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Interactive comment on "High bacterial organic carbon uptake in the Eastern Tropical South Pacific oxygen minimum zone" *by* Marie Maßmig et al.

Marie Maßmig et al.

aengel@geomar.de

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Anonymous Referee #1 (AR1): 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

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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 Comment (AC): We thank the reviewer for this comment and will include the following paragraphs in the revised version of the manuscript. Additionally, we will include a paragraph concerning the seasonality of the Peruvian system in the introduction (see comment of second Reviewer concerning line 73):

"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

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(Gerreaud 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.

AR1: 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: : :)?

AC: 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 will include figures of oxygen and Chl a concentrations and temperatures for the remaining stations in the supplement. Moreover, a more differentiated description of the study site and a comparison between cruises will be included in section 3.1:

“During our two cruises to the Peruvian upwelling system (Fig. 1), seasonal variability caused higher maximum Chl a concentrations and warmer temperatures in April compared to June 2017. 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 concentrations within the upper 10 m (M136: 3.1±2.6 μg l-1; M138: 2.8 ± 1.3 μg l-1) were not significantly different

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between the two cruises (nM136=75, n M138= 40, W=1416, p= 0.6). At depths >50 m, Chl a concentration was generally below detection limit (Fig. 2a, supplementary Figure 1). Within the upper 10 m the water was warmer in April (21.3 $\pm$1.6°C) compared to June (17.6 $\pm$ 0.6°C) (nM136=75, n M138= 40, W=2886, p<0.01) (Fig. 2b, supplementary Figure 1). Oxygen >100 μmol kg-1 was observed in the surface mixed layer, decreased steeply with depth and reached suboxic concentrations (<5μmol L-1) at > 60 $\pm$ 24 m (Fig. 3a and 4a, supplementary Figure 1). Shallowest depth with suboxic oxygen concentrations were 14 m in April (station Q) and 29 m in June (station D), probably influenced by the distance from shore (Q<D). Oxygen increased again to up to 15 μmol kg-1 at >500 m (Fig. 3a and 4a, supplementary Figure 1). TDN concentrations increased with depth from 18$\pm$8 μmol l-1 and 22$\pm$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. 2c). DOC decreased with depth from 94 $\pm$37 μmol l-1 and 69 $\pm$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. 2d) during both cruises. “

AR1: Specific Comments.

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

AC: In the revised version we will change the title: “Bacterial degradation activity in the Eastern Tropical South Pacific oxygen minimum zone”

AR1: 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.

In our study, we estimated Bacterial growth efficiency (BGE) by two independent methods as explained in chapter 2.5, line 157. One approximation includes the water temperature and uses the equation from Rivkin and Legendre (2001), the other is the

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Discussion paper

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 paragraph starting in line 332.

AR1: 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.

AC: Thank you, we will include this reference, in the revised version.

AR1: 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.

AC: Thank you, we will include this reference, in the revised version.

AR1: 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.

AC: We will modify the paragraph and focus more on previous observations of reduced carbon fluxes in OMZs:

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

Further, we will add a reference showing that the energy yield gained by denitrifying bacteria is even less than suggested by the chemical equations: “Additionally, the en-

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

AR1: 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.

AC: We will change the paragraph in the revised version:

"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). For instance, 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 waters (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 an investigation of extracellular enzyme rates in a more productive oxygen depleted system. The Peruvian upwelling system is an ideal setting in this respect, as it allows to investigate extracellular enzyme activity at shallow oxyclines, with high amounts of labile organic matter (Loginova et al. 2019)."

AR1: L61-62: Again, I disagree with the "lower efficiency of anaerobic respiration" (unless other processes different than denitrification are proved to be relevant).

AC: The term "lower efficiency of anaerobic respiration" will be changed to "energy yield"

AR1: L86-87: It is not clear for me if the filter or the ampule were rinsed with the sample.

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

AR1: L96-106: To be consistent, what is the detection limit and precision of the DHAA and DCHO analysis?

AC: We will add this information: "Detection limit of DHAA was 1.4 nmol L-1 and 10 nmol L-1 for DCHO. The precision was 2% and 5% for DHAA and DCHO, respectively."

AR1: L116: Fig. 5 is cited before Fig. 2.

AC: This will be improved in the revised version.

AR1: 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.

AC: In the revised version of the manuscript, we will 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 will be adapted.

AR1: L154: Enzymatic rates were also measured at a fixed temperature of 13_C. Could in situ temperature be taken into account?

AC: In the revised manuscript, we will apply a temperature correction for the extracellular enzyme rates to account for the differences between in situ and incubation temperature. The correction factor will be based on differences in extracellular en-

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Discussion paper

zyme rates after incubations at 22.4°C and 13°C at five stations during the cruises. All calculations, figures and discussions throughout the text will be adapted.

AR1: 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. AC: In the revised version we will provide further information in section 2.6: “For samples > 5 μmol O2 kg-1, in situ concentration, incubations were conducted under atmospheric oxygen conditions. Samples < 5 μmol O2 kg-1 in situ concentration were incubated in a gas tight incubator that had two openings to fill and flush with gas. For our experiments, the incubator was flushed and filled with N2 to reduce oxygen concentrations. Still, control measurements occasionally revealed oxygen concentrations of 8- 40 μmol O2 kg-1. Additionally, samples were in contact with oxygen during pipetting and measurement.”

Further, we will include in the discussion that results have to be interpreted with care: ” 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.”

AR1: 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?

AC: 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.

AR1: 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.

AC: We thank the reviewer for this advice. In the revised version, we will show DOC concentrations at the different stations by a line plot instead of a dotchart (Fig. 5). Since the DOC flux was calculated for each station separately, we accounted for differences between the stations (see section 2.3).

AR1: L275: DNRA might have lower energy yield, it is not so low for denitrification.

AC: 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 will justify our hypothesis of reduced bacterial activity within suboxic waters compared to the oxyclines by previous observations of reduced carbon fluxes in OMZ. In the revised manuscript, we will change this sentence to: “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)”

AR1: L291-292: I would delete “nitrous oxide” otherwise further explanation is needed as the contribution from anammox to N2O production is quite reduced.

AC: We thank the reviewer for this advice and will delete “nitrous oxide”

AR1: 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.

AC: We will include the references and will remove 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.

AR1: 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.

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

“. . .Thereby, a preferential degradation of amino acid containing organic matter in suboxic waters compared to oxic waters has been suggested (Van Mooy et al., 2002). 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 Vmax and the degradation rate of DHAA by LAPase, were more pronounced within the suboxic waters (Fig. 4b, d). Therefore, observed LAPase rates are in line with the hypothesis of preferred 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. “

AR1: 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.

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

“While labile organic matter is decreasing with depth (e.g. Loginova et al. 2019), TDN (Figure 2c), 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 O2 L-1, and microaerobic respiration might have stimulated cell-specific production or the accumulation of especially active bacterial species (Kalvelage et al. 2011, Kalvelage, 2015). “

AR1: L317-322 and L323-331: These paragraphs seem to be not finished. There are

no clear conclusion for the discussion of these results.

AC: We thank the reviewer for this remark and will finish the paragraphs with a concluding sentence in the revised version of the manuscript

" Depth distribution of cell-specific and total bacterial production was different (Fig. 3b, d); cell-specific production was reduced in suboxic waters, while total production was 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 (Figure 3c), 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.

" .... 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."

AR1: L346-347: According to M&M, BGE followed the established temperature dependence. If no other parameter was used for its calculation, I cannot see how the results of this manuscript for this calculated (but not measured) parameter suggest that oxygen availability control bacterial growth efficiency.

AC: See answer for comment concerning line 19.

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Discussion paper

AR1: L365-367: Well, this study provides estimations, but does not provide measurements for carbon and oxygen losses.

AC: In the revised version of the manuscript, we will emphasize that we only can give estimates.

AR1: L378: Why a BGE of 20% is now assumed? BGE was estimated based on in situ temperature before.

AC: We thank the reviewer for this comment. First, we will explain the choice of a BGE of 20% in the revised manuscript: "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 in Dalsgaard et al. (2012) and Kalvelage et al. (2013) suggest a bacterial production of $\leq$5 μmol C m-3 d-1, equivalent to only about 14% of total heterotrophic bacterial production in suboxic waters determined in our study."

In the revised version, we will also include a BGE of 6% into our calculations. For this we will 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 will change within the revised version because of temperature correction: "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 distributed than expected . . ..."

AR1: 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.

AC: 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 will include the following sentence to account for the uncertainty (see also remark above): "Since denitrification rates were not measured directly, the comparability of published denitrification rates and our measurements of bacterial production are limited."

AR1: 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.

AC: We thank the reviewer and will delete 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).

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