. The diapycnal flux (Φ_S) was calculated for each CTD profile (Fischer et al., 2013; Schafstall et al., 2010) assuming a constant gradient between two sampled depths for DOC and oxygen:

$$1. \quad \Phi_S = -K_\rho \nabla C_S$$

where ∇C_S is the gradient (mol m^{-4}). The diapycnal diffusivity of mass ($K\rho$) (m^2 s^{-1}) was assumed to be constant ($10^{-3}\ m^2 s^{-1}$), which is reasonable compared with turbulence measurements by a freefalling microstructure probe (see Fig. 5 and supplementary). DOC loss rates ($\nabla\Phi_{DOC}$; mmol m^{-3} d^{-1}) and oxygen loss rates ($\nabla\Phi_{DO;}$ mmol m^{-3} d^{-1}) were assumed to be equal to the negative vertical divergence of ΦS calculated from the mean diapycnal flux profile, implying all other physical supply processes to be negligible.

2.4.Bacterial abundance

Bacterial abundance was sampled in replicates at each station, whereas replicates were only analysed in exceptions. Abundance was determined by flow cytometry after Gasol and Del Giorgio (2000) from 1.6 ml sample, fixed with 0.75µl 25 % glutaraldehyde on board and stored at -80°C for maximal 3 month until analyses. Prior to analysis samples were thawed and 10 µL Flouresbrite® fluorescent beads (Polyscience, Inc.) and 10 µL Sybr Green (Invitrogen) (final concentration: 1x of the 1000x Sybr Green concentrate) were added to 400 µl sample. Cells were counted on a FACS Calibur (Becton Dickinson), calibrated with TruCount Beads ™ (BD) with a measurement error of 2 % RSD.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

2.5.Bacterial production, oxygen demand and growth efficiency

For bacterial production, the incorporation of radioactive labelled leucine (^{3}H) (specific activity 100 Ci mmol^{-1}, Biotrend) was measured (Kirchman et al., 1985; Smith and Azam, 1992) at all depths of stations G-T as replicates. For this, the radiotracer at a saturating final concentration of 20 nmol l^{-1} was added to 1.5 ml of sample and incubated for 3 hours in the dark at 13°C. Controls were poisoned with trichloracetic acid. Samples were measured with a liquid scintillation counter (Hidex 300 SL, TriathalerTM, FCI). Samples taken at *in situ* oxygen concentrations of < 5 µmol kg^{-1} were incubated under anoxic conditions by gentle bubbling with gas (0.13 % CO_2 in pure N_2). Samples from oxic waters were incubated with head space, without bubbling. All samples were shacked thoroughly in between, therefore the bubbling of just one treatment won't have any effect. ^{3}H-leucine uptake was converted to carbon units applying a conversion factor of 1.5 kg C mol^{-1} leucine (Simon and Azam, 1989). An analytical error of 5.2 % RSD was estimated with triplicate calibrations. Samples with a s.d. (standard deviation) > 30% between replicates were excluded.

The BOD is the amount of oxygen needed to fully oxygenize DOC that has been taken up and not transformed into biomass by bacterial production (BP). The BOD was calculated as the difference between the estimated bacterial DOC uptake and the bacterial production (BP) applying a respiratory quotient (*cf*) of 1 (Eq. (2)) (Del Giorgio and Cole, 1998).

$$2. \quad BOD = (DOC\ uptake - BP) \times cf$$

The DOC uptake was calculated with two different assumptions: i) the DOC uptake by bacteria equals the DOC loss rate or ii) the bacterial growth efficiency (BGE) follows the established temperature dependence, resulting in a BGE between 0.1 and 0.3 in the depth range of 10-60 m and an *in situ* temperature that varied between 14 and 19°C (Rivkin and Legendre, 2001) and can be used to estimate the bacterial DOC uptake (Eq. (3)).

$$3. \quad microbial\ DOC\ uptake = \frac{BP}{BGE}$$

2.6.Extracellular enzyme rates

Potential hydrolytic rates of LAPase and GLUCase were determined with fluorescent substrate analogues (Hoppe, 1983). L-leucine-7-amido-4-methylcoumarin (Sigma Aldrich) and 4-methylumbelliferyl-ß-D-glucopyranoside (Acros Organics) were added in final concentrations of 1, 5, 10, 20, 50, 80, 100 and 200 µmol l^{-1} in black 69 well plates (Costar) and kept frozen for at most one day until replicates of 200 µl sample were added. After 0 and 12 hours of incubation at 13°C in the dark, fluorescence was measured with a plate reader fluorometer (FLUOstar Optima, BMG labtech) (excitation: 355 nm; emission: 460 nm). An error of 2 % RSD was defined using the calibration with triplicates. Blanks with MilliQ were performed to exclude an increase in substrate decay over time.

Samples were collected in replicates (n=2) at station A-K and incubated directly after sampling under oxygen conditions resembling *in situ* oxygen conditions. For samples > 5 µmol *in situ* O_2 kg^{-1} incubations were conducted under atmospheric oxygen conditions. Samples < 5 µmol *in situ* O_2 kg^{-1} were incubated in a gas tight incubator that

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

was filled with N_2, to reduce oxygen concentrations (8 to 40 µmol O_2 kg^{-1}). To investigate the influence of the different incubation methods we additionally incubated samples > 5 µmol *in situ* O_2 kg^{-1} under reduced oxygen concentrations. On average incubations under reduced oxygen concentration yielded 2-27% higher values than those incubated under atmospheric oxygen conditions. However, the observed trends over depth remained similar (see supplementary discussion and supplementary Fig. 1).

Calibration was conducted with 7-amino-4-methylcoumarin (2 nmol l^{-1} to 1 µmol l^{-1}) (Sigma Aldrich) and 4-methylumbelliferone (Sigma Aldrich) (16 nmol l^{-1} to 1 µmol l^{-1}) in seawater at atmospheric oxygen concentrations and under N_2 atmosphere.

Maximum reaction velocity (V_{max}) at saturating substrate concentrations was calculated using both replicates at once, with the simple ligand binding function in SigmaPlot™ 12.0 (Systat Software Inc., San Jose, CA). Values for V_{max} with a s.d. >30 % were excluded from further analyses. The degradation rate (δ) [µmol C m^{-3} d^{-1}] of DHAA by LAPase and DCHO by GLUCase was calculated after Piontek et al. (2014):

$$4. \quad \delta = \frac{h_r * c}{100}$$

where h_r [% d^{-1}] is the hydrolyses turnover at 10 µmol l^{-1} substrate concentration and c is the carbon content of DHAA [µmol C m^{-3}]. Measurements of h_r with a s.d. between duplicates of more than 30% were excluded. The same procedure was conducted with the carbon content of dissolved hydrolysable leucine, instead of DHAA, to account for variations in leucine concentrations, which is the main amino acid hydrolysed by LAPase.

2.7.Data analyses

Data were plotted with Ocean Data View 4.74 (Schlitzer, 2016), MATLAB (8.3.0.532 (R2014a)) and R version 3.4.2 using the package *ggplot2* (Hadley Wickham, 2016; R Development Core Team, 2008). Statistical significances between different regimes (see supplementary Table 2 for mean and s.d. within different regimes and main text for statistical results) were tested with a *Wilcoxon test* (W) and correlation with the *Spearman Rank correlation* (S) in R version 3.4.2 (R Development Core Team, 2008) using following R packages: *FSA, car and multcomp* (Derek H. Olge, 2018; Horthorn et al., 2008; John Fox and Sanford Weisberg, 2011). For this extracellular enzyme data of station A-K and bacterial production data of station G-T were used, since not all parameters could be sampled at all depth. Diapycnal fluxes of DOC and oxygen were calculated with MATLAB (8.3.0.532 (R2014a)) and the Toolbox Gibbs SeaWater (GSW) Oceanographic Toolbox (3.05) (McDougall and Barker, 2011).

Samples were categorized into different oxygen regimes. Due to sensitivities of oxygen measurements, we did not distinguish between anoxic and suboxic regimes, but defined the "OMZ" oxygen regime by a threshold ≤ 5 µmol O_2 kg^{-1} (Gruber, 2011). We defined the oxycline as one regime (>5 to <60 µmol O_2 kg^{-1}) or separated it into "low_hypoxic" (>5 to <20 µmol O_2 kg^{-1}) and "high_hypoxic" (>20 to <60 µmol O_2 kg^{-1}) regimes, representing important thresholds of oxygen concentrations for biological processes (Gruber, 2011). Oxygen concentrations >60 µmol O_2 kg^{-1} were defined as "oxic". Moreover, we differentiated between oxygen regimes situated above and below the OMZ.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

3. Results

3.1. Biogeochemistry of the Peruvian OMZ

During our two cruises to the Peruvian upwelling system (Fig. 1), in April and June 2017 chlorophyll *a* (Chl *a*) reached maximum of 8 µg l^{-1} within the upper 25 m. At depths >50 m Chl *a* was generally below detection limit (Fig. 2a, exemplary stations). Oxygen >100 µmol kg^{-1} was observed in the surface mixed layer, decreased steeply with depth and was below detection of Winkler titration at >100 m (Fig. 3a and 4a). Oxygen increased again to up to 15 µmol kg^{-1} at >500 m (Fig. 3a and 4a). Temperatures ranged between 14 and 24 °C in the upper 100 m and decreased to 7 °C at 600 m (Fig. 2b). TDN concentrations increased with depth from 17-32 µmol l^{-1} within the upper 40 m to a maximum of 51 µmol l^{-1} at 600 m (Fig. 2c). DOC decreased with depth from 81 ±30 µmol l^{-1} at the surface to 42 µmol l^{-1} at 600 m depth, with the steepest gradient in the upper 20-60 m (Fig. 2d).

3.2. Bacterial production and enzymatic activity

Bacterial production varied strongly throughout the study region and ranged from 0.6 to 614 µmol C m^{-3} d^{-1} (Fig. 3b), decreased in general from surface to depth (except for the most coastal stations) and showed significantly higher rates in the oxygenated surface compared to the OMZ (n_{OMZ}=61, n_{oxic}=34, p<0.01, W=214) (Fig. 3b). Bacterial production did not differ between the oxyclines and the suboxic core waters, neither off-shore (suboxic: 0.6-91 µmol C m^{-3} d^{-1} ; oxyclines: 2-149 µmol C m^{-3} d^{-1}) (n_{OMZ}= 46 $n_{oxyclines}$ 25, p=0.28, W=484) nor at the most coastal stations (G and T) (suboxic: 80-160 µmol C m^{-3} d^{-1}) (oxycline: 34-195 µmol C m^{-3} d^{-1}) (n_{OMZ}=2, $n_{oxycline}$=8, p=0.4, W=12). Further, no significant correlation was observed between bacterial production and oxygen at *in situ* <20 µmol O_2 kg^{-1} (S=53904, p=0.64, r^2=0.06, n=70). No significant increase of bacterial production was observed below the OMZ (2-10.5 µmol C m^{-3} d^{-1}) compared to the core OMZ (0.6-160 µmol C m^{-3} d^{-1}) even though oxygen increased from <5 to 15 µmol kg^{-1} (Fig. 3b) (n_{OMZ}=48, $n_{bottom\_low\_hypoxic}$=5, p=0.08, W=62).

Overall, bacterial abundance ranged from 1 to 49 x 10^5 cells ml^{-1}, with highest abundance observed at the surface and close to the sediment. Cell abundance in the upper oxycline (3-16 x 10^5 cells ml^{-1}) was statistically similar to the OMZ core (1-25 x 10^5 cells ml^{-1}) ($n_{upper\_oxycline}$=36, n_{OMZ}=93, p=0.86, W=1640). However, we could observe a slight accumulation of bacterial cells in the OMZ core, compared to the upper oxycline (Fig. 3c). A sharp decrease in bacterial abundance was observed below the OMZ.

Estimates for the *in situ* degradation rate of DHAA by LAPase take into account the available concentrations of DHAA and varied between 0.7 and 31.2 µmol C m^{-3} d^{-1}. LAPase degradation rates observed within the OMZ core (5 ± 1.8 µmol C m^{-3} d^{-1}) were significantly higher than in the upper oxycline (2.5 ± 1.2 µmol C m^{-3} d^{-1}) (n_{OMZ}= 41, $n_{upper\_low\_hypoxic}$=9, p<0.001 W=331) (Fig. 4b). To exclude an influence of changing DHAA composition over depth, LAPase activity was also calculated using *in situ* concentrations of dissolved hydrolysable leucine instead of total DHAA. Degradation rates of dissolved hydrolysable leucine by LAPase (0.01-1.68 µmol C m^{-3} d^{-1}) showed the same trend with significantly higher rates in suboxic waters than in the upper oxycline (n_{OMZ}=41, $n_{upper\_low\_hypoxic}$=9, p<0.01 W=289). Thus, differences in the molecular composition of DHAA had no influence on spatial degradation patterns being higher in suboxic waters than in the upper oxycline. In contrast, degradation rates of DCHO (>1kDa) were

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

slightly reduced within the suboxic waters (0.69 ± 1.35 µmol C m^{-3} d^{-1}) compared to the upper oxycline (0.76 ± 0.51 µmol C m^{-3} d^{-1}) (n_{OMZ}= 35, $n_{upper\_low\_hypoxic}$=8, p=0.054, W=78). Since degradation rates were calculated by multiplying enzyme rates and carbon concentrations of DCHO and DHAA at *in situ* depth, differences in carbon concentrations are important for further interpretation. *In situ* carbon concentrations of DHAA were similar between the OMZ core (0.53± 0.1 µmol C L^{-1}) and the oxycline (0.57± 0.2 µmol C L^{-1}) (n_{OMZ}=42, $n_{oxycline}$=31, p=0.3 W=558). In contrast, *in situ* carbon concentrations of DCHO were reduced within the OMZ core (1.3 ± 0.4 µmol C L^{-1}) compared to the oxycline (1.5 ± 0.6 µmol C L^{-1}) (n_{OMZ}=42, $n_{oxycline}$=31, p=0.01 W=502) (Fig. 2e, f), suggesting that calculated differences between degradation rates may be influenced by different carbon concentrations. Potential hydrolytic rates at saturating substrate concentration (V_{max}) of LAPase ranged between 7 and 168 nmol l^{-1} h^{-1} and were 10 times lower for GLUCase. LAPase V_{max} was significantly higher within the suboxic waters (50 ± 22 nmol l^{-1} h^{-1}) compared to the oxycline (34 ± 18 nmol l^{-1} h^{-1}) (n_{OMZ}=49, $n_{oxycline}$=26, p<0.01 W=1045) and GLUCase V_{max} was more similar within the suboxic waters (1.6 ± 1.6 nmol l^{-1} h^{-1}) compared to the oxycline (1.1 ± 0.5 nmol l^{-1} h^{-1}) (Fig. 4d, e).

To investigate physiological effects of suboxia, we normalized bacterial production and enzymatic rates to cell abundance. Cell-specific production ranged between 2 and 286 amol C $cell^{-1}$ d^{-1} (Fig. 3d) and, in contrast to total production, was positively correlated to oxygen concentrations at <20 µmol O_2 kg^{-1} regardless of whether the most coastal stations (G and T) were included in the statistical analysis (S=31917, p<0.001, r^2=0.44, n=70) or not (S=25985, p <0.01, r^2=0.35, n=62) (Fig. 3d).

Cell-specific degradation rates of DHAA increased with depth and yielded significantly higher rates at the lower oxycline compared to all shallower depths ($n_{bottom\_low\_hypoxic}$= 6, $n_{remaining\ depths}$ =56, W=302, p <0.01). Cell-specific GLUCase degradation rate and LAPase and GLUCase V_{max} showed the same patterns (Fig. 4g-j)

3.3. Bacterial contribution to the loss of dissolved organic carbon and oxygen in the oxycline

We calculated the loss of oxygen and DOC during physical transport from below the mixed layer depth (MLD; 10-32 m) to 60 m based on observed changes in diapycnal fluxes (Eq. (1), Fig. 5). We estimated the bacterial contribution to this loss with two different assumptions (Table 1): i) We assumed that the loss of DOC over depth can be solely attributed to bacterial uptake implying that the DOC is subsequently incorporated as bacterial biomass (bacterial production) or respired to CO_2 (Eq. (2)). ii) For the second approach the amount of DOC taken up by bacteria was determined by the measured bacterial incorporation of carbon (bacterial production) and a constant ratio between carbon that is taken up and carbon that is incorporated as biomass (bacterial production) (Eq. (3)). This ratio (BGE), was here assumed to be between 10 and 30%, based on the empirical equation by Rivkin and Legendre with an *in situ* temperature that varied between 14 and 19°C (Rivkin and Legendre, 2001).

For total average DOC loss ($\nabla\Phi_{DOC}$), we calculated a range of 1.13-3.40 mmol C m^{-3} d^{-1}, with loss rates decreasing most strongly below the shallow mixed layer down to 40 m (Table 1, Fig. 5). Following the first (i) assumption all DOC that was lost over depth was taken up by bacteria and the measured bacterial production represents the fraction

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

of DOC that was incorporated as biomass. Consequently, the remaining DOC that has been taken up, in other words the difference between DOC loss and bacterial production (0.01-0.29 mmol C m^{-3} d^{-1}), was respired to CO_2 and represents the bacterial oxygen demand to account for the DOC loss (BOD_ε) (1.05-3.38 mmol O_2 m^{-3} d^{-1}) (Eq. (2)). Following this calculation, the BGE would vary between 0.5-8.6% and 1.1-7.0% in the depth range of MLD-40 m and 40-60 m, respectively, being on average constant over the two different depth ranges (2.7%). ii) Applying a BGE in the range of 10-30% and the measured bacterial production, the calculated bacterial DOC uptake was 0.04-2.9 mmol C m^{-3} d^{-1}. Hence, respiration of DOC to CO_2 accounted for a BOD_ϕ of 0.03-2.63 mmol O_2 m^{-3} d^{-1} (Table 1).

4. Discussion

We investigated bacterial degradation of dissolved organic matter by measuring bacterial production rates as an estimate for organic carbon transformation into biomass as well as rates of extracellular hydrolytic enzymes to provide information on the initial steps of organic matter degradation (Hoppe et al., 2002). We expected reduced rates of organic matter degradation within oxygen depleted waters, since anaerobic respiration has a lower energy yield than aerobic respiration. However, although bacterial production decreased with depth (Fig. 3b), this decrease was not related to oxygen concentrations. Moreover, no significant increase in bacterial production was observed at the lower oxycline, when oxygen increased again (Fig. 3b). Decreasing bacterial production with depth has also been observed for fully oxygenated regions in the Atlantic (Baltar et al., 2009) and the equatorial Pacific (Kirchman et al., 1995) and has been explained by a decrease in the amount of bioavailable organic matter over depth.

The hypothesis of reduced bacterial degradation activity within the OMZ implies reduced extracellular enzyme rates for the hydrolysis of organic matter. In our study, neither GLUCase nor LAPase V_{max} were reduced within the suboxic waters irrespective of incubation conditions (Fig. 4d, e, supplementary Fig. 1c, d). Thus, our findings show no evidence for reduced organic matter degradation in suboxic waters and are in good agreement with studies, which report similar bacterial degradation rates for oxic and suboxic waters (Cavan et al., 2017; Lee, 1992; Pantoja et al., 2009). Consequently, the hypothesis of enhanced carbon export in suboxic and anoxic waters due to reduced organic matter degradation seems fragile and alternative explanations for enhanced carbon export efficiency in suboxic and anoxic waters e.g. reduced particle fragmentation due to zooplankton avoiding hypoxia (Cavan et al., 2017) may be more likely. Moreover, a reduced degradation of organic matter in suboxic waters as it is assumed for particulate carbon in global ocean biogeochemical modelling may have to be reconsidered (Ilyina et al., 2013).

Within OMZs dissolved nitrogen fuels e.g. denitrification or anaerobic ammonium oxidation (anammox) and is reduced to e.g. dinitrogen gas, nitrous oxide that evades to the atmosphere. Current estimates result in 20-50% of the total oceanic nitrogen loss occurring in OMZs (Lam and Kuypers, 2011). Thereby, a preferential degradation of amino acid containing organic matter in suboxic waters compared to oxic waters has been suggested (Van Mooy et al., 2002). A strong degradation of nitrogen compounds (within suboxic waters) by heterotrophic bacteria (e.g. denitrifiers) enables the release of ammonia and nitrite and subsequently can maintain anammox, an autotrophic anaerobic respiratory pathway (Lam and Kuypers, 2011; Ward, 2013). This interaction between denitrifiers and

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019


Biogeosciences Discussions Open Access EGU

anammox bacteria would further fuel the loss of nitrogen to the atmosphere. Our data indeed showed enhanced degradation of amino acid containing organic matter in low oxygen waters. Indicators for protein decomposition, i.e. LAPase V_{max} and the degradation rate of DHAA by LAPase, were more pronounced within the suboxic waters (Fig. 4b, d). The close coupling between anammox and denitrifying bacteria has previously been shown for wastewater treatments. There, denitrifers directly utilize organic matter excreted by the anammox bacteria which in turn benefit from the released nitrite by respiratory nitrate reduction (Lawson et al., 2017). In the Pacific, denitrifiers and anammox bacteria coexist, but are separated in space and time (Dalsgaard et al., 2012), therefore their inter-dependency is probably weaker than in waste water treatments. Still, high protein hydrolysis rates within suboxic waters might fuel enhanced nitrogen loss due to the mutual support of anammox bacteria and heterotrophic denitrifiers.

To investigate physiological effects of suboxia, we normalized bacterial production and enzymatic rates to cell abundance and found higher cell-specific bacterial production near the oxycline compared to suboxic waters and highest cell-specific enzyme rates at the lower oxycline (Fig. 3d, 4 g-j). Higher cell-specific bacterial production at oxic-anoxic interfaces in the water column has previously been reported for the Baltic Sea (Brettar et al., 2012). Baltar et al. (2009) showed increasing cell-specific enzymatic rates and decreasing cell-specific bacterial production, with increasing depth in the subtropical Atlantic and related this pattern to decreasing organic matter lability. Besides organic matter lability, TDN might influence physiological rates and was increasing with depth (Fig. 2c). We therefore suggest that the co-occurrence of oxygen and high TDN near the oxyclines favours (micro) aerobic respiration (Kalvelage et al., 2015) and cell activity, corroborating highly active bacterial communities at oxyclines.

Depth distribution of cell-specific and total bacterial production was markedly different (Fig. 3b, d) and can be explained by higher cell abundance (Fig. 3c) partially compensating for the lower metabolic activity in the OMZ core. The reduced cell-specific production in the core of the OMZ points to either a high standing stock of bacteria with low activity or a mixed community of less active and more active cells. One reason for the accumulation of cells within the OMZ might be that some bacterivores tend to avoid the OMZ Anderson et al. (2012), highlighting the OMZ core as an ecological niche for slowly growing bacteria.

In general, bacterial community composition in OMZs has been shown to be strongly impacted by oxygen. In the OMZ near the shelf off Chile Arctic96BD-19 and SUP05 dominate heterotrophic and autotrophic groups in hypoxic waters (Aldunate et al., 2018). Next to the appearance of autotrophic bacteria that are related to sulphur (e.g. SUP05) or nitrogen cycling (e.g. Planctomycetes) also bacteria that are related to cycling of complex carbohydrates have been discovered in OMZs (Callbeck et al., 2018; Galán et al., 2009; Thrash et al., 2017), and may explain the unaltered high potential (V_{max}) of the extracellular enzymes GLUCase and heterotrophic bacterial production in suboxic waters in our study (Fig. 4e, 3b). For instance, SAR406, SAR202, ACD39 and PAUC34f have the genetic potential for the turnover of complex carbohydrates and anaerobic respiratory processes, in the Gulf of Mexico (Thrash et al., 2017).

Heterotrophic bacteria are the main users of marine dissolved organic matter (Azam et al., 1983; Carlson and Hansell, 2015) and responsible for ~79% of total respiration in the Pacific Ocean (Del Giorgio et al., 2011),

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

proposing that heterotrophic bacteria drive organic matter and oxygen cycling in the ocean and significantly contribute to the formation of the OMZ. Under the assumption that the calculated loss of DOC during diapycnal transport (<60 m) is caused solely by bacterial uptake and subtracting the amount of carbon channelled into biomass production, our study verifies the importance of bacterial DOC degradation for the formation of the OMZ. We estimated a BOD (1.05-3.38 mmol O_2 m^{-3} d^{-1}) that is in line with earlier respiration measurements in the upper oxycline off Peru (Kalvelage et al., 2015) and represents 19-33% of the oxygen loss over depth, implying a rather low average BGE (0.5-8.6%) (Table 1). Calculating the bacterial uptake of DOC from production rates and a more conservative BGE between 10 and 30% as previously suggested (Rivkin and Legendre, 2001) for the *in situ* temperature of 14 to 19 °C, 2-86% of the DOC loss and 0.4-26% of oxygen loss could be attributed to bacterial degradation of dissolved organic matter. Both approaches reveal a BGE that is still within the range of previous reports for upwelling systems of the Atlantic (<1-58%) or northeastern Pacific (<10%) (Alonso-Sáez et al., 2007; Del Giorgio et al., 2011). The high variability in BGE is a topic of ongoing research. Until now 54% of the variability could be explained by variations in temperature (Rivkin and Legendre, 2001). Our data suggest that oxygen availability may be another control of BGE leading to rather low BGE in low oxygen waters. This is especially indicated by a low and constant average BGE (2.6%), which we estimated for the water column down to 60m depth under the assumption that all DOC that is lost over depth can be attributed to bacterial uptake. A low BGE might be explained by a bacterial community that has higher energetic demands, but in return is adapted to variable oxygen conditions. Additionally, the BGE is decreasing with an increasing carbon to nitrogen ratio of the available substrate (Goldman et al., 1987). In the OMZ off Peru the ratio between DOC and dissolved organic nitrogen is frequently high (~12 to 16) (Loginova et al., 2018), and might further contribute to the low BGE. High respiration rates induced by bacterial DOC degradation contribute to sustaining the OMZ, besides oxygen consumption by bacteria that hydrolyze and degrade particulate organic matter (Cavan et al., 2017). Another, but likely minor contribution to overall respiration is made by zooplankton and higher trophic levels (e.g. Kiko et al., 2016). Additionally, physical processes such as an intrusion of oxygen depleted waters by eddies, upwelling or advection, may add to the oxygen and DOC loss over depth (Brandt et al., 2015; Llanillo et al., 2018; Steinfeldt et al., 2015).

Uncertainties of our assumption that the loss of DOC is caused solely by bacterial uptake include other processes potentially contributing to DOC removal, but not taken into consideration here like DOC adsorption onto particles, DOC uptake by eukaryotic cells or the physical coagulation of DOC into particles, e.g. by formation of gel-like particles such as transparent exopolymer particles and Coomassie stainable particles (Carlson and Hansell, 2015; Engel et al., 2004, 2005). Moreover, temporal variations in diapycnal fluxes may be large, as indicated by the confidence interval of solute fluxes (Fig. 5) during this study and by 2 to 10 times lower DOC and oxygen loss rates during other seasons (Loginova et al., 2018). However, our study is the first combining physical and microbial rate measurements for carbon and oxygen losses in the upwelling system off Peru and can help improving current biogeochemical models by constraining bacterial dissolved organic matter degradation.

In oxygen depleted waters of the Peruvian upwelling system, the chemoautotrophic process of anammox has been assumed to dominate anaerobic nitrogen cycling (Kalvelage et al., 2013), with lower but more constant rates compared to more sporadically occurring heterotrophic denitrification (Dalsgaard et al., 2012). Our study points

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

371 towards a widespread occurrence of heterotrophic denitrification processes in the Peruvian OMZ, since the here
372 applied method for measuring bacterial production is restricted to heterotrophs. Our rates for bacterial production
373 within the suboxic waters averaged to 26 µmol C m^{-3} d^{-1} (0.55-160 µmol C m^{-3} d^{-1}). To compare bacterial
374 production, i.e. rate of carbon incorporation, with denitrification rates previously reported for the South Pacific
375 (Dalsgaard et al., 2012; Kalvelage et al., 2013), we converted one mol of reduced nitrogen to 1.25 mol of oxidized
376 carbon after the reaction equation given by Lam and Kuypers (2011). This calculation indicates that on average ≤19
377 µmol C m^{-3} d^{-1} are oxidized by denitrifying bacteria in the Eastern Tropical Pacific (Dalsgaard et al., 2012; Kalvelage
378 et al., 2013). Assuming a BGE of 20% (Del Giorgio and Cole, 1998) then suggests that denitrification supports a
379 bacterial production of ≤4 µmol C m^{-3} d^{-1}, thus only about 15% of total heterotrophic bacterial production in suboxic
380 waters determined in this study. For the sum of anaerobic carbon oxidation rates including denitrification, DNRA
381 and simple nitrate reduction, 109 µmol C m^{-3} d^{-1} (6-515 µmol C m^{-3} d^{-1}) may be expected for the Peruvian shelf, with
382 the reduction of nitrate to nitrite representing the largest proportion (2-505 µmol C^{-1} m^{-3} d^{-1}), based on the relative
383 abundance of the different N-functional genes (Kalvelage et al., 2013). These anaerobic respiration measurements
384 are equivalent to a bacterial production of ~ 22 µmol C m^{-3} d^{-1} (1-103 µmol C m^{-3} d^{-1}) and are thus in good agreement
385 with our direct measurements of bacterial production rates. However, the reduction of nitrate, could not be detected
386 at every depth and incubation experiments partly showed huge variations over depth (Kalvelage et al., 2013),
387 whereas we were able to measure bacterial production in every sample. Our data therefore suggest that the carbon
388 oxidation potential off Peru is more evenly distributed than expected and also corroborate earlier suggestions of
389 unexpectedly high rates of heterotrophic nitrogen cycling in the OMZ off Peru based on observations of high
390 concentrations of atmospheric nitrous oxide (Bourbonnais et al., 2017).

391 **5. Conclusion**

392 Our study suggests that suboxia does not reduce enzymatic degradation of organic matter and bacterial production in
393 the Eastern Tropical South Pacific off Peru. Bacterial production in suboxic waters points towards a dominance of
394 heterotrophic anaerobic respiratory pathways, and may fuel high nitrogen loss rates to the atmosphere including
395 climate relevant nitrous oxide as previously observed for this system. Clear differences between cell-specific and
396 total rates of bacterial activity allude to different controls of cell abundance in suboxic systems and highlight the
397 OMZ as a specific ecological niche. The combination of bacterial and physical rate measurements suggests that low
398 BGEs in the upper oxycline contribute to sustaining the OMZ. Our study highlights the need for a better
399 understanding and quantification of processes responsible for oxygen and dissolved organic matter loss in OMZs that
400 is inevitable to predict future patterns of deoxygenation in a warming climate.

401 *Data Availability.* PANGEA: 10.1594/PANGAEA.891247
402

403 *Author contributions.* M.M. and A.E. designed the scientific study, analysed the data and wrote the manuscript. J.L.
404 calculated DOC and oxygen fluxes, G.K. sampled and calibrated the CTD data and both helped writing the
405 manuscript.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgments: We thank Jon Roa, Tania Klüver and Ruth Flerus for the sampling and/or analysis of DOC/TDN; cell abundance, bacterial production and DHAA. Moreover, we would like to thank Judith Piontek, Sören Thomsen, Carolina Cisternas-Novoa and Frédéric A.C. Le Moigne who helped and gave advice for sampling during the cruises. We are grateful to the working group of Hermann Bange and Stefan Sommer who provided Winkler measurements. We thank the cruise leaders Hermann Bange and Marcus Dengler, crew, officers and the captains of the F.S. Meteor for the support on board and the successful cruises. This study was supported by the Helmholtz Association and by the Collaborative Research Center 754 / SFB Sonderforschungsbereich 754 'Climate-Biogeochemistry Interactions in the Tropical Ocean'.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

415 **References**

416 Aldunate, M., De la glesia, R., Bertagnolli, A. D. and Ulloa, O.: Oxygen modulates bacterial community composition
417 in the coastal upwelling waters off central Chile, Deep. Res. Part II, in press, 1–12, doi:10.1016/j.dsr2.2018.02.001,
418 2018.

419 Alonso-Sáez, L., Gasol, J. M., Arístegui, J., Vilas, J. C., Vaqué, D., Duarte, C. M. and Agustí, S.: Large-scale
420 variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean,
421 Limnol. Oceanogr., 52(2), 533–546, doi:10.4319/lo.2007.52.2.0533, 2007.

422 Anderson, R., Winter, C. and Jürgens, K.: Protist grazing and viral lysis as prokaryotic mortality factors at Baltic Sea
423 oxic−anoxic interfaces, Mar. Ecol. Prog. Ser., 467, 1–14, doi:10.3354/meps10001, 2012.

424 Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstadt, F.: The ecological role of water-
425 column microbes in the sea., Mar. Ecol. Prog. Ser., 10(3), 257–263, 1983.

426 Baltar, F., Arístegui, J., Sintes, E., van Aken, H. M., Gasol, J. M. and Herndl, G. J.: Prokaryotic extracellular
427 enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the
428 (sub)tropical Atlantic, Environ. Microbiol., 11(8), 1998–2014, doi:10.1111/j.1462-2920.2009.01922.x, 2009.

429 Benner, R. and Amon, R. M. W.: The size-reactivity continuum of major bioelements in the ocean, Ann. Rev. Mar.
430 Sci., 7(1), 185–205, doi:10.1146/annurev-marine-010213-135126, 2015.

431 Boetius, A. and Lochte, K.: Effect of organic enrichments on hydrolytic potentials and growth of bacteria in deep-sea
432 sediments., Mar. Ecol. Prog. Ser., 140, 239–250, doi:10.3354/meps140239, 1996.

433 Bourbonnais, A., Letscher, R. T., Bange, H. W., Échevin, V., Larkum, J., Mohn, J., Yoshida, N. and Altabet, M. A.:
434 N2O production and consumption from stable isotopic and concentration data in the Peruvian coastal upwelling
435 system, Global Biogeochem. Cycles, 31(4), 678–698, doi:10.1002/2016GB005567, 2017.

436 Brandt, P., Bange, H. W., Banyte, D., Dengler, M., Didwischus, S., Fischer, T., Greatbatch, R. J., Hahn, J., Kanzow,
437 T., Karstensen, J., Körtzinger, A., Krahmann, G., Schmidtko, S., Stramma, L., Tanhua, T. and Visbeck, M.: On the
438 role of circulation and mixing in the ventilation of oxygen minimum zones with a focus on the eastern tropical North
439 Atlantic, Biogeoscience, 12, 489–512, doi:10.5194/bg-12-489-2015, 2015.

440 Brettar, I., Christen, R. and Höfle, M. G.: Analysis of bacterial core communities in the central Baltic by comparative
441 RNA–DNA-based fingerprinting provides links to structure–function relationships., ISME J., 6(1), 195–212,
442 doi:10.1038/ismej.2011.80, 2012.

443 Callbeck, C. M., Lavik, G., Ferdelman, T. G., Fuchs, B., Gruber-vodicka, H. R., Hach, P. F., Littmann, S.,
444 Schoffelen, N. J., Kalvelage, T., Thomsen, S., Schunck, H., Löscher, C. R., Schmitz, R. A. and Kuypers, M. M. M.:
445 Oxygen minimum zone cryptic sulfur cycling sustained by offshore transport of key sulfur oxidizing bacteria, Nat.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

446 Commun., 9(1729), 1–11, doi:10.1038/s41467-018-04041-x, 2018.

447 Carlson, C. A. and Hansell, D. A.: DOM Sources, Sinks, Reactivity, and Budgets, in Biogeochemistry of Marine
448 Dissolved Organic Matter, pp. 65–126, Elsevier., 2015.

449 Cavan, E. L., Trimmer, M., Shelley, F. and Sanders, R.: Remineralization of particulate organic carbon in an ocean
450 oxygen minimum zone, Nat. Commun., 8(May 2016), 14847, doi:10.1038/ncomms14847, 2017.

451 Cole, J. J. and Pace, M. L.: Bacterial secondary production in oxic and anoxic freshwaters, Limnol. Oceanogr., 40(6),
452 1019–1027, doi:10.4319/lo.1995.40.6.1019, 1995.

453 Czeschel, R., Stramma, L., Schwarzkopf, F. U., Giese, B. S., Funk, A. and Karstensen, J.: Middepth circulation of
454 the eastern tropical South Pacific and its link to the oxygen minimum zone, J. Geophys. Res., 116(C01015), 1–13,
455 doi:10.1029/2010JC006565, 2011.

456 Dalsgaard, T., Thamdrup, B., Farías, L. and Revsbech, N. P.: Anammox and denitrification in the oxygen minimum
457 zone of the eastern South Pacific, Limnol. Oceanogr., 57(5), 1331–1346, doi:10.4319/lo.2012.57.5.1331, 2012.

458 Derek H. Olge: FSA: Fisheries Stock Analysis, 2018.

459 Devol, A. H. and Hartnett, H. E.: Role of the oxygen-deficient zone in transfer of organic carbon to the deep ocean,
460 Limnol. Oceanogr., 46(7), 1684–1690, doi:10.4319/lo.2001.46.7.1684, 2001.

461 Dittmar, T., Cherrier, J. and Ludichowski, K. U.: The analysis of amino acids in seawater., in Practical guidelines for
462 the analysis of seawater., edited by O. Wurl, pp. 67–78, CRC Press, Boca Raton., 2009.

463 Engel, A. and Galgani, L.: The organic sea-surface microlayer in the upwelling region off the coast of Peru and
464 potential implications for air–sea exchange processes, Biogeosciences, 13(4), 989–1007, doi:10.5194/bg-13-989-
465 2016, 2016.

466 Engel, A. and Händel, N.: A novel protocol for determining the concentration and composition of sugars in
467 particulate and in high molecular weight dissolved organic matter (HMW-DOM) in seawater., Mar. Chem., 127(1),
468 180–191, doi:10.1016/j.marchem.2011.09.004, 2011.

469 Engel, A., Thoms, S., Riebesell, U., Rochelle-Newall, E. and Zondervan, I.: Polysaccharide aggregation as a
470 potential sink of marine dissolved organic carbon, Nature, 428(6986), 929–932, doi:10.1038/nature02453, 2004.

471 Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J.-P., Harlay, J.,
472 Heemann, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M.-D., Rochelle-Newall, E., Schneider, U.,
473 Terbrueggen, A. and Riebesell, U.: Testing the direct effect of CO2 concentration on a bloom of the coccolithophorid
474 Emiliania huxleyi in mesocosm experiments, Limnol. Oceanogr., 50(2), 493–507, doi:10.4319/lo.2005.50.2.0493,
475 2005.

476 Fischer, T., Banyte, D., Brandt, P., Dengler, M., Krahmann, G., Tanhua, T. and Visbeck, M.: Diapycnal oxygen
477 supply to the tropical North Atlantic oxygen minimum zone, Biogeosciences, 10(7), 5079–5093, doi:10.5194/bg-10-
478 5079-2013, 2013.

479 Galán, A., Molina, V., Thamdrup, B., Woebken, D., Lavik, G., Kuypers, M. M. M. and Ulloa, O.: Anammox bacteria
480 and the anaerobic oxidation of ammonium in the oxygen minimum zone off northern Chile, Deep. Res. II, 56, 1021–
481 1031, doi:10.1016/j.dsr2.2008.09.016, 2009.

482 Gasol, J. M. and Del Giorgio, P. A.: Using flow cytometry for counting natural planktonic bacteria and
483 understanding the structure of planktonic bacterial communities, Sci. Mar., 64(2), 197–224,
484 doi:10.3989/scimar.2000.64n2197, 2000.

485 Del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu. Rev. Ecol. Syst,
486 29(May), 503–541, 1998.

487 Del Giorgio, P. A., Condon, R., Bouvier, T., Longnecker, K., Bouvier, C., Sherr, E. and Gasol, J. M.: Coherent
488 patterns in bacterial growth, growth efficiency, and leucine metabolism along a northeastern Pacific inshore-offshore
489 transect, Limnol. Oceanogr., 56(1), 1–16, doi:10.4319/lo.2011.56.1.0001, 2011.

490 Goldman, J. C., Caron, D. A. and Dennett, M. R.: Regulation of gross growth efficiency and ammonium regeneration
491 in bacteria by substrate C : N ratio., Limnol. Oceanogr., 32(6), 1239–1252, doi:10.4319/lo.1987.32.6.1239, 1987.

492 Graco, M. I., Purca, S., Dewitte, B., Castro, C. G., Morón, O., Ledesma, J., Flores, G. and Gutiérrez, D.: The OMZ
493 and nutrient features as a signature of interannual and low-frequency variability in the Peruvian upwelling system,
494 Biogeosciences, 14(20), 4601–4617, doi:10.5194/bg-14-4601-2017, 2017.

495 Grossart, H., Allgaier, M., Passow, U. and Riebesell, U.: Testing the effect of CO_2 concentration on the dynamics of
496 marine heterotrophic bacterioplankton, Limnol. Oceanogr., 51(1), 1–11, doi:10.4319/lo.2006.51.1.0001, 2006.

497 Gruber, N.: Warming up, turning sour, losing breath : ocean biogeochemistry under global change., Phili. Trans. R.
498 Soc., 369(1943), 1980–1996, doi:10.1098/rsta.2011.0003, 2011.

499 Hadley Wickham: ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag, New York., 2016.

500 Hoppe, H.-G.: Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of
501 methylumbelliferyl-substrates., Mar. Ecol. Prog. Ser., 11, 299–308, 1983.

502 Hoppe, H.-G., Gocke, K. and Kuparinen, J.: Effect of H2S on heterotrophic substrate uptake, extracellular enzyme
503 activity and growth of brackish water bacteria., Mar. Ecol. Prog. Ser., 64, 157–167 [online] Available from:
504 http://www.int-res.com/articles/meps/64/m064p157.pdf, 1990.

505 Hoppe, H.-G., Arnosti, C. and Herndl, G. F.: Ecological significance of bacterial enzymes in the marine
506 environment, in Enzymes in the environment: activity, ecology, and applications, edited by R. Burns and R. Dick,

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

507 pp. 73–108, Marcel Dekker, Inc., New York., 2002.

508 Horthorn, T., Bretz, F. and Westfall, P.: Simultaneous Inference in General Parametric Models, Biometrical J., 50(3),
509 346–363, 2008.

510 Ilyina, T., Six, K. D., Segschneider, J., Maier-Reimer, E., Li, H. and Nunez-Riboni, I.: Global ocean biogeochemistry
511 model HAMOCC : Model architecture and performance as component of the MPI-Earth system model in different
512 CMIP5 experimental realizations, J. Adv. Model. earth Syst., 5, 1–29, doi:10.1029/2012MS000178, 2013.

513 John Fox and Sanford Weisberg: An {R} Companion to Applied Regression, 2nd ed., SAGE Publications Ltd,
514 Thousant OAk {CA}., 2011.

515 Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Löscher, C. R., Oschlies, A., Paulmier, A., Stramma, L.
516 and Kuypers, M. M. M.: Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum
517 zone, Nat. Geosci., 6(3), 228–234, doi:10.1038/ngeo1739, 2013.

518 Kalvelage, T., Lavik, G., Jensen, M. M., Revsbech, N. P., Löscher, C., Schunck, H., Desai, D. K., Hauss, H., Kiko,
519 R., Holtappels, M., LaRoche, J., Schmitz, R. A., Graco, M. I. and Kuypers, M. M. M.: Aerobic microbial respiration
520 in oceanic oxygen minimum zones, edited by Z.-X. Quan, PLoS One, 10(7), e0133526,
521 doi:10.1371/journal.pone.0133526, 2015.

522 Kämpf, J. and Chapman, P.: Upwelling Systems of the World, Springer International Publishing Switzerland, Cham.
523 [online] Available from: http://link.springer.com/10.1007/978-3-319-42524-5, 2016.

524 Kiko, R., Hauss, H., Buchholz, F. and Melzner, F.: Ammonium excretion and oxygen respiration of tropical
525 copepods and euphausiids exposed to oxygen minimum zone conditions, Biogeoscience, 13, 2241–2255,
526 doi:10.5194/bg-13-2241-2016, 2016.

527 Kirchman, D., K'nees, E. and Hodson, R.: Leucine incorporation and its potential as a measure of protein synthesis
528 by bacteria in natural aquatic systems., Appl. Environm. Microbiol., 49(3), 599–607, 1985.

529 Kirchman, D. L., Rich, J. H. and Barber, R. T.: Biomass and biomass production of heterotrophic bacteria along
530 140°W in the equatorial Pacific: Effect of temperature on the microbial loop, Deep Sea Res. Part II Top. Stud.
531 Oceanogr., 42(2–3), 603–619, doi:10.1016/0967-0645(95)00021-H, 1995.

532 Lam, P. and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum zones., Annu. Rev. Mar.
533 Sci, 3, 317–348, doi:10.1146/annurev-marine-120709-142814, 2011.

534 Lawson, C. E., Wu, S., Bhattacharjee, A. S., Hamilton, J. J., Mcmahon, K. D., Goel, R. and Noguera, D. R.:
535 Metabolic network analysis reveals microbial community interactions in anammox granules., Nat. Commun.,
536 8(15416), 1–12, doi:10.1038/ncomms15416, 2017.

537 Lee, C.: Controls on organic carbon preservation : the use of stratified water bodies to compare intrinsic rates of

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

538 decomposition in oxic and anoxic systems., Geochim. Cosmochim. Acta, 56(8), 3323–3335, doi:10.1016/0016-
539 7037(92)90308-6, 1992.

540 Lindroth, P. and Mopper, K.: High performance liquid chromatographic determination of subpicomole amounts of
541 amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde., Anal. Chem., 51(11), 1667–1674,
542 doi:10.1021/ac50047a019, 1979.

543 Llanillo, P. J., Pelegrí, J. L., Talley, L. D., Pena-Izquirdo, J. and Cordero, R. R.: Oxygen Pathways and Budget for
544 the Eastern South Pacific Oxygen Minimum Zone, J. Geophys. Res., 123, 1722–1744, doi:10.1002/2017JC013509,
545 2018.

546 Loginova, A. N., Thomsen, S., Dengler, M., Lüdke, J. and Engel, A.: Diapycnal dissolved organic matter supply into
547 the upper Peruvian oxycline, Biogeosciences Discuss., 2(June), 1–23, doi:10.5194/bg-2018-284, 2018.

548 McDougall, T. J. and Barker, P. M.: Getting started with TEOS-10 and the Gibbs Seawater (GSW) oceanographic
549 toolbox, SCOR/IAPSO WG 127, , 28, 2011.

550 Van Mooy, B. A. S., Keil, R. G. and Devol, A. H.: Impact of suboxia on sinking particulate organic carbon:
551 Enhanced carbon flux and preferential degradation of amino acids via denitrification., Geochim. Cosmochim. Acta,
552 66(3), 457–465, doi:10.1016/S0016-7037(01)00787-6, 2002.

553 Pantoja, S., Rossel, P., Castro, R., Cuevas, L. A., Daneri, G. and Córdova, C.: Microbial degradation rates of small
554 peptides and amino acids in the oxygen minimum zone of Chilean coastal waters, Deep Sea Res. Part, 56(16), 1055–
555 1062, doi:10.1016/j.dsr2.2008.09.007, 2009.

556 Piontek, J., Sperling, M., Nöthig, E. M. and Engel, A.: Regulation of bacterioplankton activity in Fram Strait (Arctic
557 Ocean) during early summer: The role of organic matter supply and temperature., J. Mar. Syst., 132, 83–94,
558 doi:10.1016/j.jmarsys.2014.01.003, 2014.

559 R Development Core Team: R: A language and environment for statistical computing, [online] Available from:
560 http://www.r-project.org, 2008.

561 Rivkin, R. B. and Legendre, L.: Biogenic carbon cycling in the upper ocean: Effects of microbial respiration, Science
562 ., 291(5512), 2398–2400, doi:10.1126/science.291.5512.2398, 2001.

563 Roullier, F., Berline, L., Guidi, L., Durrieu De Madron, X., Picheral, M., Sciandra, A., Pesant, S. and Stemmann, L.:
564 Particle size distribution and estimated carbon flux across the Arabian Sea oxygen minimum zone, Biogeosciences,
565 11(16), 4541–4557, doi:10.5194/bg-11-4541-2014, 2014.

566 Schafstall, J., Dengler, M., Brandt, P. and Bange, H.: Tidal-induced mixing and diapycnal nutrient fluxes in the
567 Mauritanian upwelling region, J. Geophys. Res., 115(C10), C10014, doi:10.1029/2009JC005940, 2010.

568 Schlitzer, R.: Ocean Data View, 2016.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

569 Simon, M. and Azam, F.: Protein content and protein synthesis rates of planktonic marine bacteria., Mar. Ecol. Prog.
570 Ser., 51(3), 201–213, 1989.

571 Smith, D. C. and Azam, F.: A simple , economical method for measuring bacterial protein synthesis rates in seawater
572 using 3H-leucine, Mar. Microb. Food Web, 6(2), 107–114, 1992.

573 Steinfeldt, R., Sültenfuß, J., Dengler, M., Fischer, T. and Rhein, M.: Coastal upwelling off Peru and Mauritania
574 inferred from helium isotope disequilibrium, Biogeoscience, 12, 7519–7533, doi:10.5194/bg-12-7519-2015, 2015.

575 Stramma, L., Schmidtko, S., Levin, L. A. and Johnson, G. C.: Ocean oxygen minima expansions and their biological
576 impacts, Deep Sea Res. Part I Oceanogr. Res. Pap., 57(4), 587–595, doi:10.1016/j.dsr.2010.01.005, 2010.

577 Sugimura, Y. and Suzuki, Y.: A high-temperature catalytic oxidation method for the determination of non-volatile
578 dissolved organic carbon in seawater by direct injection of a liquid sample, Mar. Chem., 24(2), 105–131,
579 doi:10.1016/0304-4203(88)90043-6, 1988.

580 Taylor, G. T., Thunell, R., Varela, R., Benitez-Nelson, C. and Scranton, M. I.: Hydrolytic ectoenzyme activity
581 associated with suspended and sinking organic particles within the anoxic Cariaco Basin, Deep Sea Res. I, 56(8),
582 1266–1283, doi:10.1016/j.dsr.2009.02.006, 2009.

583 Thamdrup, B., Dalsgaard, T. and Revsbech, N. P.: Widespread functional anoxia in the oxygen minimum zone of the
584 Eastern South Pacific, Deep Sea Res. Part I Oceanogr. Res. Pap., 65, 36–45, doi:10.1016/j.dsr.2012.03.001, 2012.

585 Thrash, C. J., Seitz, K. W., Baker, B. J., Temperton, B., Gillies, L. E., Rabalais, N. N., Henrissat, B. and Mason, U.:
586 Metabolic roles of uncultivated bacterioplankton lineages in the northern Gulf of Mexico “Dead Zone,” MBio, 8(5),
587 1–20, doi:10.1128/mBio.01017-17, 2017.

588 Ward, B. B.: How nitrogen is lost, Science ., 341(6144), 352–353, doi:10.1126/science.1240314, 2013.

589 Weiss, M., Abele, U., Weckesser, J., Welte, W., Schiltz, E. and Schulz, G.: Molecular architecture and electrostatic
590 properties of a bacterial porin, Science ., 254(5038), 1627–1630, doi:10.1126/science.1721242, 1991.

591 Winkler, W. L.: Die Bestimmung des im Wasser gelösten Sauerstoffes., Berichte der Dtsch. Chem. Gesellschaft,
592 21(2), 2843–2854, doi:10.1002/cber.188802102122, 1888.

593

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

Figure legends

Figure 1: Station map. All presented stations in the Eastern Tropical South Pacific off Peru sampled in 2017. For detailed informations about the stations see supplementary Table 1.

Figure 2: Biotic and abiotic conditions at selected stations exemplary for the sampling conditions. Chlorophyll **(a)**, temperature **(b)**, total dissolved nitrogen (TDN) **(c)**, dissolved organic carbon (DOC) **(d),** carbon content of dissolved hydrolysable amino acids (DHAA) **(e)** and carbon content of high molecular weight dissolved carbohydrates (DCHO) **(f)** over depth at different stations from on- to offshore off Peru.

Figure 3: Bacterial growth activity at different *in situ* oxygen concentrations from on- to offshore off Peru. Oxygen concentrations **(a)**, total bacterial production (BP) **(b)**, bacterial abundance **(c)** cell-specific BP **(d)** over the upper 800 m depth with a zoom in the upper 100 m (small plots).

Figure 4: Extracellular enzyme rates at different *in situ* oxygen concentrations. Oxygen concentrations **(a)**, degradation rates of dissolved amino acids (DHAA) by leucine-aminopeptidase (LAPase) **(b)**, degradation rates of high molecular weight dissolved carbohydrates (DCHO) by ß-glucosidase (GLUCase) **(c)** total potential LAPase rates (V_{max}) **(d)**, Glucase V_{max} **(e)**, cell abundance **(f)**, cell-specific degradation rates DHAA by LAPase **(g)**, cell-specific degradation rates of DCHO by GLUCase **(h)**, cell-specific LAPase V_{max} **(i)** and cell-specific Glucase V_{max} **(j)** at different oxygen regimes off Peru.

Figure 5: Measured concentrations and calculated proxies for the change of dissolved organic carbon (DOC) and dissolved oxygen (DO) flux over depth for stations G-T: The average diapycnal diffusivity of mass (K_ρ) over depth with confidence interval and the constant K_ρ ($1 \times 10^{-3}\ m^2 s^{-1}$) that was used for further calculations **(a)**. Concentrations of DOC in the upper 100 m and the resulting change of DOC flux over depth ($\nabla\Phi$) **(b)**. Concentrations of DO in the upper 100 m and the resulting change of DO flux over depth ($\nabla\Phi$) **(c)**.

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

620 **Tables**

621 **Table 1:** Estimates of oxygen and DOC loss over depth based on *in situ* physical observations and bacterial rate measurements. Oxygen and DOC loss rates were estimated from the
622 change in oxygen and DOC fluxes over depth. The bacterial uptake of DOC was calculated from bacterial production based on a growth efficiency of 10 and 30% (DOC uptake$_{\phi}$). The
623 bacterial oxygen demand (BOD) and bacterial growth efficiency (BGE$_{\varepsilon}$) was calculated from bacterial production and the assumption that DOC loss can be completely explained by
624 bacterial uptake (BOD$_{\varepsilon}$) or estimated based on a BGE of 10 and 30% (BOD$_{\phi}$).

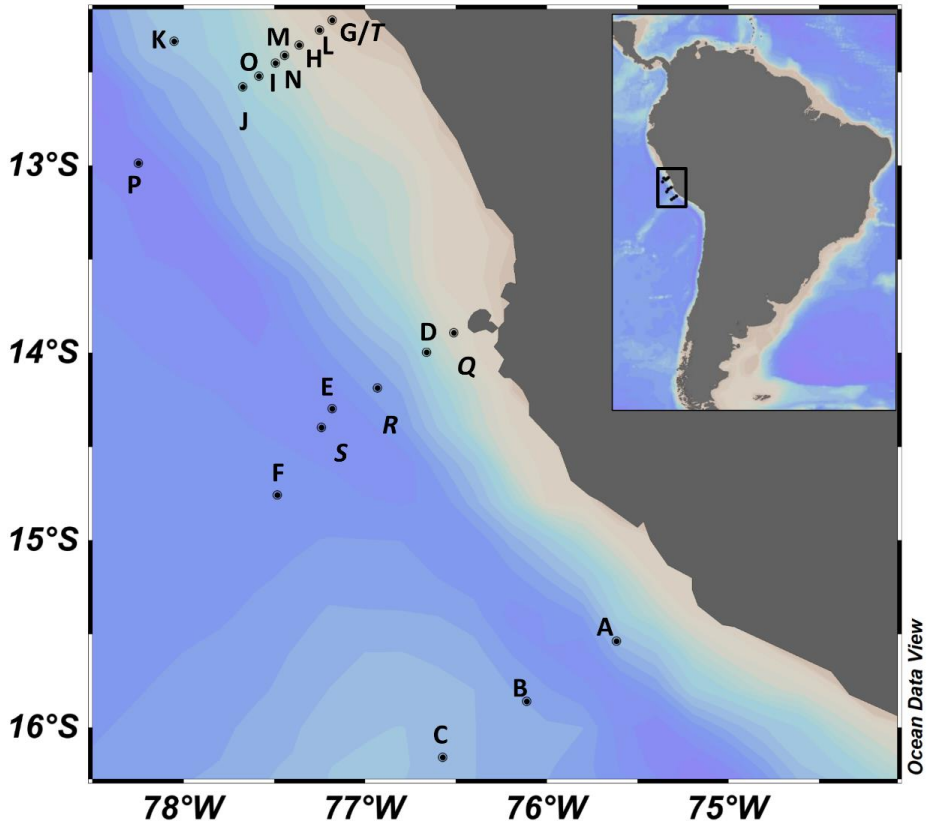
625

Depth	oxygen loss	DOC loss	DOCuptake$_{\phi 10}$			DOC uptake$_{\phi 30}$			Bacterial Production			BOD $_{\varepsilon}$			BOD$_{\phi 10}$			BOD$_{\phi 30}$			BGE $_{\varepsilon}$		
[m]	[mmol m^{-3} d^{-1}]																				%		
	avg	avg	avg	min	max	avg	min	max	avg	min	max	avg	min	max	avg	min	max	avg	min	max	avg	min	max
MLD-40	10.23	3.4	0.90	0.17	2.92	0.30	0.06	0.97	0.09	0.02	0.29	3.3	3.1	3.38	0.81	0.15	2.63	0.21	0.04	0.68	2.65	0.50	8.62
40-60	5.55	1.13	0.30	0.12	0.79	0.10	0.04	0.26	0.03	0.01	0.08	1.1	1.05	1.12	0.27	0.11	0.72	0.07	0.03	0.19	2.67	1.10	7.04

626

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

627 **Figures**



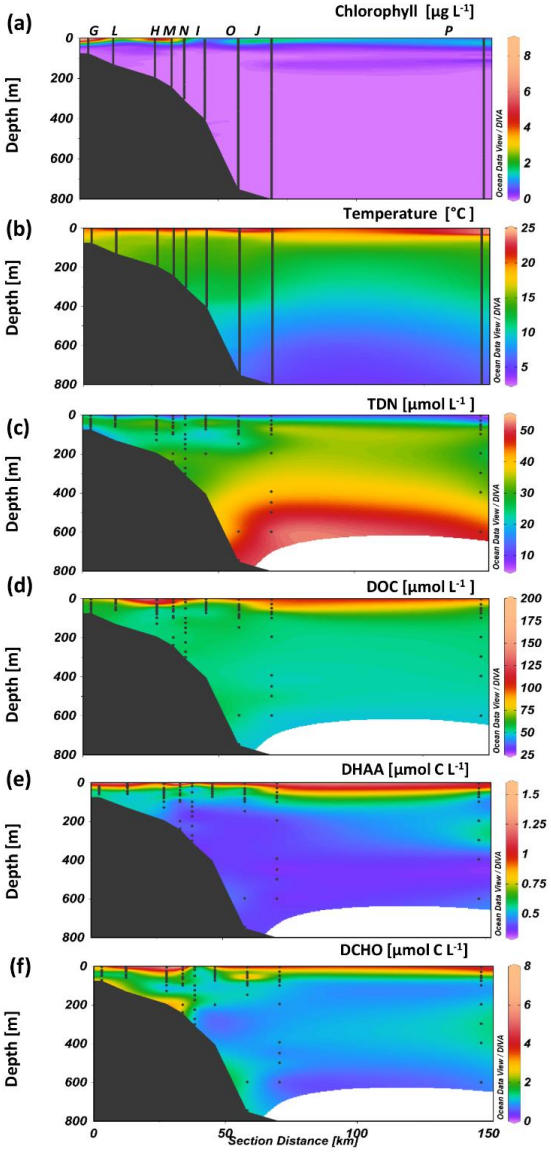
628

629 **Figure 1**

630

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

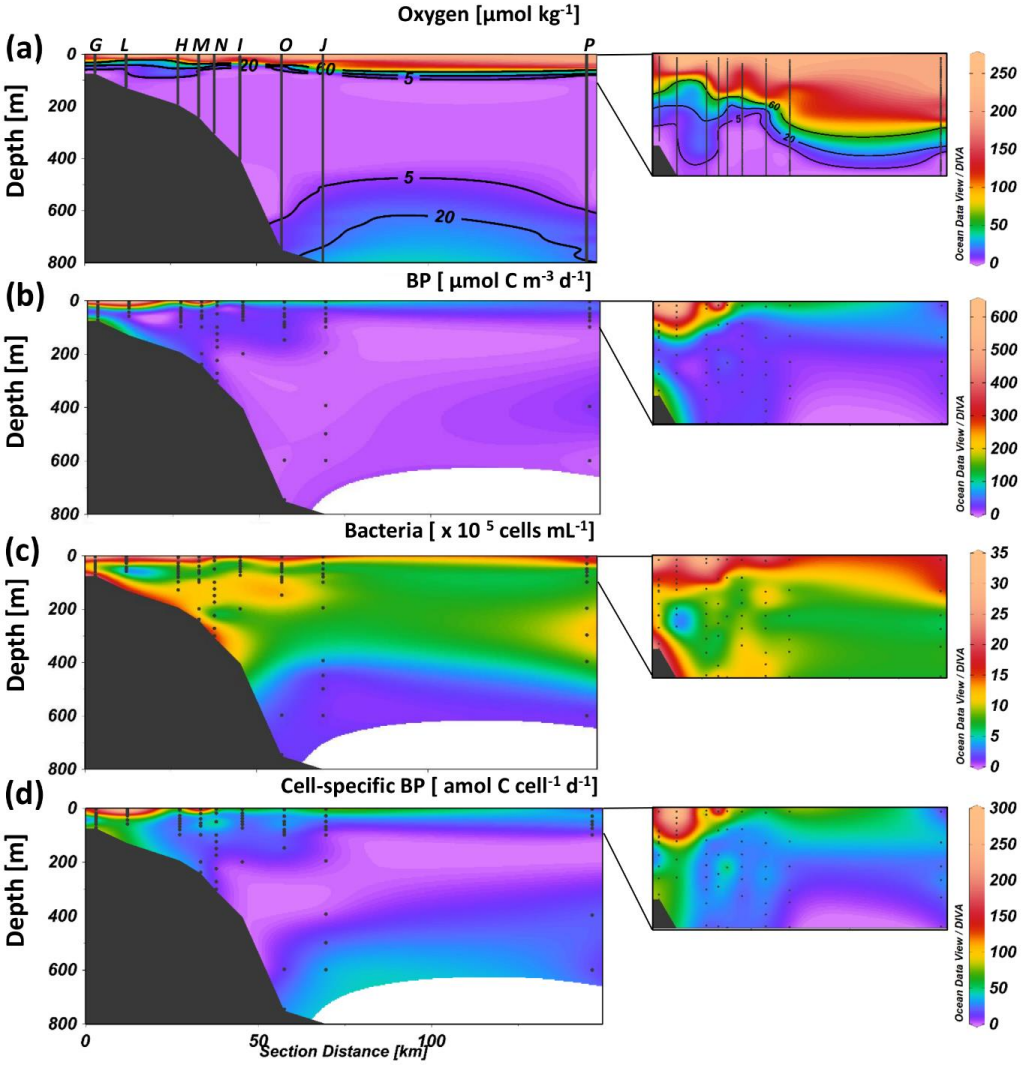
Biogeosciences
Discussions
Open Access
EGU



631

632 **Figure 2**

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019



633

634 **Figure 3**

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019

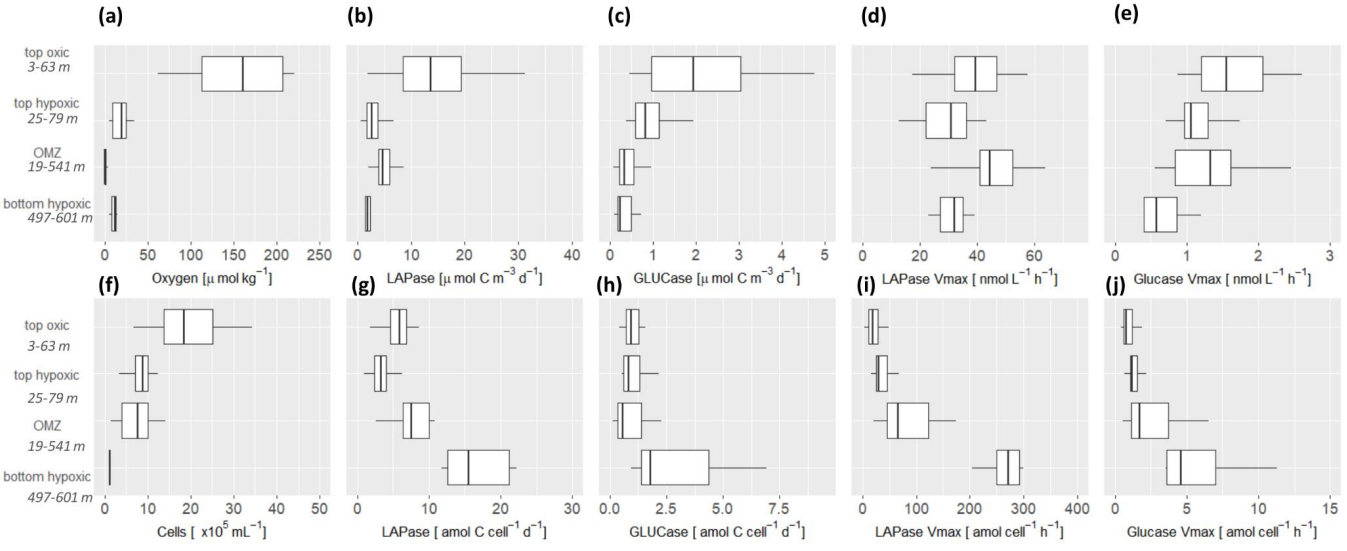
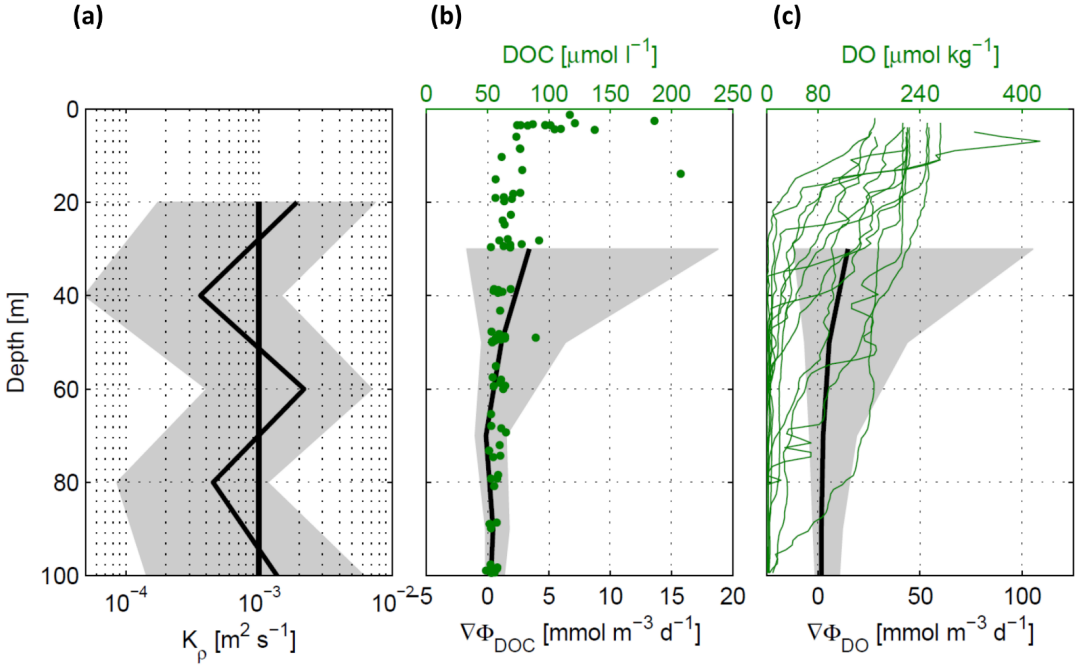


Figure 4

https://doi.org/10.5194/bg-2019-237
Preprint. Discussion started: 17 June 2019



641

642

643

644 **Figure 5**