Answers to the first Referee

comments from referees/public:

The manuscript by Maßmig et al. shows interesting results from two cruises in the ETSP OMZ off Peru. The combination of DOC, TDN, DHAA and DCHO with bacterial production and extracellular enzyme rates provides a nice overview of the microbial activity in general terms. Authors also show diapycnal fluxes for oxygen and DOC, including the potential role of microbial processes into those total fluxes. A similar manuscript has been recently published by the same authors (Loginova et al. 2019 Biogeosciences, 16). DOC, TDN, DON, DHAA, DCHO and diapycnal DOC and oxygen fluxes were also measured/estimated in a previous cruise in the same area. It is clear that the present study includes other data but discussion lacks a comparison between both studies and some results/conclusions seems to be repeated. For instance, the 33% of oxygen loss over depth attributed to bacterial oxygen demand is quite similar than in the previous study (38%). Please extend the discussion and comparison with the previous manuscript.

author's response:

We thank the reviewer for this comment and included a comparison between our study and Loginova et al. 2019 in the revised version of the manuscript. Additionally, we included a paragraph concerning the seasonality of the Peruvian system in the introduction (see comment of second Reviewer concerning line 73):

author's changes in manuscript (page and line numbers refer to the revised manuscript pdf):

page 14 line 445

"Loginova et al. (2019) conducted similar physical rate measurements in the same study area with ~2 and ~10 times lower DOC and oxygen loss in the upper ~40 m compared to our study. Differences in loss rates were mainly caused by a ~ 10 times higher diapycnal diffusivity of mass in our study. This may have been caused by weaker stratification in the upper 100 m depth or differences in the turbulence conditions. Loginova et al. (2019) estimated a contribution of bacterial DOM degradation to oxygen loss (38 %) based on the loss of labile DOC (DHAA and DCHO). This value agrees well with our estimates of 18-33% of total oxygen loss, calculated under the assumption that DOC loss is solely attributed to bacterial degradation. However, the comparison of DOC and oxygen loss within each study revealed different patterns. Loginova et al. (2019) found a loss of DOC that clearly exceeded the loss of oxygen within the upper ~40 m. Hence, respiration of DOC could fully explain the observed oxygen loss in that study. In our study, more oxygen than DOC was lost over depth (Table 1). This loss of oxygen needs additional explanations such as degradation of particulate organic matter and physical mixing processes. One reason for the observed differences between the two studies that have been conducted in the same region might be seasonality. The study by Loginova et al. (2019) took place in austral summer, whereas our data were gained during austral winter. Water temperature was quite similar during both studies, probably due to the coastal El Niño one month before our sampling campaign (Garreaud, 2018). Still, the study by Loginova et al. (2019) included more stations with high Chl *a* concentrations (~8 µg L^{-1}), as typical for the austral summer, indicating a more productive system with more labile DOM (DCHO and DHAA). Prevalence of more labile DOM might explain the higher contribution of microbial DOM respiration to oxygen loss in the study by Loginova et al. (2019). Additionally, Loginova et al. (2019)

sampled with a much higher vertical resolution within the upper 140 m, restricting the direct comparability with our study. “

comments from referees/public:

The stations were sampled in two cruises (April and June) and distributed in three transects perpendicular to the coast: Lima, Paracas and Puerto Caballas (approx.). Spatial and temporal variability is however not considered in the manuscript. Some data correspond to some transects and cruise and other data correspond to other but no clear differentiation is included. Substances concentrations and fluxes were measured in Lima and Paracas transects in April, but enzymatic activity was measured in Paracas and Puerto Caballas in June. These data are however pooled and used for all the later estimations without any further consideration of spatiotemporal differences. Only one transect (Lima) is shown in Figures 2-3, are the conditions equal in the other transects (Temperature, Oxygen, Chlorophyl: : :)?

author's response:

With our approach we focus on possible differences between oxygen regimes. Hence, statistics of bacterial production and of extracellular enzyme rates are always related to the different oxygen concentrations. However, we included figures of oxygen and Chl *a* concentration and temperature for the remaining stations in the supplement (supplementary Fig. 1). Moreover, a more differentiated description of the study site and a comparison between cruises has been included in section 3.1:

author's changes in manuscript:

page 8 line 244

“During our two cruises to the Peruvian upwelling system (Fig. 1), maximum Chl *a* concentration was higher and temperatures were warmer in April compared to June 2017, probably representing seasonal variability. Chl *a* concentration reached up to 11 and 4 µg l^{-1} within the upper 25 m in April and June, respectively. Still, average Chl *a* concentration at depth <10 m (M136: 3.1±2.6 µg l^{-1}; M138: 2.8 ± 1.3 µg l^{-1}) were not significantly different between the two cruises. At depths >50 m, Chl *a* concentration was generally below detection limit (Fig. 3a, supplementary Fig. 1). At depth < 10 m the water was warmer in April (21.3 ±1.6°C) than in June (17.6 ± 0.6°C) (Fig. 3b, supplementary Fig. 1). Oxygen concentration >100 µmol kg^{-1} was observed in the surface mixed layer. Oxygen decreased steeply with depth, reached suboxic concentrations (<5 µmol kg^{-1}) at > 60 ± 24 m (Fig. 2c, 4a and 5a, supplementary Fig.1) and fell below detection of Winkler titration. For further analysis and within the text *in situ* oxygen concentrations <5 µmol O_2 kg^{-1} are referred to as “suboxic”. Shallowest depth with suboxic oxygen concentrations was 14 m in April (station Q) and 29 m in June (station D), probably representing that station Q was situated closer to the shore than station D. Oxygen increased again to up to 15 µmol kg^{-1} at >500 m (Fig. 4a and 5a, supplementary Fig. 1). TDN concentrations increased with depth from 18±8 µmol l^{-1} and 22±7 µmol l^{-1} within the upper 20 m in April and June, respectively, and reached a maximum of 54 µmol l^{-1} at 850 m (Fig. 3c). DOC decreased with depth from 94 ±37 µmol l^{-1} and 69 ±12 µmol l^{-1} in the upper 20 m in April and June, respectively, to lowest values of 37 µmol l^{-1} at 850 m. The steepest gradient in DOC concentration was observed in the upper 20-60 m (Fig. 2b and 3d) during both cruises.”

comments from referees/public:

Title: It does not reflect the measurements performed in the study. “Bacterial organic carbon uptake” was not measured.

author's response:

In the revised version we changed the title.

author's changes in manuscript:

page 1 line 1

“Bacterial degradation activity in the Eastern Tropical South Pacific oxygen minimum zone”

comments from referees/public:

L19: Bacterial growth efficiency was taken from Rivkin and Legrende (2001) as a simple function of temperature. It should not be considered as a result from the present study.

author's response:

In our study, we estimated bacterial growth efficiency (BGE) by two independent methods as explained in chapter 2.5. One approximation includes the water temperature and uses the equation from Rivkin and Legendre (2001), the other is based on measured bacterial production and DOC loss rates. The BGE referred to within the abstract was calculated with latter method and is therefore a result of this study and independent from Rivkin and Legendre (2001). The results are described in section 3.3 and discussed in the discussion.

comments from referees/public:

L25: Gruber et al. is a good reference for global scale processes and future conditions, however, a better reference for the measurement of anoxic conditions in the ETNP OMZs would be: Tiano et al. 2014. Deep-Sea Res. Part I. 94, 173-183.

author's response:

Thank you, we included this reference, in the revised version.

comments from referees/public:

L28: One classical reference dealing with the extention and volumens of the different OMZs is Paulmier & Ruiz-Pino 2009. Progress in Oceanography 80, 113-128.

author's response:

Thank you, we included this reference, in the revised version.

comments from referees/public:

L36-37: DNRA might result in lower metabolic energy yield, but it is not a mayor pathway in OMZs. Although it has been found in the ETSP, it showed sporadic and low rates (Kalvelage et al. 2013). On the other hand, denitrification might be considered one of the main anaerobic heterotrophic process but it is yielding 99% of the energy compared to aerobic respiration, i.e. it is almost equally efficient. This paragraph seems to be biased to introduce the idea of inefficient anaerobic metabolism, but it is not proved.

author's response:

We modified the paragraph and focus more on previous observations of reduced carbon fluxes in OMZs.

author's changes in manuscript:

page 2 line 44

"Within OMZs, enhanced vertical carbon export has been observed (Devol and Hartnett, 2001; Roullier et al., 2014) and explained by potentially reduced remineralization of organic matter in suboxic and anoxic waters."

author's response:

Further, we added a reference showing that the energy yield gained by denitrifying bacteria is even less than suggested by the chemical equations:

author's changes in manuscript:

page 2 line 49

"Additionally, the energy yield available for the production of cell mass seems to be less than suggested by the chemical equations (Strohm et al., 2007)."

comments from referees/public:

L51-58: The effect of oxygen concentration on bacterial production and extracellular enzymes activity was ambiguous before the comment of G.Taylor. When the differential particulate organic matter was considered, hydrolytic rates were similar. This paragraph needs then some rewording because the study is not clearly justified now.

author's response:

We changed the paragraph in the revised version:

author's changes in manuscript:

page 3 line 66

"Investigations of hydrolysis rates as the initial step of organic matter degradation, may help to unravel possible adaptation strategies of bacterial communities to suboxic and anoxic conditions (Hoppe et al., 2002). High extracellular enzyme rates might compensate a putative lower energy yield of anaerobic respiration and the subsequent biogeochemical effects. However, very few studies have investigated the effect of oxygen on hydrolytic rates, so far. Hoppe et al. (1990) did not find differences between oxic and anoxic incubations of Baltic Sea water. In the Cariaco Basin, hydrolytic rates were significantly higher in oxic compared to anoxic water (Taylor et al., 2009). However, this difference did not persist after rates were normalized to particulate organic matter concentration. The dependence of hydrolysis rates on organic matter concentrations described by Taylor et al. (2009), suggest that productivity may play a role for extracellular enzymatic rates in oxygen depleted systems. The Peruvian upwelling system displays high amounts of labile organic matter (Loginova et al., 2019) at shallow oxyclines and thus allows for studying effects of low oxygen on extracellular enzyme rates under substrate replete conditions."

comments from referees/public:

L61-62: Again, I disagree with the "lower efficiency of anaerobic respiration" (unless other processes different than denitrification are proved to be relevant).

author's response:

The term “lower efficiency of anaerobic respiration” has been changed to “energy yield”

comments from referees/public:

L86-87: It is not clear for me if the filter or the ampule were rinsed with the sample.

author's response:

In the revised version, we clarified that the filter was rinsed with the sample. The ampules were combusted (500°C/ 8 h) and should not contain any organic carbon.

author's changes in manuscript:

page 4 line 100

“Samples were filtered through a syringe filter (0.45 µm glass microfiber GD/X membrane, Whatman 115 ™), that was rinsed with 50 ml sample, into a combusted glass ampoule (8 h, 500 °C).”

comments from referees/public:

L96-106: To be consistent, what is the detection limit and precision of the DHAA and DCHO analysis?

author's response:

We added this information:

author's changes in manuscript:

page 5 line 119

“Detection limit of DHAA was 1.4 nmol L^{-1} depending on amino acid and 10 nmol L^{-1} for DCHO. The precision was 2% and 5% for DHAA and DCHO, respectively.”

comments from referees/public:

L116: Fig. 5 is cited before Fig. 2.

author's response:

This has been improved in the revised version.

author's changes in manuscript:

Figure order has been changed.

comments from referees/public:

L131: Bacterial Production was measured at 13°C for all samples. Considering the range of temperatures found along the water column (7-24 °C), incubation temperature was up to 12°C off the in situ temperature. There were no compensation for the temperature variation, probably leading to significant deviation from in situ estimates. Considering the relevance of these results for the discussion, authors should correct measured rates with in situ temperature.

author's response:

In the revised version of the manuscript, we take *in situ* temperature into account when calculating bacterial production following the approach by López-Urrutia and Morán (2007). All calculations, figures and discussions throughout the text have been adapted.

author's changes in manuscript:

page 5 line 151

"The incubation of samples at a constant temperature of 13°C resulted in deviations of max. 11°C between incubation ($T_{incubation}$) and *in situ* temperatures (T_{insitu}). In order to estimate *in situ* bacterial production from measured bacterial production during incubations, measured temperature differences were taken into account following the approach of López-Urrutia and Morán (2007). First, the temperature difference between T_{insitu} and $T_{incubation}$ (δT) was computed in electron volt (ev^{-1}), after T_{insitu} and $T_{incubation}$ (K) had been multiplied with the Boltzmann's constant *k (8.62x10^{-5} eV K^{-1}):*

1. $$\delta T\ [ev^{-1}] = \frac{1}{T_{incubation}[K]\ x\ k\ [evK^{-1}]} - \frac{1}{T_{insitu}[K]\ x\ k\ [evK^{-1}]}$$

The decadal logarithm of *in situ* bacterial production ($\log_{10} BP_{insitu}$) was then calculated from the decadal logarithm of measured bacterial production during incubations ($\log_{10} BP_{incubation}$). Therefore we applied three different factors (*F*) depending on *in situ* Chl *a* concentration as proposed by López-Urrutia and Morán (2007); with *F* being -0.583, -0.5 and -0.42 [$fgCcell^{-1}d^{-1}ev$] for <0.5, 0.5-2 and >2 µg Chl *a* L^{-1}, respectively:

2. $$log_{10}BP_{insitu}[fgCcell^{-1}d^{-1}] =$$

$$log_{10}BP_{incubation}[fgCcell^{-1}d^{-1}]\ +\ \delta T\ [ev^{-1}]x\ F\ [fgCcell^{-1}d^{-1}ev]$$

Within the text, figures, equations and statistic results it is always referred to temperature corrected *in situ* bacterial production. Temperature corrected bacterial production and original bacterial production measured during incubation can be compared in supplementary Table 2."

comments from referees/public:

L154: Enzymatic rates were also measured at a fixed temperature of 13°C. Could in situ temperature be taken into account?

author's response:

In the revised manuscript, we applied a temperature correction for the extracellular enzyme rates to account for the differences between *in situ* and incubation temperature. The correction factor was based on differences in extracellular enzyme rates after incubations at 22.4°C and 13°C at five stations during the cruises. All calculations, figures and discussions throughout the text were adapted.

author's changes in manuscript:

.**page 7 line 209**

"Similar to bacterial production, *in situ* extracellular enzyme rates were estimated based on extracellular enzyme rates measured during incubation. To account for the differences between *in situ* and incubation temperatures a correction factor (*F*) was applied based on differences in extracellular enzyme rates after additional incubations at 22.4°C next to the regular incubations at 13°C at five stations during the cruises. The fluorescence signals at different substrate concentrations increased on average by a factor of 0.05 and 0.03 ($°C^{-1}$) for GLUCase and LAPase, respectively. Under the assumption that the increase in rates with temperature was linear, measured enzyme rates were adapted to *in situ* temperature, with (EER_{insitu}*; nmol*

$L^{-1}\ h^{-1}$) and ($EER_{incubation}$) being the *in situ* extracellular enzyme rates and extracellular enzyme rates during incubation, respectively:

1. $\delta T\ [°C] = T_{insitu}[°C] - T_{incubation}\ [°C]$

2. $EER_{insitu}[nmolL^{-1}h^{-1}] =$

$$EER_{incubation}[nmolL^{-1}h^{-1}] + EER_{incubation}[nmolL^{-1}h^{-1}]\ x\ F\ [°C^{-1}]\ x\ \delta T\ [°C]$$

Within the text, figures, equations and statistic results it is always referred to the temperature corrected *in situ* extracellular enzyme rates. Temperature corrected extracellular enzyme rates and original extracellular enzyme rates measured during incubation can be compared in supplementary Table 2."

comments from referees/public:

L159-160: Please improve the description of the "Gas tight incubator". Considering the oxygen concentration values in your "low oxygen" incubations (8-40 umol/kg), how realistic are the conclusions applied to the anoxic core from these incubations? Oxygen concentrations of 8 uM are way above the Km for microbial processes such as Oxygen respiration, ammonium and nitrite oxidation, for instance, and above the inhibition values for anammox and denitrification. Please, include in the discussion the possible limitation of the measurements considering the high oxygen values achieved in the incubations.

author's response:

In the revised version we provide further information in section 2.6:

author's changes in manuscript:

page 6 line 188

"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 had two openings to fill and flush it with gas. For our experiment the incubator was flushed and filled with N_2, to reduce oxygen concentrations. Still control measurements occasionally revealed oxygen concentrations of 8 to 40 µmol O_2 kg^{-1}. Additionally, samples were in contact with oxygen during pipetting and measurement."

author's response:

Further, we included in the discussion that results have to be interpreted with care

author's changes in manuscript:

page 11 line 344

"The extracellular enzymes rates of our study have to be interpreted carefully since incubation was not fully anoxic and the remaining oxygen might have biased the results. Still, we assume that most extracellular enzymes were present at the time of sampling and thus oxygen contamination during the incubations did not strongly influence the rate measurements."

comments from referees/public:

L201 (and L314): TDN includes the inorganic fraction. Nitrate in OMZs increases with depth, and might reach values up to 30-40 uM (example: Lam et al. 2009. Proceedings of the National Academy of Sciences 106, 4752-

4757), which might represent 80-100% of the measured TDN. Could the authors include inorganic nutrients and use DON instead?

author's response:

Because the fraction of DON in TDN is low compared to DIN, especially at depth, DON obtained by subtracting DIN from TDN has a relatively high error. Moreover, bacteria may also use DIN. We therefore think that for the purpose of this study, TDN is the more accurate value (see also answer to comment on line 314).

comments from referees/public:

L261-270: It is not clear how the parameters (DOC loss) have been calculated, only ranges are shown and it feels like the ranges have been subtracted without including the apparent heterogeneity of the different stations. Based on the data shown in Fig. 5, the large differences in the oxyclines must result in large differences in diapycnal oxygen fluxes. Some separation in the data shown in Fig. 5 would be advisable. Anoxic conditions are reached at depths varying from 20 to 100 m, probably with very different values for the measured variables (Chl a, DOC: : :) too. Contrary, DOC values change quickly in the first 10 m, but seems to be relatively constant below. Dots are not connected with lines so it seems to be a pool of data without a clear pattern. All the station seems to be the same.

author's response:

We thank the reviewer for this advice. In the revised version, we now show DOC concentrations at the different stations by a line plot instead of a dotchart (new Fig. 2). Since the DOC flux was calculated for each station separately, we accounted for differences between the stations (see section 2.3).

comments from referees/public:

L275: DNRA might have lower energy yield, it is not so low for denitrification.

author's response:

We thank the reviewer for this comment. We found a study showing a lower energy yield of denitrification than expected (see answer to comment concerning 36-37). Still, we justify our hypothesis of reduced bacterial activity within suboxic waters compared to the oxyclines by previous observations of reduced carbon fluxes in OMZ.

author's changes in manuscript:

page 11 line 335

"We expected reduced rates of organic matter degradation within oxygen depleted waters, since reduced bacterial degradation activity might explain enhanced carbon fluxes in suboxic and anoxic waters (Devol and Hartnett 2001)"

comments from referees/public:

L291-292: I would delete "nitrous oxide" otherwise further explanation is needed as the contribution from anammox to N2O production is quite reduced.

author's response:

We thank the reviewer for this advice and deleted “nitrous oxide”

comments from referees/public:

L296-297: Remove “respiratory” from “autotrophic anaerobic respiratory pathway”. Babbin et al (2014, Science 344, 406-408) and Kalvelage et al. (2013. Nature Geosciences 6, 228-234) are also appropriate references for that quote. In addition, I would delete the sentence in L298-299, denitrification+anammox are included in the global estimations for N losses.

author's response:

We included the references and removed the word “respiratory”. However, we do not understand the ambition to remove the sentence: ”Our data indeed showed enhanced degradation of amino-acid-containing organic matter in low oxygen waters”. This sentence does not indicate that denitrification and anammox are not included in the global estimation of N loss. It only indicates that our data are in line with the theory of high degradation of nitrogen compounds that might fuel anaerobic respiratory processes.

comments from referees/public:

L301-307: This section exceed the results obtained in the present manuscript. A possible link to N cycle could be pointed, but the connection between hydrolysis and coupled denitrification-anammox is not supported.

author's response:

In the revised version of the manuscript, we strictly separate the direct interpretation of our results and possible implications:

author's changes in manuscript:

page 11 line 358

“. . . Meanwhile, a preferential degradation of amino acid containing organic matter in suboxic waters compared to oxic waters has been suggested (Van Mooy et al., 2002). Degradation of nitrogen compounds by heterotrophic bacteria (e.g. denitrifiers) in suboxic waters enables the release of ammonia and nitrite and subsequently may support anammox, an autotrophic anaerobic pathway (Babbin et al., 2014; Kalvelage et al., 2013; Lam and Kuypers, 2011; Ward, 2013). This interaction between denitrifiers and anammox bacteria could 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. 5b, d). Therefore, observed LAPase rates are in line with the hypothesis of preferential degradation of nitrogen compounds under suboxia. However, simultaneous rate measurements of protein hydrolysis, nitrate reduction (e.g. denitrification) and anammox are needed to prove an indirect stimulation of anammox by protein hydrolysis via denitrification. A close coupling between anammox and nitrate reducing bacteria has previously been shown for wastewater treatments. There, nitrate reducers directly take up 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 are separated in space and time (Dalsgaard et al., 2012), potentially weakening a direct inter-dependency."

comments from referees/public:

L314-316: Inorganic nitrogen might be the mayor fraction of TDN. This fact must be taken into account, especially if any stimulation of metabolism is considered.

author's response:

We would like to stick to TDN (see explanation above). However, we included more detailed information about a possible contribution of TDN to cell growth and activity:

author's changes in manuscript:

page 12 line 383

"While labile organic matter is decreasing with depth (e.g. Loginova et al., 2019), TDN (Fig. 3c), especially inorganic nitrogen is increasing with depth. Thus, high concentrations of inorganic nitrogen at the lower oxycline are available for heterotrophic and chemoautotrophic energy gains. For instance, the co-occurrence of nitrate reduction, that was still detected at 25 µmol O_2 L^{-1}, and microaerobic respiration might have stimulated cell-specific production or the accumulation of especially active bacterial species (Kalvelage et al., 2011, 2015)."

comments from referees/public:

L317-322 and L323-331: These paragraphs seem to be not finished. There are no clear conclusion for the discussion of these results.

author's response:

We thank the reviewer for this remark and finished the paragraphs with a concluding sentence in the revised version of the manuscript

author's changes in manuscript:

page 13 line 389

"Depth distribution of cell-specific and total bacterial production was different (Fig. 4b, d and supplementary Fig. 2); cell-specific production was significantly reduced in suboxic waters, while total production was more similar in suboxic waters compared to the oxycline. This suggests that lower cell-specific production was compensated by higher cell abundance within the suboxic waters (Fig. 4c), resulting in an overall unhampered bacterial organic matter cycling in the OMZ core. One reason for the accumulation of cells within the OMZ might be reduced predation, suggesting the OMZ core as an ecological niche for slowly growing bacteria. Reduced grazing by bacterivores thus preserves bacterial biomass in suboxic waters from entering into the food chain. This way of bacterial biomass preservation has been suggested as possible explanation for enhanced carbon preservation in anoxic sediments by Lee (1992), and may also explain our observations for the anoxic water column."

author's changes in manuscript:

page 13 line 404

389 ” For instance, SAR406, SAR202, ACD39 and PAUC34f have the genetic potential for the turnover of
390 complex carbohydrates and anaerobic respiratory processes, in the Gulf of Mexico (Thrash et al., 2017).
391 Consequently, our findings of active bacterial degradation of DOM are supported by molecular biological
392 studies. Still, simultaneous measurements of bacterial degradation and production have to be combined
393 with molecular analysis, in future studies off Peru..”
394

395 **comments from referees/public:**

396 L346-347: According to M&M, BGE followed the established temperature dependence. If no other parameter was
397 used for its calculation, I cannot see how the results of this manuscript for this calculated (but not measured)
398 parameter suggest that oxygen availability control bacterial growth efficiency.
399 **author's response:**
400 Please see answer to comment concerning line 19.
401

402 **comments from referees/public:**

403 L365-367: Well, this study provides estimations, but does not provide measurements for carbon and oxygen losses.
404 **author's response:**
405 In the revised version of the manuscript, we emphasize that we only can give estimates.
406 **author's changes in manuscript:**
407 **page 14 line 443**
408 “…gives estimates for carbon and oxygen losses…”
409

410 **comments from referees/public:**

411 L378: Why a BGE of 20% is now assumed? BGE was estimated based on in situ temperature before.
412 **author's response:**
413 We thank the reviewer for this comment. First, we explain the choice of a BGE of 20% in the revised
414 manuscript.
415 **author's changes in manuscript:**
416 **page 15 line 477**
417 ” The amount of carbon oxidized by denitrification based on the studies of Dalsgaard et al. (2012) and
418 Kalvelage et al. (2013) can be converted into bacterial production applying a BGE. The average
419 temperature dependent BGE was 20%. A BGE of 20% agrees well with other studies (Del Giorgio and
420 Cole, 1998). Assuming a BGE of 20%, the denitrification rates of Dalsgaard et al. (2012) and Kalvelage
421 et al. (2013) suggest a bacterial production of ≤5 µmol C m^{-3} d^{-1}, equivalent to only about 14% of total
422 average heterotrophic bacterial production in suboxic waters determined in our study.”
423 **author's response:**
424 In the revised version, we also included calculations that are based on a BGE of 6%. For this we did not
425 focus on the denitrification rates mentioned in the paragraph above, but on the sum of anaerobic carbon
426 oxidation rates including denitrification, DNRA and simple nitrate reduction, as it is also discussed within

the manuscript for a BGE of 20%. Absolut values changed within the revised version because of temperature correction:

author's changes in manuscript:

page 15 line 489

"The same calculation can be repeated assuming a BGE of 6%, which is the average BGE within this study based on DOC loss and bacterial production. Assuming a BGE of 6%, the estimated 109 µmol C m^{-3} d^{-1} that are respired by anaerobic carbon oxidation (Kalvelage et al., 2013) would represent 94% of the carbon uptake. Consequently, 7 µmol C m^{-3} d^{-1}, i.e. 6% of the carbon uptake, are incorporated into the bacterial biomass. A bacterial biomass production of 7 µmol C m^{-3} d^{-1} is even lower than the bacterial production of 27 µmol C m^{-3} d^{-1}, based on a BGE of 20% and cannot explain the average bacterial production measured in suboxic waters during our study (37 µmol C m^{-3} d^{-1}). Therefore, this estimation suggests higher rates of heterotrophic anaerobic respiratory processes than previously measured. Since denitrification rates were not measured directly, the comparability of published denitrification rates and our measurements of bacterial production are limited. However, our data suggest that the carbon oxidation potential off Peru is more evenly horizontally and vertically distributed than expected ..."

comments from referees/public:

L383-390: The presented data for bacterial production can not be directly attributed to denitrification as it was not directly measured and the high oxygen levels during the BP measurements could have inhibited denitrification. The last and conclusive sentence seems to be pretentious.

author's response:

Samples of bacterial production were incubated in closed vials and bubbled with a N2/CO2 mixture (section 2.5). Therefore, we may assume ongoing anoxic respiratory processes such as denitrification. However, we included the following sentence to account for the uncertainty (see also remark above):

author's changes in manuscript:

page 15 line 496

"Since denitrification rates were not measured directly, the comparability of published denitrification rates and our measurements of bacterial production are limited."

comments from referees/public:

L392-400: Conclusions should be more attached to the obtained and proved results of the measurements. The measurements of bacterial production do not allow to prove the dominance of individual pathways and even less to link it with the production of nitrous oxide.

author's response:

We thank the reviewer and deleted the questionable part of the conclusion and instead refer to the search of alternative explanations for the enhanced carbon fluxes in OMZs compared to the oxygenated water (see also comment of the second reviewer line 399).

Answers to the second Referee

comments from referees/public:

This is a useful study investigating the complicated microbial dynamics within oxygen minimum zones with many different biogeochemical and physical measurements made. The authors focus on calculating bacterial production predominantly associated with carbon cycling, but then also use other studies to consider the input of nitrogen cycling and anoxic processes. The manuscript is very well written, generally clear and detailed. I have a few suggestions to revise the text and figures to make some of the points clearer and to hopefully clarify some uncertainties. One point that was not mentionned was that the bacteria were collected from suboxic concentrations but rates measured in oxic conditions I assume? How might the fact the microbes are being oxidised affect your results? It is difficult to work in OMZs and many of the studies cited would have done a similar thing but i think this should be discussed.

author's response:

We thank the reviewer for this comment. Regarding the extracellular enzyme rates, we are aware of having conducted a challenging method. This is because of the trade-off between feasible measurements of extracellular enzyme rates at different substrate concentrations to calculate Vmax and possible contamination of the sample with oxygen. In the revised version, we included an additional sentence to emphasize that results have to be interpreted with care.

author's changes in manuscript:

page 11 line 344

" The extracellular enzymes rates of our study have to be interpreted carefully since incubation was not fully anoxic and the remaining oxygen might have biased the results. Still, we assume that most extracellular enzymes were present at the time of sampling and thus oxygen contamination during the incubations did not strongly influence the rate measurements."

author's response:

Samples of bacterial production were incubated in closed vials and bubbled with a N2/CO2 mixture (section 2.5)., avoiding oxygen contamination during the incubation time. Therefore, we assume that bacterial production was not affected by oxygenation.

comments from referees/public:

Also the authors seemed to switch between top/bottom hypoxic and upper/lower oxycline, which i took to mean the same thing. If not this should be clarified.

author's response:

The term low_hypoxic is defined in the method section 2.7 (>5 to <20 µmol O_2 kg^{-1}). Therefore, it is correct that "bottom_low_hypoxic" is identical to the lower oxycline, since at the lower oxycline oxygen concentrations only increased up to 15 µmol. kg^{-1} The term "upper_low_hypoxic" differs from the term "upper oxycline" since the upper oxycline includes waters with oxygen concentrations between 5 to 60 µmol kg^{-1}. Within the revised version of the manuscript we replaced the statistical test that were until now only done for the "upper_low_hypoxic" waters by statistical tests with samples from the entire oxycline, to be consistent.

comments from referees/public:

Line 16 - Change to 'from the upper AND lower oxyclines', using 'or' makes it seem negative and I had to read it a few times to understand you were saying production was high.

author's response:

In the revised version, we changed the sentence.

author's changes in manuscript:

page 1 line 15

"Nevertheless, high cell-specific bacterial production was observed in samples from oxyclines and cell-specific extracellular enzyme rates were especially high at the lower oxycline, corroborating earlier findings of highly active and distinct micro-aerobic bacterial communities. "

comments from referees/public:

Line 73 - I noticed the transects had data from both cruises. They are quite close together temporally, but even so some discussion on how the data is aggregated and if temporal affects are accounted for is needed. Did you look at the data separately per cruise too? Which data/transects are used in the figures? What is the seasonality like in the region?

author's response:

We thank the reviewer for his/her advice and agree with the proposal to include more information about the different cruises. Therefore, we i) describe the seasonality within the sampling region in the introduction of the revised manuscript, ii) describe the study area in more detail for each cruise and iii) show the oxygen content, chlorophyll *a* concentrations and temperatures for each station in the revised supplement (see also first comment of the first reviewer). However, we prefer not to distinguish between cruises for statistical tests of bacterial production and extracellular enzyme rates, since we focus on possible differences between oxygen regimes. Moreover, bacterial production was only sampled during April and combining extracellular enzyme rates of both cruises increases sampling size. Still, in the revised manuscript we included the represented cruises in the subtitle of the figures.

author's changes in manuscript:

page 2 line 34

i) "In austral winter, upwelling and subsequently the nutrient supply to the surface waters increase (Bakund and Nelson, 1991; Echevin et al., 2008). However, chlorophyll *a* (Chl *a*) concentration is highest in austral summer, with the seasonal amplitude being stronger for surface than for depth averaged Chl *a* concentrations (Echevin et al., 2008). In winter, phytoplankton growth is, next to iron, mainly limited by light due to the deeper mixing, whereas in summer macronutrients can become a limiting factor (Echevin et al., 2008). Further, El Niño–Southern Oscillation may affect organic matter cycling in the area since it affects the depth of the oxycline and therefore the extent of anaerobic processes in the upper water column (Llanillo et al., 2013). During the year of this study (2017), neither a strong La Niña nor a strong El Niño was detected (https://ggweather.com/enso/oni.htm). However, in January, February and March 2017 there was a strong coastal El Niño with enhanced warming (+1.5°C) of sea surface temperatures in the eastern Pacific (Garreaud, 2018)."

author's changes in manuscript:

page 8 line 244

"During our two cruises to the Peruvian upwelling system (Fig. 1), maximum Chl *a* concentration was higher and temperatures were warmer in April compared to June 2017, probably representing seasonal variability. Chl *a* concentration reached up to 11 and 4 µg l^{-1} within the upper 25 m in April and June, respectively. Still, average Chl *a* concentration at depth <10 m (M136: 3.1±2.6 µg l^{-1}; M138: 2.8 ± 1.3 µg l^{-1}) were not significantly different between the two cruises. At depths >50 m, Chl *a* concentration was generally below detection limit (Fig. 3a, supplementary Fig. 1). At depth < 10 m the water was warmer in April (21.3 ±1.6°C) than in June (17.6 ± 0.6°C) (Fig. 3b, supplementary Fig. 1). Oxygen concentration >100 µmol kg^{-1} was observed in the surface mixed layer. Oxygen decreased steeply with depth, reached suboxic concentrations (<5 µmol kg^{-1}) at > 60 ± 24 m (Fig. 2c, 4a and 5a, supplementary Fig.1) and fell below detection of Winkler titration. For further analysis and within the text *in situ* oxygen concentrations <5 µmol O_2 kg^{-1} are referred to as "suboxic". Shallowest depth with suboxic oxygen concentrations was 14 m in April (station Q) and 29 m in June (station D), probably representing that station Q was situated closer to the shore than station D. Oxygen increased again to up to 15 µmol kg^{-1} at >500 m (Fig. 4a and 5a, supplementary Fig. 1). TDN concentrations increased with depth from 18±8 µmol l^{-1} and 22±7 µmol l^{-1} within the upper 20 m in April and June, respectively, and reached a maximum of 54 µmol l^{-1} at 850 m (Fig. 3c). DOC decreased with depth from 94 ±37 µmol l^{-1} and 69 ±12 µmol l^{-1} in the upper 20 m in April and June, respectively, to lowest values of 37 µmol l^{-1} at 850 m. The steepest gradient in DOC concentration was observed in the upper 20-60 m (Fig. 2b and 3d) during both cruises."

comments from referees/public:

Line 139 - citation for first use of BOD and write in full first time used in main text

author's response:

In the revised version, we improved this sentence.

author's changes in manuscript:

page 6 line 168

"The bacterial oxygen demand (BOD; mmol O_2 m^{-3} d^{-1}) is the amount of oxygen needed to fully oxygenize organic carbon that has been taken up and not transformed into biomass by bacterial production (mmol C m^{-3} d^{-1})."

comments from referees/public:

Line 145 - what temperature dependance? Is a conversion used? Cite

author's response:

In the revised version, we included the formula for temperature dependence of the cited study:

author's changes in manuscript:

page 6 line 175

". . . ii) the bacterial growth efficiency (BGE) follows the established temperature dependence (BGE=0.374[±0.04] -0.0104[±0.002]T [°C]), resulting in a BGE between 0.1 and 0.3 in the depth range of 10-60 m and an *in situ* temperature of 14 to 19°C (Rivkin and Legendre, 2001)."

comments from referees/public:

Line 198 - Perhaps place oxygen concentration in Fig. 2 with the other 'standard' oceanographic measurements, as it is a key plot for this paper. I would also add on horizontal layers onto the transect images, i.e. by using black lines to show oxycline/ omz/hypoxic/oxic layers.

author's response:

We appreciate the suggestion of the reviewer. However, we placed the oxygen concentration on purpose as first panel of the new Figure 4, since all statistical analysis are referred to this parameter and therefore it should appear together with the biological rates. This also enables an overview about the respective oxygen concentrations at sampling depth that we further indicated by black lines in the revised version of the manuscript.

comments from referees/public:

Also, what was lowest oxygen concentration, was it 5 umol/L so only suboxic or even lower to maybe anoxic as set by your definitions in the introduction? The study refers to 'suboxic' throughout which makes me think outside of the OMZ, but actually this is the OMZ. Being clear about this early on in the results would help.

author's response:

We thank the reviewer for this advice. Indeed, we only distinguished between suboxic and hypoxic conditions. We are confident that the OMZ core includes zones with oxygen concentrations below our detection limit, as it is described in section 3.1. This is also indicated by increased nitrate concentrations (~6µmol L^{-1}) in the OMZ core, suggesting anaerobic reduction of nitrate (data not included in the manuscript). In the revised version we included a sentence in section 3.1 to clarify which oxygen concentrations were relevant for our statistical analysis.

author's changes in manuscript:

page 8 line 250

"Oxygen decreased steeply with depth, reached suboxic concentrations (<5 µmol kg^{-1}) at > 60 ± 24 m (Fig. 2c, 4a and 5a, supplementary Fig.1) and fell below detection of Winkler titration. For further analysis and within the text *in situ* oxygen concentrations <5 µmol O_2 kg^{-1} are referred to as "suboxic"."

comments from referees/public:

Line 199 - Does this mean OMZ is 100-500 m depth, be explicit

author's response:

Within the revised version, we are more explicit and reformulate this paragraph (see also comment concerning line 73) :

author's changes in manuscript:

page 8 line 250

"Oxygen decreased steeply with depth, reached suboxic concentrations (<5 µmol kg^{-1}) at > 60 ± 24 m (Fig. 2c, 4a and 5a, supplementary Fig.1) and fell below detection of Winkler titration. For further analysis and within the text *in situ* oxygen concentrations <5 µmol O_2 kg^{-1} are referred to as "suboxic". Shallowest depth with suboxic oxygen concentrations was 14 m in April (station Q) and 29 m in June (station D), probably representing that station Q was situated closer to the shore than station D. Oxygen increased again to up to 15 µmol kg^{-1} at >500 m (Fig. 4a and 5a, supplementary Fig. 1)."

author's response:

Consequently, the suboxic waters was between 60 and 500 m.

comments from referees/public:

Line 206 - 'except for most coastal stations' - what happened at these stations?

author's response:

In the revised version we included an additional sentence (bacterial production differs from the submitted manuscript, since bacterial production was corrected for differences between incubation and in situ temperature; see comments of first reviewer):

author's changes in manuscript:

page 9 line 262

"Bacterial production varied strongly throughout the study region and ranged from 0.2 to 2404 µmol C m^{-3} d^{-1} (Fig. 4b), decreased in general from surface to depth (except for the most coastal station) and showed significantly higher rates in the oxygenated surface compared to the OMZ (Fig. 4b). At the most coastal station (G) bacterial production remained high near the bottom depth of 75 m (280 µmol C m^{-3} d^{-1} at 72 m) (Fig. 4b)."

comments from referees/public:

Line 235 - full statistical results in parentheses is great to see and the correct way to present results, however with so many tests and parts of the text in parentheses it stops the flow when reading. Can you shorten the statistical results in some way? Or add a table to the supplementary material?

author's response:

We agree, that the flow of reading is disturbed and included the statistical results in the supplement.

author's changes in manuscript:

see new supplementary Table 2

comments from referees/public:

Line 242 - normalisation completely changes the pattern of production with depth and oxygen, reverses it compared to un-normalised data. It would be good to show this and discuss further, perhaps using scatter plots too.

author's response:

We very much appreciate this comment of the reviewer. Producing the scatter plot helped us to see that the trends between cell-specific and total production are not completely invers. Still, cell-specific production is correlating more strongly with oxygen than total production. Thus, as described in the manuscript, cell abundance seems to counteract the lower cell-specific bacterial production at suboxic oxygen concentrations compared to the oxyclines. A further statistical test revealed that cell-specific production is more similar between suboxic waters and the oxycline, at the coastal stations (G and T). This suggests that the supply of organic matter stimulates bacterial production under suboxia. We included this thought within the discussion of the revised manuscript.

author's changes in manuscript:

page 12 line 376

"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. In our study, differences in cell-specific bacterial production between suboxic waters and the oxycline did not persist at the most coastal stations (G and T). This indicates the stimulation of bacterial activity, including anaerobic respiratory processes, by the high input of labile organic matter. Therefore, our study suggests that a possible impairment of cell-specific bacterial production under suboxia is reduced by supply of organic matter. However, this hypothesis is restricted to a very limited number of samples and should be tested in further studies."

author's response:

The scatter plot with cell-specific and total bacterial production rates in relation to oxygen is included in the supplement of the revised manuscript and referred to within the results.

author's changes in manuscript:

page 10 line 305

"A detailed view at total- and cell-specific bacterial production in dependence of *in-situ* oxygen concentrations, reveals a stronger increase of cell-specific bacterial production, especially at <10 µmol O_2 kg^{-1} at different stations (supplementary Fig. 2)."

comments from referees/public:

Line 243 - Units of 'amol per cell per day' are incredibly low as one may expect from a 'per cell' measurement, but is this comparable with results from other studies?

author's response:

We agree with the reviewer that these rates seem low. Baltar et al. (2009) presents cell-specific production rates in the subtropical Atlantic (Figure 1 c) that varied between ~0.006-0.03 fmol C cell d^{-1} (corresponding to 6-30 amol C cell d^{-1}) between 96 and 503 m depth. Our original measurements ranged between 2-286 amol C cell d^{-1} between surface waters and ~650 m depth. After temperature correction, cell-specific production rates ranged between 1 and 1120 amol C cell d^{-1}. Consequently, our data include the measurement range of the former study in the Atlantic.

comments from referees/public:

Line 284 - Is this finding because experiments were run in oxic conditions, as were some of the studies you cited too. But should consider the affects of exposing microbes from OMZ to oxygen.

author's response:

In the revised version, we included in the discussion that results have to be interpreted with care (see also answer to the first comment of this reviewer).

author's changes in manuscript:

page 11 line 344

"The extracellular enzymes rates of our study have to be interpreted carefully since incubation was not fully anoxic and the remaining oxygen might have biased the results. Still, we assume that most extracellular enzymes were present at the time of sampling and thus oxygen contamination during the incubations did not strongly influence the rate measurements."

comments from referees/public:

Line 375 - where did the data of amount of reduced nitrogen come from, was it Kalvelage? I found this section, whilst interesting, a little hard to follow which numbers were from this study and which from others. For instance, why use BGE from Del Giorgio 1998 when you calculated it in this study?

author's response:

We thank the reviewer for this comment that includes one comment of the first reviewer. First, we explain the choice of a BGE of 20% in the revised manuscript:

author's changes in manuscript:

page 15 line 477

" The amount of carbon oxidized by denitrification based on the studies of Dalsgaard et al. (2012) and Kalvelage et al. (2013) can be converted into bacterial production applying a BGE. The average temperature dependent BGE was 20%. A BGE of 20% agrees well with other studies (Del Giorgio and Cole, 1998). Assuming a BGE of 20%, the denitrification rates of Dalsgaard et al. (2012) and Kalvelage et al. (2013) suggest a bacterial production of ≤5 µmol C m^{-3} d^{-1}, equivalent to only about 14% of total average heterotrophic bacterial production in suboxic waters determined in our study."

author's response:

Second, we clearly indicated the source of data:

author's changes in manuscript:

page 14 line 472

"We compared bacterial production, i.e. rates of carbon incorporation, with denitrification rates previously reported for the South Pacific. Therefore, we converted one mol of reduced nitrogen that were measured by Dalsgaard et al. (2012) and Kalvelage et al. (2013) to 1.25 mol of oxidized carbon after the reaction equation given by Lam and Kuypers (2011). This calculation indicates that on average ≤19 µmol C m^{-3} d^{-1} are oxidized by denitrifying bacteria in the Eastern Tropical Pacific (Dalsgaard et al., 2012; Kalvelage et al., 2013)....."

author's response:

Third, we expanded the calculation and include a BGE of 6%. For this we did not focus on the denitrification rates mentioned in the paragraph above, but on the sum of anaerobic carbon oxidation rates including denitrification, DNRA and simple nitrate reduction, as it is also discussed within the manuscript for a BGE of 20% (line 380). Absolut values changed within the revised version because of temperature correction.

author's changes in manuscript:

page 15 line 489

"The same calculation can be repeated assuming a BGE of 6%, which is the average BGE within this study based on DOC loss and bacterial production. Assuming a BGE of 6%, the estimated 109 µmol C m^{-3} d^{-1} that are respired by anaerobic carbon oxidation (Kalvelage et al., 2013) would represent 94% of the carbon uptake. Consequently, 7 µmol C m^{-3} d^{-1}, i.e. 6% of the carbon uptake, are incorporated into the bacterial biomass. A bacterial biomass production of 7 µmol C m^{-3} d^{-1} is even lower than the bacterial production of 27 µmol C m^{-3} d^{-1}, based on a BGE of 20% and cannot explain the average bacterial production measured in suboxic waters during our study (37 µmol C m^{-3} d^{-1}). Therefore, this estimation

suggests higher rates of heterotrophic anaerobic respiratory processes than previously measured. Since denitrification rates were not measured directly, the comparability of published denitrification rates and our measurements of bacterial production are limited. However, our data suggest that the carbon oxidation potential off Peru is more evenly horizontally and vertically distributed than expected ..."

comments from referees/public:

Line 388 - Do you mean distributed evenly vertically or horizontally, or both?

author's response:

We were able to measure heterotrophic bacterial production at every depth and station. Therefore, we here suggest a more evenly horizontal and vertical distribution of heterotrophic anaerobic production, than indicated by heterotrophic anaerobic respiration measurements. We added the words "horizontally " and "vertically", within the revised version.

comments from referees/public:

Line 399 - I agree with final sentence of paper but not mentioned anywhere how can improve understanding or quantification processes, so on its own this final sentence is a bit weak for such a thorough study.

author's response:

We thank the reviewer and added suggestions for future studies in the conclusion of the revised manuscript.

author's changes in manuscript:

page 16 line 501

"Our study suggests that suboxia does not reduce bacterial degradation of organic matter in the Eastern Tropical South Pacific off Peru. Bacterial species are seemingly adapted to these environments and higher cell abundance compensates for hampered cell-specific bacterial production under suboxia. Therefore, the previously observed enhanced carbon export in OMZs compared to oxygenated waters requires alternative explanations. Differences between cell-specific and total rates of bacterial activity allude to different controls of cell abundance in suboxic systems, highlighting the OMZ as a specific ecological niche. The combination of bacterial and physical rate measurements suggests that low BGEs in the upper oxycline contribute to sustaining the OMZ. Meanwhile, new findings during our study call for additional studies: i) DOC loss differed strongly between our investigation and the study of Loginova et al. (2019). Therefore, combined physical and biological rate measurements in the Peruvian upwelling system should be repeated during austral summer, to learn more about the interplay of DOC loss and bacterial production during different seasons. ii) Integrated measurements of denitrification, microaerobic respiration and bacterial production are needed to estimate the fractions of incorporated and respired carbon under suboxia. The BGE received in that way could support or disprove the low BGE estimate, which was calculated from DOC loss and bacterial production in our study. Consequently, our study highlights the need for a better mechanistic understanding and quantification of processes responsible for oxygen and DOM loss in OMZs that is inevitable to predict future patterns of deoxygenation in a warming climate."

comments from referees/public:

Figures: Fig. 2 and Fig.3 - show horizontal oxygen regions as suggested above. Also, reduce extrapolation with ODV, large gap _ 100 km where no station/data between coastal and offshelf. Which interpolation did you use in ODV? The stations are running from the coast which is more east than offshore according to figure 1, so perhaps flip horizontally to reflect the east to west/coast to offshore nature of the spatial distribution. Having longitude instead of distance from coast (or both preferably) may be more useful, and make clear the inset is top 100 m.

author's response:

In the revised version, we indicated the oxygen concentration at the sampling depths in new Figure 3 and 4. Until now we used “Diva setting” with automatic scale adjustment, but in the revised version we reduced the extrapolation to visualize the gap between stations. Further we flipped the y axis as well as indicate the longitude. Furthermore, we indicated the depth in the insets.

author's changes in manuscript:

see new Figures 3 and 4

comments from referees/public:

Fig 4 - labels of oxygen regimes different to text where instead oxyclines often referred to, is that the same as top and bottom hypoxic? Continuity throughout would be helpful.

author's response:

In the revised version, we replaced the words “top hypoxic” and “bottom hypoxic” by “upper-“ and “lower oxycline” in new Figure 5.

author's changes in manuscript:

see new Figure 5

comments from referees/public:

Fig. 5. Add a title for each panel so do not need to refer to legend as much.

author's response:

In the revised version, we added subheadings to each panel of new Figure 2.

author's changes in manuscript:

see new Figure 2

~~High bacterial organic carbon uptake~~Bacterial degradation activity in the Eastern Tropical South Pacific oxygen minimum zone

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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, including the upper and lower oxycline, 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~~3 to ~~160~~281 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~~were significantly higher in suboxic waters compared to ~~the upper oxycline~~waters of 5-60 µmol O_2 kg^{-1}, suggesting no impairment of bacterial organic matter degradation in the core of the OMZ. Nevertheless, high cell-specific bacterial production was observed in samples from oxyclines and cell-specific extracellular enzyme rates were ~~observed in samples from~~ especially high at the ~~upper or~~ lower ~~oxyclines~~oxycline, corroborating earlier findings of highly active and distinct micro-aerobic bacterial communities. To assess the impact of bacterial degradation of dissolved organic matter (DOM) 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 low bacterial growth efficiencies of ~~0.5-8.6~~1-21% 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.

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).~~(Gruber, 2011; Thamdrup et al., 2012; Tiano et al., 2014). 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).~~(Kämpf and Chapman, 2016; Paulmier and Ruiz-Pino, 2009; Thamdrup et al., 2012). There, nutrient-rich water is upwelled and supports high rates of primary production and accumulation of 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). In austral winter, upwelling and subsequently the nutrient supply to the surface waters increase (Bakund and Nelson, 1991; Echevin et al., 2008). However, chlorophyll *a* (Chl *a*) concentration is highest in austral summer, with the seasonal amplitude being stronger for surface than for depth averaged Chl *a* concentrations (Echevin et al., 2008). In winter, phytoplankton growth is, next to iron, mainly limited by light due to the deeper mixing, whereas in summer macronutrients can become a limiting factor (Echevin et al., 2008). Further, El Niño–Southern Oscillation may affect organic matter cycling in the area since it affects the depth of the oxycline and therefore the extent of anaerobic processes in the upper water column (Llanillo et al., 2013). During the year of this study (2017), neither a strong La Niña nor a strong El Niño was detected (https://ggweather.com/enso/oni.htm). However, in January, February and March 2017 there was a strong coastal El Niño with enhanced warming (+1.5°C) of sea surface temperatures in the eastern Pacific (Garreaud, 2018).

~~Within OMZs,~~Within OMZs, enhanced vertical carbon export has been observed (Devol and Hartnett, 2001; Roullier et al., 2014) and explained by a potentially reduced remineralization of organic matter in suboxic and anoxic waters. This is possibly because 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). Additionally, the energy yield available for the production of cell mass seems to be less than expected from the chemical equations (Strohm et al., 2007). 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).~~Thus, microbial activity and consequently organic matter remineralization in suboxic and anoxic waters might be reduced, possibly explaining enhanced export of carbon. 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~~DOM (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~~. For instance, 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~~DOM decomposition rates did not change in relation to oxygen concentrations (Lee, 1992; Pantoja et al., 2009). ~~Further measurements~~Investigations of ~~microbial activity such as extracellular enzyme~~hydrolysis rates~~,~~ as the initial step of organic matter degradation, may help to unravel possible adaptation strategies of bacterial communities to suboxic and anoxic conditions (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~~. High extracellular enzyme rates might compensate a putative lower energy yield of anaerobic respiration and the subsequent biogeochemical effects. However, very few studies have investigated the effect of oxygen on hydrolytic rates, so far. Hoppe et al. (1990) did not find differences between oxic and anoxic incubations of Baltic Sea water. In the Cariaco Basin, hydrolytic rates were significantly higher in oxic compared to anoxic water (Taylor et al., 2009). However, this difference did not persist after rates were normalized to particulate organic matter concentration. The dependence of hydrolysis rates on organic matter concentrations described by Taylor et al. (2009), suggest that productivity may play a role for extracellular enzymatic rates in oxygen depleted systems. The Peruvian upwelling system displays high amounts of labile organic matter (Loginova et al., 2019) at shallow oxyclines and thus allows for studying effects of low oxygen on extracellular enzyme rates under substrate replete conditions. In general, combined investigations of extracellular enzyme 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. ChlorophyllChl *a* fluorescence was detected with a WETStar chlorophyllChl *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 ™),™) that was 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).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) 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).

At each station replicate 4 ml and 16 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). Detection limit of DHAA was 1.4 nmol L^{-1} depending on amino acid and 10 nmol L^{-1} for DCHO. The precision was 2% and 5% for DHAA and DCHO, respectively.

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

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 methods and Fig. 2a). 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.~~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.

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.~~SD (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 incubation of samples at a constant temperature of 13°C resulted in deviations of max. 11°C between incubation ($T_{incubation}$) and *in situ* temperatures (T_{insitu}). In order to estimate *in situ* bacterial production from measured bacterial production during incubations, measured temperature differences were taken into account following the approach of López-Urrutia and Morán (2007). First, the temperature difference between T_{insitu} and $T_{incubation}$ (δT) was computed in electron volt (ev^{-1}), after T_{insitu} and $T_{incubation}$ (K) had been multiplied with the Boltzmann's constant k *(8.62x10^{-5} eV K^{-1}):*

2. $$\delta T\ [ev^{-1}] = \frac{1}{T_{incubation}[K]\ x\ k\ [evK^{-1}]} - \frac{1}{T_{insitu}[K]\ x\ k\ [evK^{-1}]}$$

The decadal logarithm of *in situ* bacterial production ($\log_{10} BP_{insitu}$) was then calculated from the decadal logarithm of measured bacterial production during incubations ($\log_{10} BP_{incubation}$). Therefore we applied three different factors (F) depending on *in situ* Chl *a* concentration as proposed by López-Urrutia and Morán (2007); with F being -0.583, -0.5 and -0.42 $[fgCcell^{-1}d^{-1}ev]$ for <0.5, 0.5-2 and >2 µg Chl *a* L^{-1}, respectively:

3. $$log_{10}BP_{insitu}[fgCcell^{-1}d^{-1}] =$$

$$log_{10}BP_{incubation}[fgCcell^{-1}d^{-1}] + \delta T\ [ev^{-1}]x\ F\ [fgCcell^{-1}d^{-1}ev]$$

Within the text, figures, equations and statistic results it is always referred to temperature corrected *in situ* bacterial production. Temperature corrected bacterial production and original bacterial production measured during incubation can be compared in supplementary Table 2.

The bacterial oxygen demand (BOD; mmol O_2 m^{-3} d^{-1}) is the amount of oxygen needed to fully oxygenize organic carbon that has been taken up and not transformed into biomass by bacterial production (mmol C m^{-3} d^{-1}). 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~~4)) (Del Giorgio and Cole, 1998).

~~2.~~4. $$BOD = ~~(DOC\ uptake - BP)~~(DOC\ uptake - bacterial\ production) \times cf$$

The bacterial DOC uptake was calculated ~~with~~under two different assumptions: i) the DOC uptake by bacteria equals the DOC loss rate over depth or ii) the bacterial growth efficiency (BGE) follows the established temperature dependence~~,~~ (BGE=0.374[±0.04] -0.0104[±0.002]T [°C]), 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~~of 14 ~~and~~to 19°C (Rivkin and Legendre, 2001) and can be used to estimate the bacterial DOC uptake from bacterial production (Eq. (~~3~~5)).

~~3.~~5. ~~*microbial*~~ *bacterial DOC uptake* $= \frac{\sim\sim BP \sim\sim}{\sim\sim BGE \sim\sim} \frac{bacterial\ production}{BGE}$

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 ~~was~~had two openings to fill and flush it with gas. For our experiment the incubator was flushed and filled with N_2, to reduce oxygen concentrations ~~(~~. Still control measurements occasionally revealed oxygen concentrations of 8 to 40 µmol O_2 kg^{-1}~~).~~. Additionally, samples were in contact with oxygen during pipetting and measurement. 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.~~SD >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.~~6. $\delta = \frac{h_T * c}{100}$

where h_r [% d^{-1}] is the hydrolyses turnover at ~~10~~10^3 µmol ~~l~~$^{-1}$ m^{-3} substrate concentration and *c* is the carbon content of DHAA [µmol C m^{-3}]. Measurements of h_r with a ~~s.d.~~SD 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.

Similar to bacterial production, *in situ* extracellular enzyme rates were estimated based on extracellular enzyme rates measured during incubation. To account for the differences between *in situ* and incubation temperatures a correction factor (*F*) was applied based on differences in extracellular enzyme rates after additional incubations at 22.4°C next to the regular incubations at 13°C at five stations during the cruises. The fluorescence signals at different substrate concentrations increased on average by a factor of 0.05 and 0.03 ($°C^{-1}$) for GLUCase and LAPase, respectively. Under the assumption that the increase in rates with temperature was linear, measured enzyme rates were adapted to *in situ* temperature, with (EER_{insitu}; *nmol* L^{-1} h^{-1}) and ($EER_{incubation}$) being the *in situ* extracellular enzyme rates and extracellular enzyme rates during incubation, respectively:

7. $$\delta T\ [°C] = T_{insitu}[°C] - T_{incubation}\ [°C]$$

8. $$EER_{insitu}[nmolL^{-1}h^{-1}] =$$

$$EER_{incubation}[nmolL^{-1}h^{-1}] + EER_{incubation}[nmolL^{-1}h^{-1}]\ x\ F\ [°C^{-1}]\ x\ \delta T\ [°C]$$

Within the text, figures, equations and statistic results it is always referred to the temperature corrected *in situ* extracellular enzyme rates. Temperature corrected extracellular enzyme rates and original extracellular enzyme rates measured during incubation can be compared in supplementary Table 2.

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.~~SD 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 suboxic "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}) including the upper and lower oxycline 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 partly differentiated between oxygen regimes situated above and below the OMZ~~.~~ (see supplementary Table 2 for results).

3. Results

3.1. Biogeochemistry of the Peruvian OMZ

During our two cruises to the Peruvian upwelling system (Fig. 1), maximum Chl *a* concentration was higher and temperatures were warmer in April ~~and~~compared to June 2017~~ chlorophyll *a* (Chl *a*)~~, probably representing seasonal variability. Chl *a* concentration reached ~~maximum of 8~~up to 11 and 4 µg l^{-1} within the upper 25 m~~.~~ in April and June, respectively. Still, average Chl *a* concentration at depth <10 m (M136: 3.1±2.6 µg l^{-1}; M138: 2.8 ± 1.3 µg l^{-1}) were not significantly different between the two cruises. At depths >50 m, Chl *a* concentration was generally below detection limit (Fig. ~~2a, exemplary stations).~~3a, supplementary Fig. 1). At depth < 10 m the water was warmer in April (21.3 ±1.6°C) than in June (17.6 ± 0.6°C) (Fig. 3b, supplementary Fig. 1). Oxygen concentration >100 µmol kg^{-1} was observed in the surface mixed layer~~.~~. Oxygen decreased steeply with depth~~ and was ~~, reached suboxic concentrations (<5 µmol kg^{-1}) at > 60 ± 24 m (Fig. 2c, 4a and 5a, supplementary Fig.1) and fell below detection of Winkler titration ~~at >100 m (Fig. 3a and 4a).~~. For further analysis and within the text *in situ* oxygen concentrations <5 µmol O_2 kg^{-1} are referred to as "suboxic". Shallowest depth with suboxic oxygen concentrations was 14 m in April (station Q) and 29 m in June (station D), probably representing that station Q was situated closer to the shore than station D. 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).~~4a and 5a, supplementary Fig. 1). TDN concentrations increased with depth from ~~17–32~~18±8 µmol l^{-1} and 22±7 µmol l^{-1} within the upper ~~40~~20 m ~~to~~in April and June, respectively, and reached a maximum of ~~51~~54 µmol l^{-1} at ~~600~~850 m (Fig. ~~2c~~3c). DOC decreased with depth from ~~81 ±30~~94 ±37 µmol l^{-1} and 69 ±12 µmol l^{-1} in the upper 20 m in April and June, respectively, to lowest values of 37 µmol l^{-1} at ~~the surface to 42 µmol l^{-1} at 600 m depth, with the~~ 850 m. The steepest gradient in DOC concentration was observed in the upper 20-60 m (Fig. ~~2d).~~ 2b and 3d) during both cruises.

~~3.2.~~ 3.2. Bacterial production and enzymatic activity

Bacterial production varied strongly throughout the study region and ranged from 0.~~62~~ to ~~614~~2404 µmol C m^{-3} d^{-1} (Fig. ~~3b~~4b), decreased in general from surface to depth (except for the most coastal ~~stations~~station) 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).~~Fig. 4b). At the most coastal station (G) bacterial production remained high near the bottom depth of 75 m (280 µmol C m^{-3} d^{-1} at 72 m) (Fig. 4b). Bacterial production did not differ significantly between the oxyclines and the suboxic core waters, neither off-shore (suboxic: 0.~~6–91~~3-127 µmol C m^{-3} d^{-1} ; oxyclines: ~~2–149~~1-304 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~~146-281 µmol C m^{-3} d^{-1}) (oxycline: ~~34–195~~74-452 µmol C m^{-3} d^{-1}) (~~n_{OMZ}=~~see supplementary Table 2~~, $n_{oxycline}$=8, p=0.4, W=12~~ for all statistical results). 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~~. Additionally, significantly lower bacterial production was observed ~~below~~within the ~~OMZ (2–10.5~~lower oxycline (0.7-3.3 µmol C m^{-3} d^{-1}) compared to the core OMZ (0.~~6–~~

~~160~~3-281 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).~~ 4a, b). Trends between oxygen regimes were similar between temperature corrected bacterial production (presented throughout the text) and original bacterial production measured during incubation (supplementary Table 2).

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~~oxyclines (1-16 x 10^5 cells ml^{-1}) was ~~statistically similar to~~significantly lower than in 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~~4c). 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~~39.7 µmol C m^{-3} d^{-1}. LAPase degradation rates observed within the OMZ core (5~~ ±~~.5 ± 2.1~~.8~~ µmol C m^{-3} d^{-1}) were significantly higher than in the ~~upper oxycline (~~oxyclines (3.1 ± 2.~~5 ± 1.2~~3 µmol C m^{-3} d^{-1}) (~~n_{OMZ}= 41, $n_{upper\_low\_hypoxic}$=9, p<0.001 W=331) (~~Fig. ~~4b~~5b). 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~~92 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).~~oxyclines. 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 slightly reduced within the suboxic waters (0.69 ± 1.~~35~~30 µmol C m^{-3} d^{-1}) compared to the ~~upper oxycline (~~oxyclines (1.1 ± 1.0~~.76 ± 0.51~~ µmol C m^{-3} d^{-1}) (~~n_{OMZ}= 35, $n_{upper\_low\_hypoxic}$=8, p=0.054, W=78~~Fig. 5c). 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~~Fig. 3e, 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~~9 and ~~168~~158 nmol l^{-1} h^{-1} and were ~~10~~~30 times lower for GLUCase. LAPase V_{max} was significantly higher within the suboxic waters (50 ± ~~22~~21 nmol l^{-1} h^{-1}) compared to the oxycline (~~34 ± 18~~36 ± 20 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~~5 nmol l^{-1} h^{-1}) compared to the oxycline (1.~~1~~2 ± 0.~~5~~6 nmol l^{-1} h^{-1}) (Fig. ~~4d~~5d, e). ~~Trends between oxygen regimes were similar between temperature corrected extracellular enzyme rates (presented throughout the text) and extracellular enzyme rates measured during incubation (supplementary Table 2).~~

To investigate physiological effects of suboxia, we normalized bacterial production and enzymatic rates to cell abundance. Cell-specific production ranged between ~~2~~1 and ~~286~~1120 amol C $cell^{-1}$ d^{-1} (Fig. ~~3d) and, in~~4d). In contrast to total production, cell-specific production was ~~positively~~significantly higher at the oxyclines compared to suboxic

core waters at the off-shore stations (suboxic: 1-102 µmol C m^{-3} d^{-1}, oxyclines: 6-219 µmol C m^{-3} d^{-1}). At the most coastal stations (G and T) cell-specific rates were more similar between suboxic waters and the oxyclines (suboxic: 129-135 µmol C m^{-3} d^{-1}) (oxycline: 72-284 µmol C m^{-3} d^{-1}). Further, cell-specific bacterial production was slightly correlated (spearman rank correlation =0.36) to oxygen concentrations at ~~<~~≤20 µmol O_2 kg^{-1} ~~regardless of whether~~and as long as 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)~~this correlation was significant (Fig. ~~3d).~~ 4d, supplementary Table 2). A detailed view at total- and cell-specific bacterial production in dependence of *in-situ* oxygen concentrations, reveals a stronger increase of cell-specific bacterial production, especially at <10 µmol O_2 kg^{-1} at different stations (supplementary Fig. 2).

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 LAPase V_{max}, GLUCase V_{max} and GLUCase degradation rate ~~and LAPase and GLUCase V_{max}~~ showed the same ~~patterns~~trends, however for the latter this trend was not significant (Fig. ~~4g~~5g-j, supplementary Table 2)

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~~2b, c). We estimated the bacterial contribution to this loss ~~with~~using two different ~~assumptions~~approaches (Table 1): i) We assumed that the loss of DOC over depth ~~can be solely attributed to~~equalled the bacterial uptake implying that the DOC is subsequently incorporated as bacterial biomass (bacterial production) or respired to CO_2 (Eq. (~~2)).~~4)) 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)).~~5)) (see section 2.5 for details). This ratio (BGE), was here assumed to be ~~between~~ 10 ~~and~~or 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~~2c). 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 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~~03- 0.~~29~~71 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~~0.98-3.~~38~~36 mmol O_2 m^{-3} d^{-1}) (Eq. (~~2~~4)). Following this calculation, the BGE would vary between ~~0.5-8.6~~1-21 % and ~~1.1-7.0~~2 -13 % in the depth range of MLD-40 m and 40-60 m, respectively, being on average almost constant over the two different depth ranges (~~2.7~~6.6 and 5.0%). ii) Applying a BGE in the range of 10- and 30% and the measured bacterial production, the calculated bacterial DOC uptake$_\phi$ was 0.~~04-2.9~~08-7.10 mmol C m^{-3} d^{-1}. Hence, respiration of DOC to CO_2 accounted for a BOD_ϕ of 0.~~03-2.63~~06-6.39 mmol O_2 m^{-3} d^{-1} (Table 1).

4. Discussion

We investigated bacterial degradation of ~~dissolved organic matter~~DOM 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~~We expected reduced rates of organic matter degradation within oxygen depleted waters, since reduced bacterial degradation activity might explain enhanced carbon fluxes in suboxic and anoxic waters (Devol and Hartnett, 2001). However, although bacterial production decreased with depth (Fig. 4b), this decrease was not related to oxygen concentrations. Moreover, no significant increase in bacterial production was observed at the lower oxycline, when oxygen concentration increased again (Fig. ~~3b~~4b). 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 also implies reduced extracellular enzyme rates for the hydrolysis of organic matter. The extracellular enzymes rates of our study have to be interpreted carefully since incubation was not fully anoxic and the remaining oxygen might have biased the results. Still, we assume that most extracellular enzymes were present at the time of sampling and thus oxygen contamination during the incubations did not strongly influence the rate measurements. In our study, neither GLUCase nor LAPase V_{max} were reduced within the suboxic waters compared to the oxyclines irrespective of incubation conditions (Fig. ~~4d~~5d, e, supplementary Fig. ~~1c, d~~3 and 4). 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).~~(Cavan et al., 2017; Lee, 1992; Pantoja et al., 2009). Consequently, the hypothesis of enhanced carbon export in ~~suboxic and anoxic~~OMZ 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)~~(Cavan et al., 2017) may be more likely. ~~Moreover~~Likewise, a reduced degradation of particulate organic ~~matter~~carbon in suboxic waters as it is often assumed ~~for particulate carbon~~ in global ocean biogeochemical ~~modelling~~models 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~~Meanwhile, 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~~Degradation of nitrogen compounds ~~(within suboxic waters)~~ by heterotrophic bacteria (e.g. denitrifiers) in suboxic waters enables the release of ammonia and nitrite and subsequently ~~can maintain~~may support anammox, an autotrophic anaerobic ~~respiratory~~ pathway ~~(Lam and Kuypers, 2011; Ward, 2013). This interaction between denitrifiers and anammox bacteria would further fuel the loss of nitrogen to the atmosphere. Our data indeed showed enhanced degradation of amino acid~~ (Babbin et al., 2014; Kalvelage et al., 2013;

Lam and Kuypers, 2011; Ward, 2013). This interaction between denitrifiers and anammox bacteria could 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. 4b5b, d). TheTherefore, observed LAPase rates are in line with the hypothesis of preferential degradation of nitrogen compounds under suboxia. However, simultaneous rate measurements of protein hydrolysis, nitrate reduction (e.g. denitrification) and anammox are needed to prove an indirect stimulation of anammox by protein hydrolysis via denitrification. A close coupling between anammox and denitrifyingnitrate reducing bacteria has previously been shown for wastewater treatments. There, denitrifersnitrate reducers directly utilizetake up 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 denitrifierspotentially weakening a direct inter-dependency.

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 g4d, 5g-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.

. 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. In our study, differences in cell-specific bacterial production between suboxic waters and the oxycline did not persist at the most coastal stations (G and T). This indicates the stimulation of bacterial activity, including anaerobic respiratory processes, by the high input of labile organic matter. Therefore, our study suggests that a possible impairment of cell-specific bacterial production under suboxia is reduced by supply of organic matter. However, this hypothesis is restricted to a very limited number of samples and should be tested in further studies. While labile organic matter is

decreasing with depth (e.g. Loginova et al., 2019), TDN (Fig. 3c), especially inorganic nitrogen is increasing with depth. Thus, high concentrations of inorganic nitrogen at the lower oxycline are available for heterotrophic and chemoautotrophic energy gains. For instance, the co-occurrence of nitrate reduction, that was still detected at 25 µmol O_2 L^{-1}, and microaerobic respiration might have stimulated cell-specific production or the accumulation of especially active bacterial species (Kalvelage et al., 2011, 2015).

Depth distribution of cell-specific and total bacterial production was different (Fig. 4b, d and supplementary Fig. 2); cell-specific production was significantly reduced in suboxic waters, while total production was more similar in suboxic waters compared to the oxycline. This suggests that lower cell-specific production was compensated by higher cell abundance within the suboxic waters (Fig. 4c), resulting in an overall unhampered bacterial organic matter cycling in the OMZ core. One reason for the accumulation of cells within the OMZ might be reduced predation, suggesting the OMZ core as an ecological niche for slowly growing bacteria. Reduced grazing by bacterivores thus preserves bacterial biomass in suboxic waters from entering into the food chain. This way of bacterial biomass preservation has been suggested as possible explanation for enhanced carbon preservation in anoxic sediments by Lee (1992), and may also explain our observations for the anoxic water column.

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)~~(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)~~(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. ~~4c, 3b~~5e, 4b). 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). Consequently, our findings of active bacterial degradation of DOM are supported by molecular biological studies. Still, simultaneous measurements of bacterial degradation and production have to be combined with molecular analysis, in future studies off Peru.

Heterotrophic bacteria are the main users of marine ~~dissolved organic matter~~DOM ~~(Azam et al., 1983; Carlson and Hansell, 2015)~~(Azam et al., 1983; Carlson and Hansell, 2015) and responsible for ~79% of total respiration in the Pacific Ocean (Del Giorgio et al., 2011), 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~~0.98-3.~~38~~36 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~~18-33% of the oxygen loss over depth, implying a rather low average BGE (~~0~~6.5~~ 8.6~~ and 5.0 %) (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~~3-209% of the DOC loss and ~~0.4 26~~1-62% of oxygen loss could be attributed to bacterial degradation of ~~dissolved organic matter. Both approaches reveal a BGE~~DOM. The first approach reveals an average BGE (6.5 and 5.0%) that is still within the range of previous reports for upwelling systems of the Atlantic (<1-58%) ~~or~~and 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~~but rather constant average BGE (~~2.~~6.5 and 5.0%), 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)~~(Loginova et al., 2019), 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)~~(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)~~(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)~~(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~~2b, c) 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~~(Loginova et al., 2019). However, our study is the first combining physical and microbial rate measurements and gives estimates for carbon and oxygen losses in the upwelling system off Peru and can help improving current biogeochemical models by constraining bacterial ~~dissolved organic matter~~DOM degradation.

Loginova et al. (2019) conducted similar physical rate measurements in the same study area with ~2 and ~10 times lower DOC and oxygen loss in the upper ~40 m compared to our study. Differences in loss rates were mainly caused by a ~ 10 times higher diapycnal diffusivity of mass in our study. This may have been caused by weaker stratification in the upper 100 m depth or differences in the turbulence conditions. Loginova et al. (2019) estimated a contribution of bacterial DOM degradation to oxygen loss (38 %) based on the loss of labile DOC (DHAA and DCHO). This value agrees well with our estimates of 18-33% of total oxygen loss, calculated under the assumption that DOC loss is solely attributed to bacterial degradation. However, the comparison of DOC and oxygen loss within each study revealed

different patterns. Loginova et al. (2019) found a loss of DOC that clearly exceeded the loss of oxygen within the upper ~40 m. Hence, respiration of DOC could fully explain the observed oxygen loss in that study. In our study, more oxygen than DOC was lost over depth (Table 1). This loss of oxygen needs additional explanations such as degradation of particulate organic matter and physical mixing processes. One reason for the observed differences between the two studies that have been conducted in the same region might be seasonality. The study by Loginova et al. (2019) took place in austral summer, whereas our data were gained during austral winter. Water temperature was quite similar during both studies, probably due to the coastal El Niño one month before our sampling campaign (Garreaud, 2018). Still, the study by Loginova et al. (2019) included more stations with high Chl *a* concentrations (~8 µg L^{-1}), as typical for the austral summer, indicating a more productive system with more labile DOM (DCHO and DHAA). Prevalence of more labile DOM might explain the higher contribution of microbial DOM respiration to oxygen loss in the study by Loginova et al. (2019). Additionally, Loginova et al. (2019) sampled with a much higher vertical resolution within the upper 140 m, restricting the direct comparability with our study.

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 towards a widespread occurrence of heterotrophic denitrification processes in the Peruvian OMZ, since the here applied method for measuring bacterial production is restricted to heterotrophs. Our rates for bacterial production 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 production, i.e. rate of carbon incorporation, with denitrification rates previously reported for the South Pacific (Dalsgaard et al., 2012; Kalvelage et al., 2013), we converted one mol of reduced nitrogen~~37 µmol C m^{-3} d^{-1} (0.3-281 µmol C m^{-3} d^{-1}).

We compared bacterial production, i.e. rates of carbon incorporation, with denitrification rates previously reported for the South Pacific. Therefore, we converted one mol of reduced nitrogen that were measured by Dalsgaard et al. (2012) and Kalvelage et al. (2013) to 1.25 mol of oxidized carbon after the reaction equation given by ~~Lam and Kuypers (2011).~~Lam and Kuypers (2011). This calculation indicates that on average ≤19 µmol C m^{-3} d^{-1} are oxidized by denitrifying bacteria in the Eastern Tropical Pacific (Dalsgaard et al., 2012; Kalvelage et al., 2013). ~~Assuming a BGE of 20%~~

The amount of carbon oxidized by denitrification based on the studies of Dalsgaard et al. (2012) and Kalvelage et al. (2013) can be converted into bacterial production applying a BGE. The average temperature dependent BGE was 20%. A BGE of 20% agrees well with other studies (Del Giorgio and Cole, 1998) ~~then suggests that denitrification supports a bacterial production of ≤4 µmol C m^{-3} d^{-1}, thus only about 15% of total heterotrophic bacterial production in suboxic waters determined in this study.~~. Assuming a BGE of 20%, the denitrification rates of Dalsgaard et al. (2012) and Kalvelage et al. (2013) suggest a bacterial production of ≤5 µmol C m^{-3} d^{-1}, equivalent to only about 14% of total average heterotrophic bacterial production in suboxic waters determined in our study. For the sum of anaerobic carbon oxidation rates including denitrification, DNRA 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 the reduction of nitrate to nitrite representing the largest proportion (2-505 µmol C^{-1} m^{-3} d^{-1}), based on the relative abundance of the different N-functional genes (Kalvelage et al., 2013).

These anaerobic respiration measurements are equivalent to a bacterial production of ~ ~~22~~27 µmol C m^{-3} d^{-1} (1-~~103~~129 µmol C m^{-3} d^{-1}) and are thus ~~in good agreement with~~lower than our direct measurements of bacterial production rates. ~~However~~Moreover, the reduction of nitrate, could not be detected at every depth and incubation experiments partly showed huge variations over depth (Kalvelage et al., 2013), whereas we were able to measure bacterial production in every sample. ~~Our data therefore suggest that the carbon oxidation potential off Peru is more evenly~~The same calculation can be repeated assuming a BGE of 6%, which is the average BGE within this study based on DOC loss and bacterial production. Assuming a BGE of 6%, the estimated 109 µmol C m^{-3} d^{-1} that are respired by anaerobic carbon oxidation (Kalvelage et al., 2013) would represent 94% of the carbon uptake. Consequently, 7 µmol C m^{-3} d^{-1}, i.e. 6% of the carbon uptake, are incorporated into the bacterial biomass. A bacterial biomass production of 7 µmol C m^{-3} d^{-1} is even lower than the bacterial production of 27 µmol C m^{-3} d^{-1}, based on a BGE of 20% and cannot explain the average bacterial production measured in suboxic waters during our study (37 µmol C m^{-3} d^{-1}). Therefore, this estimation suggests higher rates of heterotrophic anaerobic respiratory processes than previously measured. Since denitrification rates were not measured directly, the comparability of published denitrification rates and our measurements of bacterial production are limited. However, our data suggest that the carbon oxidation potential off Peru is more evenly horizontally and vertically distributed than expected and also corroborate earlier suggestions of unexpectedly high rates of heterotrophic nitrogen cycling in the OMZ off Peru based on observations of high concentrations of atmospheric nitrous oxide (Bourbonnais et al., 2017).

5. Conclusion

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

Our study suggests that suboxia does not reduce bacterial degradation of organic matter in the Eastern Tropical South Pacific off Peru. Bacterial species are seemingly adapted to these environments and higher cell abundance compensates for hampered cell-specific bacterial production under suboxia. Therefore, the previously observed enhanced carbon export in OMZs compared to oxygenated waters requires alternative explanations. Differences between cell-specific and total rates of bacterial activity allude to different controls of cell abundance in suboxic systems, highlighting the OMZ as a specific ecological niche. The combination of bacterial and physical rate measurements suggests that low BGEs in the upper oxycline contribute to sustaining the OMZ. Meanwhile, new findings during our study call for additional studies: i) DOC loss differed strongly between our investigation and the study of Loginova et al. (2019). Therefore, combined physical and biological rate measurements in the Peruvian upwelling system should be repeated

during austral summer, to learn more about the interplay of DOC loss and bacterial production during different seasons. ii) Integrated measurements of denitrification, microaerobic respiration and bacterial production are needed to estimate the fractions of incorporated and respired carbon under suboxia. The BGE received in that way could support or disprove the low BGE estimate, which was calculated from DOC loss and bacterial production in our study. Consequently, our study highlights the need for a better mechanistic understanding and quantification of processes responsible for oxygen and DOM loss in OMZs that is inevitable to predict future patterns of deoxygenation in a warming climate.

Data Availability. PANGEA: 10.1594/PANGAEA.891247

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

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

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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**~~**Figure 2**: 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)**.

Figure 3: 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~~4: Bacterial growth activity at different *in situ* oxygen concentrations from on- to offshore off Peru~~.~~ during April 2017 (M136). 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~~5: Extracellular enzyme rates at different *in situ* oxygen concentrations~~.~~ during April and June 2017 (M136, M138). 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).~~

Tables

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 (mmol m^{-3} d^{-1}) were estimated from the change in oxygen and DOC fluxes over depth. The bacterial uptake of DOC (mmol m^{-3} d^{-1}) was calculated from bacterial production (mmol m^{-3} d^{-1}) based on a growth efficiency of 10 and 30% (DOC uptake$_{\phi}$). The bacterial oxygen demand (BOD, mmol m^{-3} d^{-1}) and bacterial growth efficiency (BGE$_{\varepsilon}$, %) was calculated from bacterial production and the assumption that DOC loss can be completely explained by bacterial uptake (BOD$_{\varepsilon}$) or estimated based on a BGE of 10 and 30% (BOD$_{\phi}$).

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 2.22	0.1 735	2.92 7.10	0.3 074	0.0 612	0.97 2.37	0.0 922	0.0 203	0.2 971	3.3 17	3.12. 68	3.3 836	0.81 2.00	0.1 531	2.63 6.39	0.2 152	0.0 408	0.68 1.66	2.65 6.55	0.50 1.02	8.622 0.92
40-60	5.55	1.13	0.3056	0.1 225	0.79 1.46	0.1 019	0.0 408	0.2649	0.0 306	0.0 103	0.0 815	1.1 07	1.05 0.98	1.1 210	0.2751	0.1 123	0.72 1.32	0.0 713	0.0 306	0.1934	2.67 5.00	1.10 2.26	7.041 2.97

Figures

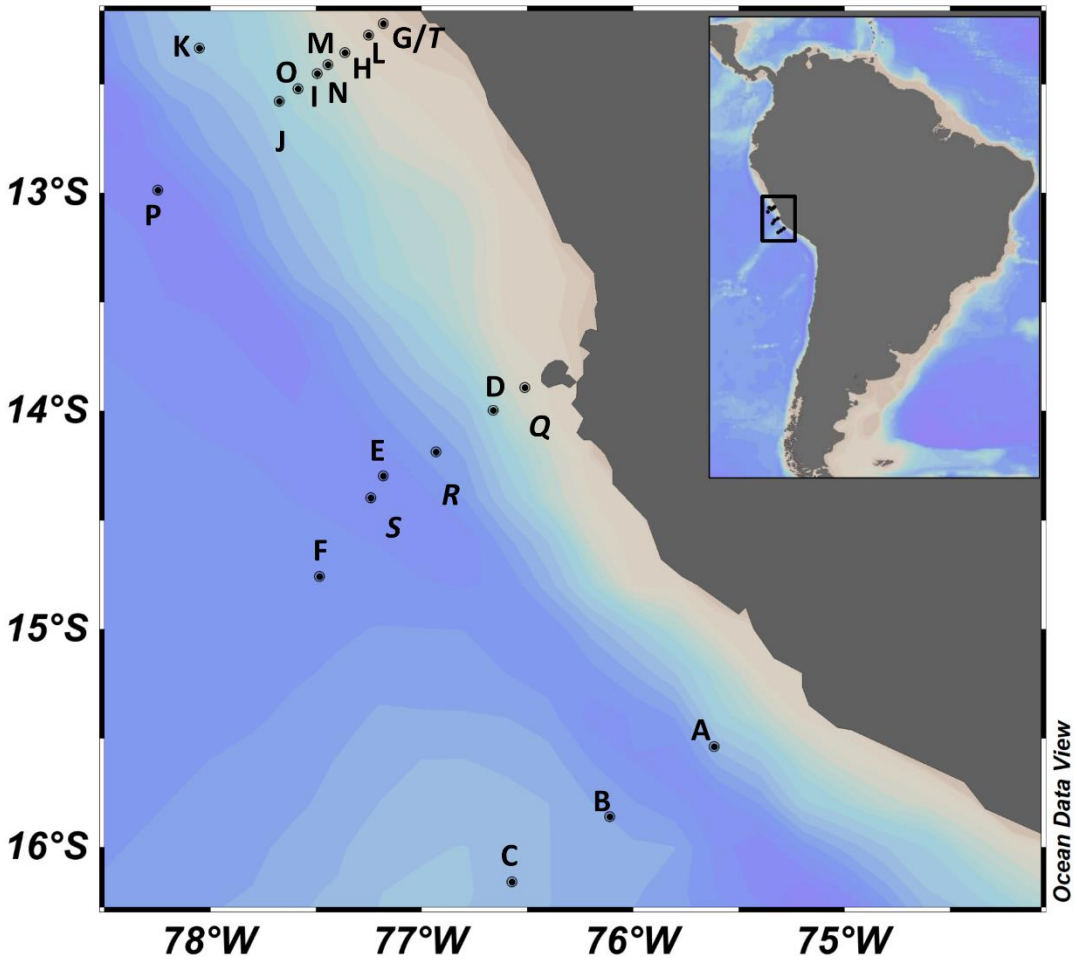
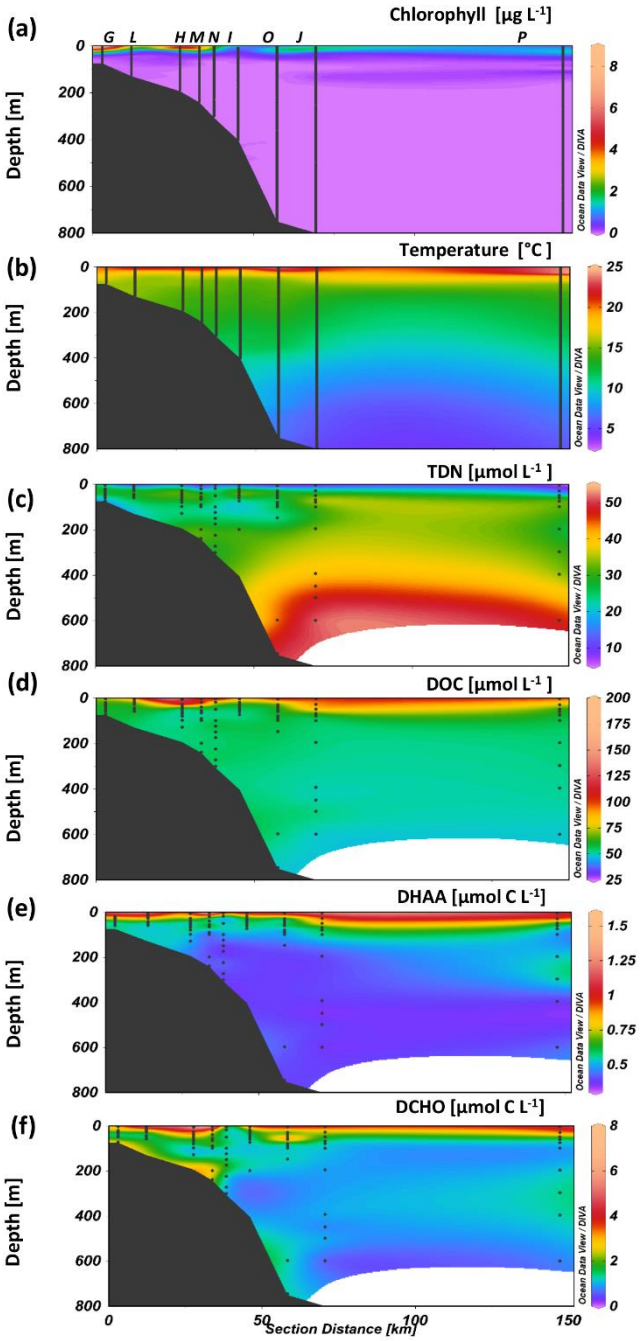
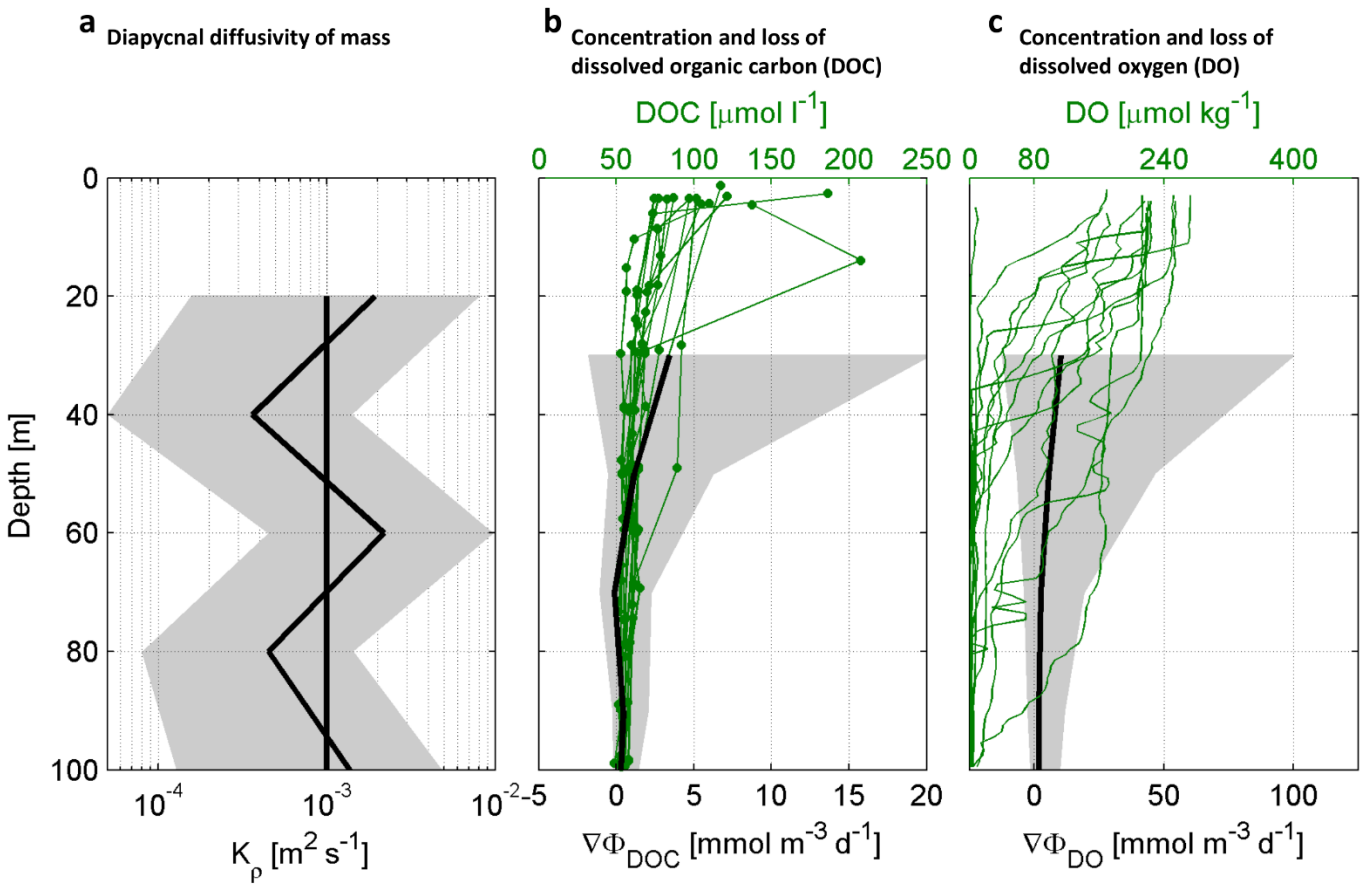


Figure 1

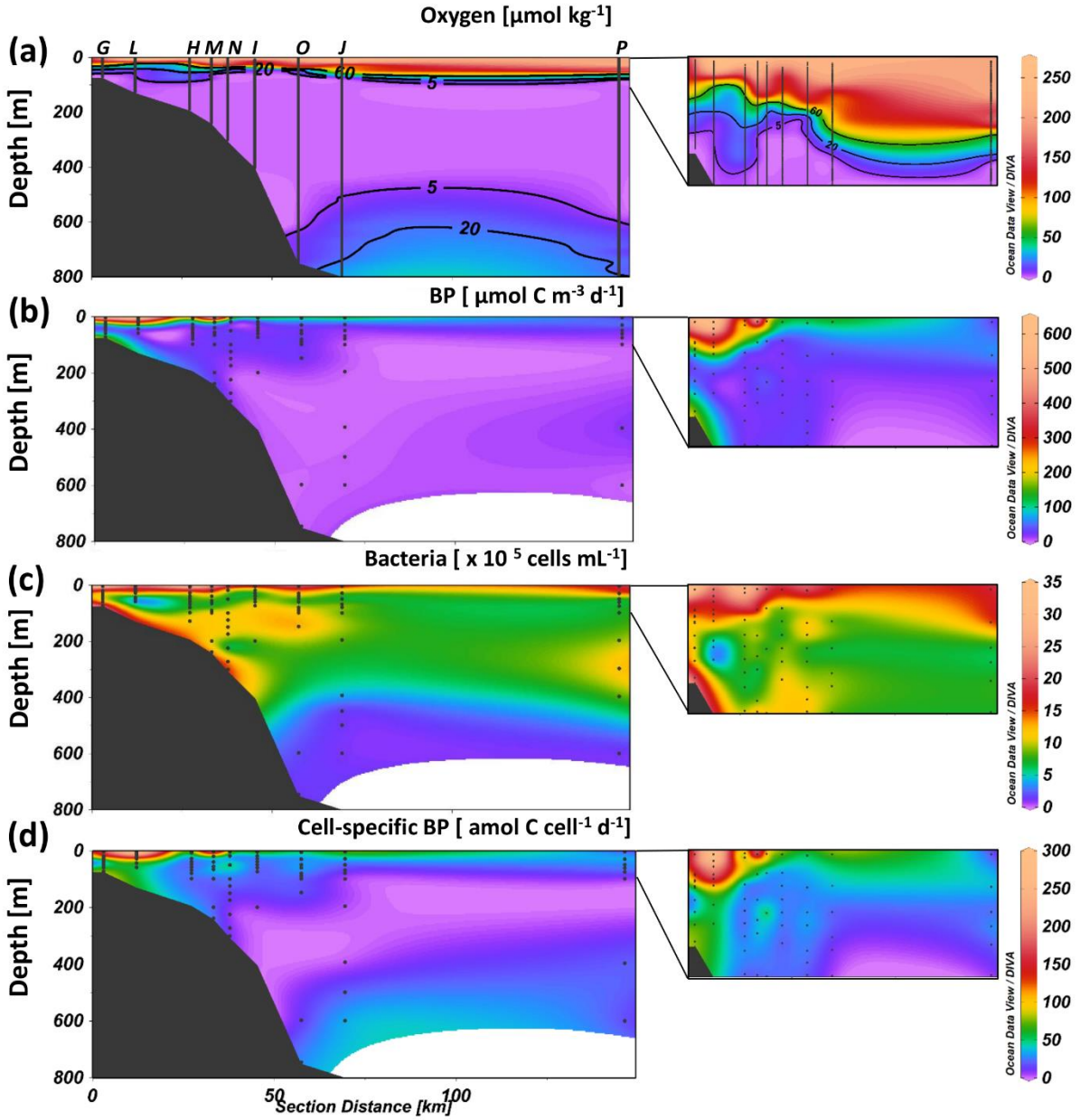

(a)
Chlorophyll [µg L-1]
G L H M N I O J P
Depth [m]
0 200 400 600 800
8 6 4 2 0
Ocean Data View / DIVA
(b)
Temperature [°C]
25 20 15 10 5
(c)
TDN [µmol L-1]
50 40 30 20 10
(d)
DOC [µmol L-1]
200 175 150 125 100 75 50 25
(e)
DHAA [µmol C L-1]
1.5 1.25 1 0.75 0.5
(f)
DCHO [µmol C L-1]
8 6 4 2 0
0 50 100 150
Section Distance [km]

866



867

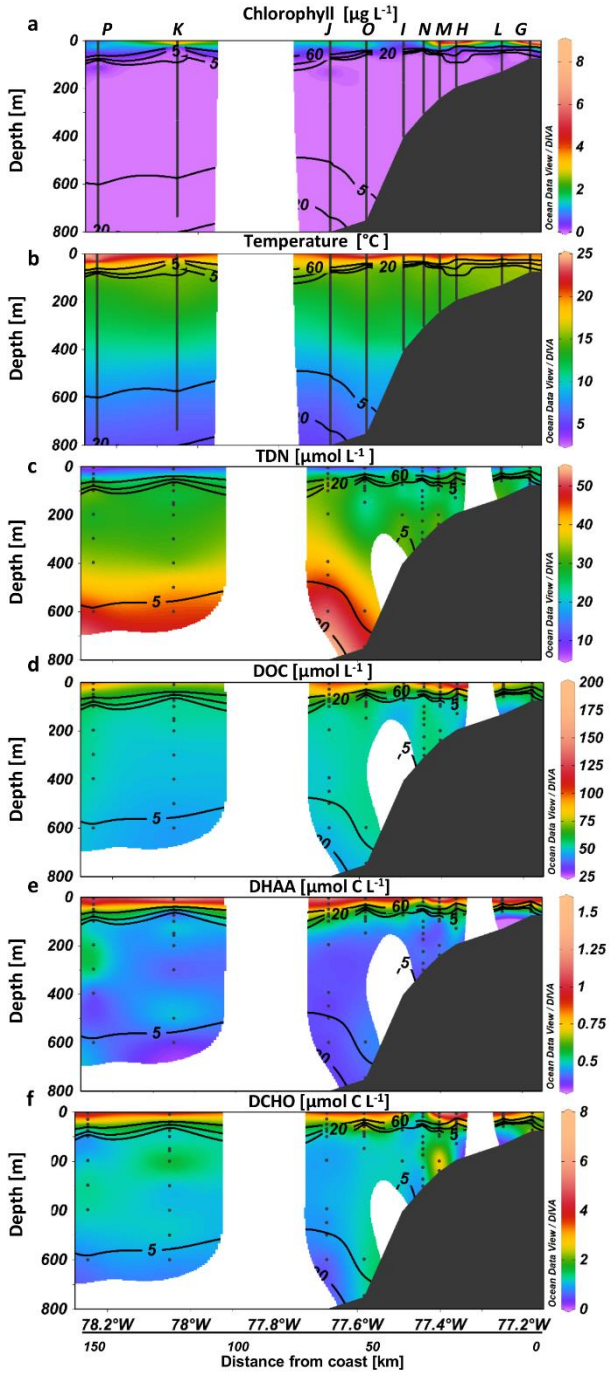
868 **Figure 2**


Oxygen [µmol kg-1]
(a)
G L H M N I O J P
Depth [m]
0
200
400
600
800
20
60
5
5
20
Ocean Data View / DIVA
250
200
150
100
50
0
BP [µmol C m-3 d-1]
(b)
Depth [m]
600
500
400
300
200
100
0
Bacteria [x 10 5 cells mL-1]
(c)
Depth [m]
35
30
25
20
15
10
5
0
Cell-specific BP [amol C cell-1 d-1]
(d)
Depth [m]
300
250
200
150
100
50
0
0
50
100
Section Distance [km]

869

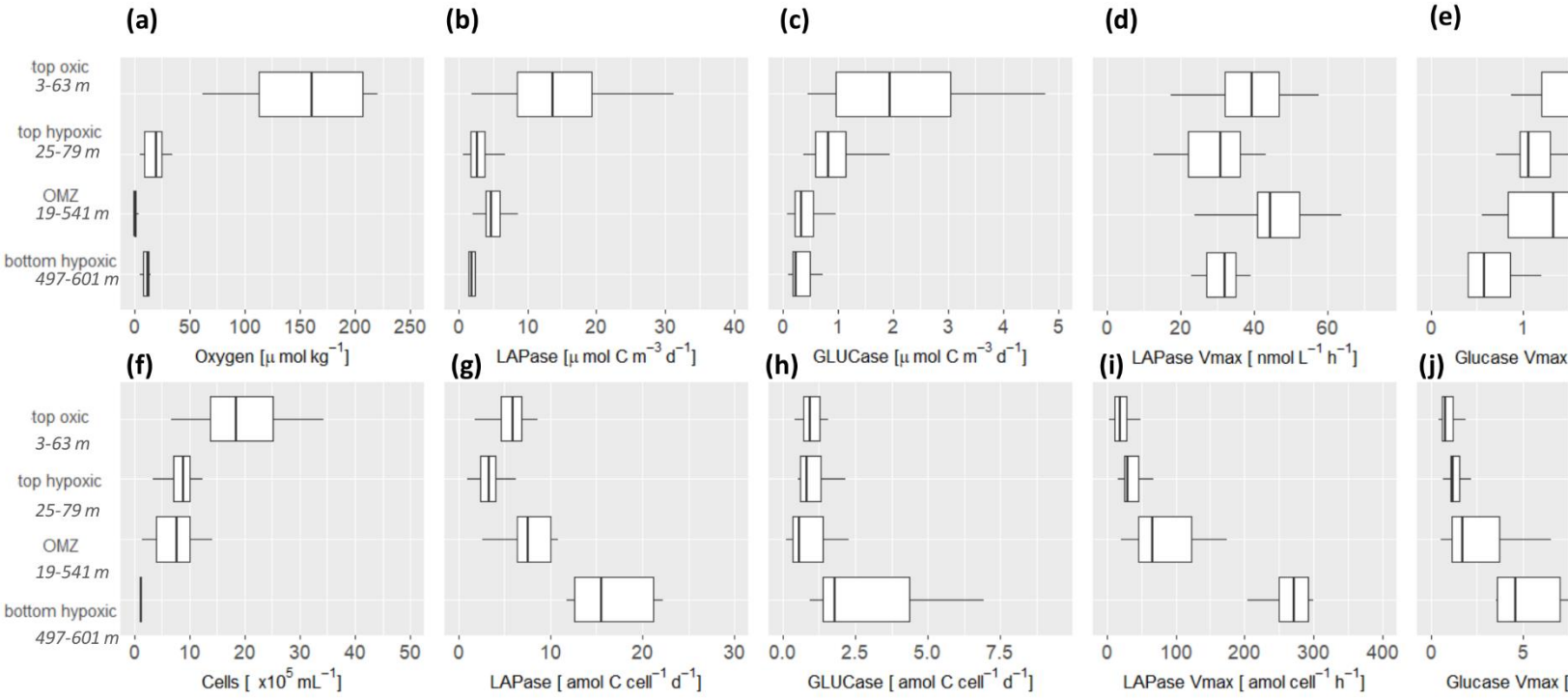
870

871

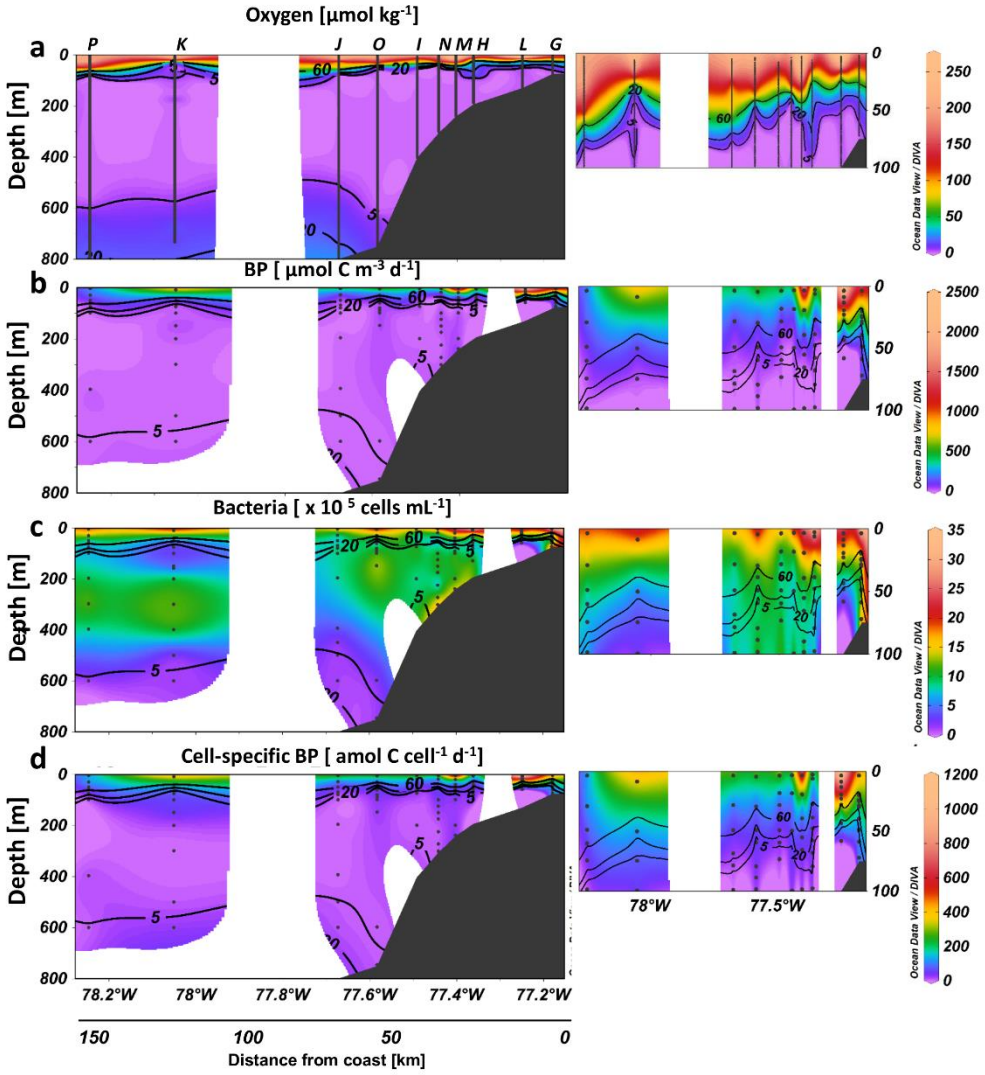


872

873 **Figure 3**

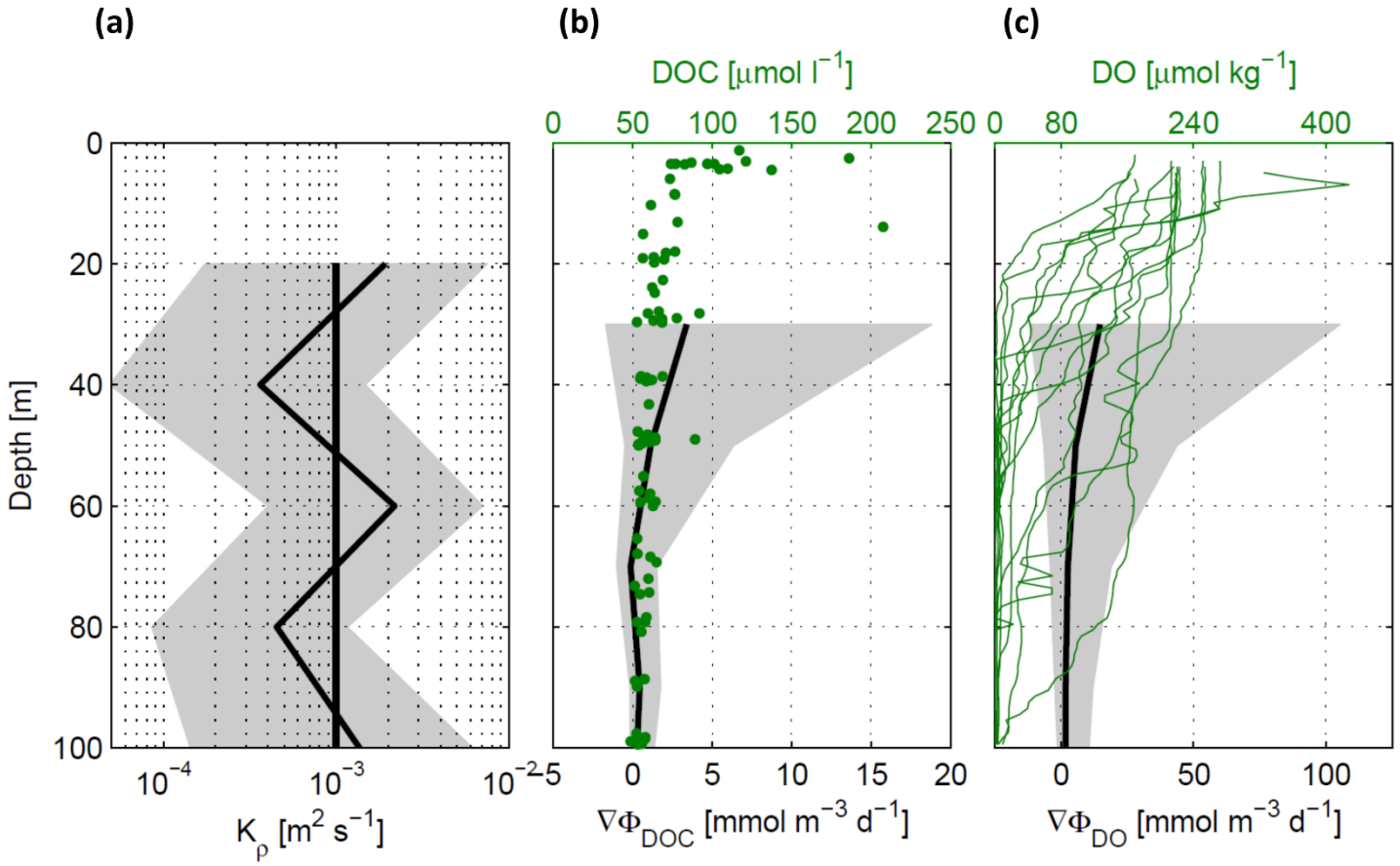

(a)
(b)
(c)
(d)
(e)
top oxic
3-63 m
top hypoxic
25-79 m
OMZ
19-541 m
bottom hypoxic
497-601 m
Oxygen [μ mol kg^{-1}]
LAPase [μ mol C m^{-3} d^{-1}]
GLUCase [μ mol C m^{-3} d^{-1}]
LAPase Vmax [nmol L^{-1} h^{-1}]
Glucase Vmax
(f)
(g)
(h)
(i)
(j)
Cells [x10^{5} mL^{-1}]
LAPase [amol C cell^{-1} d^{-1}]
GLUCase [amol C cell^{-1} d^{-1}]
LAPase Vmax [amol cell^{-1} h^{-1}]
Glucase Vmax [

874



875

876 **Figure 4**


(a)
(b)
(c)
DOC [µmol l^{-1}]
DO [µmol kg^{-1}]
Depth [m]
K_ρ [m^2 s^{-1}]
$\nabla\Phi_{DOC}$ [mmol m^{-3} d^{-1}]
$\nabla\Phi_{DO}$ [mmol m^{-3} d^{-1}]

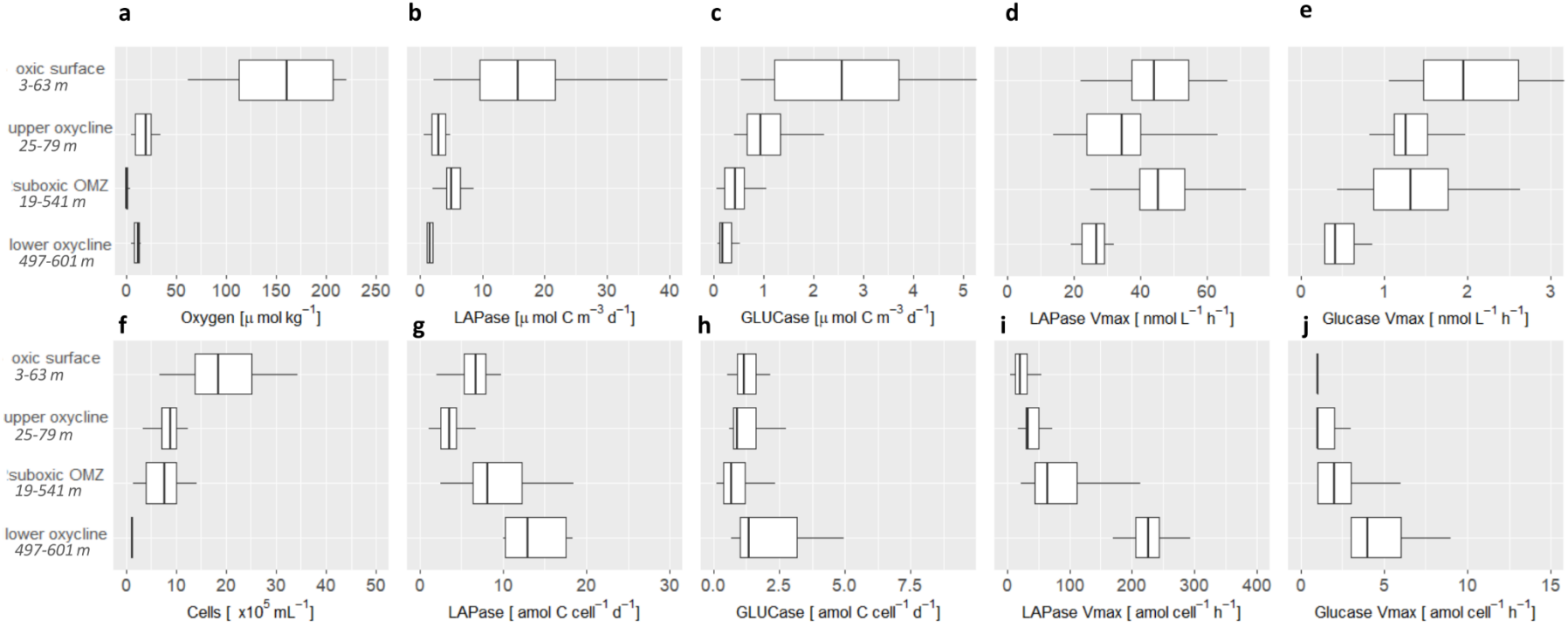

a
b
c
d
e
oxic surface
3-63 m
upper oxycline
25-79 m
suboxic OMZ
19-541 m
lower oxycline
497-601 m
Oxygen [µ mol kg^{-1}]
LAPase [µ mol C m^{-3} d^{-1}]
GLUCase [µ mol C m^{-3} d^{-1}]
LAPase Vmax [nmol L^{-1} h^{-1}]
Glucase Vmax [nmol L^{-1} h^{-1}]
f
g
h
i
j
Cells [x10^{5} mL^{-1}]
LAPase [amol C cell^{-1} d^{-1}]
GLUCase [amol C cell^{-1} d^{-1}]
LAPase Vmax [amol cell^{-1} h^{-1}]
Glucase Vmax [amol cell^{-1} h^{-1}]

Figure 5