Dear Editor,

Attached is a revised version of our manuscript. Changes made according to the comments of Referee #1 are highlighted in yellow; changes based on the comments of Referee # 2 are highlighted in green.

In addition, we had to slightly revise the oxygen consumption budget for the eddy over time. We previously used oxygen concentrations from the coast off Mauritania in an area from 18.10°-19.22°N. However, based on a refined eddy track calculation, we had to correct the average oxygen concentration at σ_T= 26.3 ± 0.15 kg m^{-3} from 35.12 ± 4.08 µmol kg^{-1} to 36.69 ± 6.91 µmol kg^{-1} using only the data from 18.10°-18.25°N. Further, we excluded the glider based measurements from the budget calculation in order to avoid comparing two different detection methods. Therefore, we used the Winkler-measurements from Meteor M105 survey with a minimum oxygen concentration of 4.8µmol kg^{-1}, instead of the glider-based minimum of 1.2 µmol kg^{-1}. This translates into a revised slightly more conservative oxygen consumption rate of 0.18 µmol kg^{-1} d^{-1} (in the previous version of the manuscript we calculated 0.19 µmol kg^{-1} d^{-1}). A very detailed explanation on the oxygen development over time will, however, be subject of the manuscript of Fiedler et al., this issue.

Additional discussion of the manuscript with Prof. Beman, University of California, Merced, led to the following additional changes:

1. p. 14190, 13-16: Numerical information on the differences of the SAR11 cluster was added.
2. p. 14195, 1-5: A statement on N_2O production was included.

Despite not specifically requested by the referees, we also included a description of the qPCR detection limits.

All changes in addition to the referees' comments are highlighted in light blue in the main text. A marked-up version of the revised manuscript is attached.

Thank you for editing this manuscript!

All the best,

Carolin

Response to Referee #1

I would like to thank Referee 1 for considering this manuscript being adequate for publication in Biogeosciences. The comments and thoughts provided by Referee 1 were particularly helpful to improve this manuscript. Questions and comments were addressed as described in the following; modifications are highlighted in yellow in the main text:

General comments:

Referee comment (RC)1: I am fairly surprised to not see organisms of the SUP05 clade being present/ abundant here as they are normally found in OMZs. Was the amplicon dataset screened for them?

Author comment (AC)1: SUP05 was indeed present in the dataset; we presented the diversity on the phylum level, SUP05 would only show up on the family level and clusters within the phylum of Proteobacteria, as shown in figure 4. In order to provide a deeper insight into the distribution of SUP05 and other organism groups, we now included a table and a plot including all taxa on the family level to the supplement and added the information of SUP05 distribution to the text.

RC2: If the figures are already the size they later should appear in the manuscript, then I would suggest increasing it for the sake of readability. They are really nice, so no need to make them so small.

AC2: Thanks for this nice remark- we will increase the figures in the final manuscript.

RC3: While the manuscript is generally well written, sometimes complicated sentence structure with multiple sub-sentences makes reading bothersome. Streamlining this a little could enhance the reading flow significantly.

AC3: The manuscript has now been checked by a native speaker in order to increase the reading flow.

Specific comments:

RC4: p. 14178, l. 10 ff: "...at depth around 40 to 100 m..." sounds weird. Is "...at depths of around 40..." meant?

AC4: Changed as suggested.

RC5: p. 14180, l. 6-13: Why state the aim and then the ultimate aim? Wouldn't it make more sense to clearly define what the aim was? To identify differences in microbial community structure between eddy and surrounding waters was just a step towards the aim, or?

AC5: This is true, the paragraph has been revised:

'In this study, we investigated differences in microbial community structure in an O_2-depleted eddy, surrounding ETNA open waters, and upwelled waters on the Mauritanian shelf. This was achieved using a combined high-throughput 16S rDNA amplicon sequencing/qPCR approach along with carbon uptake rate measurements and hydrochemical observations. This study aimed to understand the microbial community response to O_2 depleted conditions with regard to primary production and remineralization in these poorly described anomalies to further understand the sensitivity of the ETNA biogeochemistry to future ocean deoxygenation.'

RC6: p. 14190, l. 15: It would be nice to cite the original papers here.

AC6: We replaced the cited overview paper by the original publication of Giovannoni et al., 1990 in Nature.

RC7: p. 14190, l. 21 ff: It is not clear if the methanogens detected are free living in the water column or are particle associated. Judging from the collection of biomass samples on 0.2 µm filters, both options could be possible. Were free living methanogens detected in OMZs previously?

AC7: Sure, as a result of our sampling strategy both options are possible; however, it would not matter with regard to the difference in community structure between samples inside and outside the eddy. Ganesh et al. (2015 in ISME j) show in the supplement of their study that a certain proportion of euryarchaeota are present in the filter fraction of 0.2-1.6 µm. The authors consider this fraction as free-living organisms. They, however, do not show more details on the phylogeny of this group so that it remains unclear whether these are methanogens or not.

RC8: p. 14192, l. 12-15: To confirm if the upper chl a maximum is caused by eukaryotic phototrophs, it might be an idea to look into the sequences classified as cyanobacterial 16S rRNA and see if 16S genes from plastids were detected and are hidden in there.

AC8: The applied sequence analysis method removes sequences of chloroplasts and mitochondria in the initial step. So, they are not hidden in the cyanobacterial part of the dataset. However, it is possible to recover them, and we added the information to the text. Actually, the idea of recovering information on eukaryotic phototrophs from the plastids is interesting (although a characterization of eukaryotes remains impossible from plastid sequences) - still the extraction protocol for nucleic acids may not be as efficient for eukaryotes as it is for microbes. This has to be considered. In addition, we tested the applied primer set *in silico* on the SILVA database and determined a relatively low coverage of only 38% for chloroplast sequences. Therefore, the information obtained from chloroplast sequence analysis can only be taken indicative for the presence and abundance of eukaryotic phototrophs; however, using it as a quantitative tool is only possible to a limited extent. Particularly, it can't be quantitatively compared to the abundance of cyanobacteria because of the different coverage as described above.

RC9: p. 14193, l. 23-25: Judging from the phylogenetic information on the metabolisms of the organisms is not always possible, so the statement is fairly strong here. While I think the authors are right I still would word it a little bit more careful.

AC9: I agree, the sentence has been rephrased:

'The close proximity of increased abundances of the HNLC-PCC maximum to the O_2 minimum in the eddy may thus point towards a beneficial relation between the HNLC-PCC and the eddy core water microbial community that largely consists of heterotrophic organisms.'

RC10: p. 14194, l. 7: original studies are missing in the references (Morris 2002, Rappe 2002)

AC10: We added the suggested references.

RC11: p. 14195, l. 4: How do the 3000 copies/L and 100 copies/L compare to the bacterial 16S rRNA gene copy numbers? What part of the population has the capability for denitrification? Because of differences in cell numbers between eddy and the outside the actual difference might not be as high as suggested.

AC11: The part of the community containing *nirS* is very minor in all samples, which does not mean that the corresponding process is not active or important. (For comparison, N2-fixers are considered to account for only 2% in ocean surface metagenomes; however, they contribute up to 80% of new nitrogen to these waters). The absolute number of *nirS* sequences measured against a standard dilution series cannot be compared to 16S rRNA copy numbers from the amplicon sequencing, as this is would mean comparing two different methods.

However, we see a highly pronounced effect of denitrification on nitrous oxide (N_2O) production within the eddy core waters based on molecular and isotopic data (Grundle, Löscher et al.' Extreme N_2O activity in an oxygenated ocean', under review). This effect is not present outside the eddy, where N_2O is supposed to be exclusively produced by nitrification (see, e.g., Löscher et al., 2012, Ryabenko et al., 2012). Therefore, we are convinced that our *nirS* dataset mirrors the presence/activity of the denitrification process, which is the key finding, here. We added the reference (Grundle et al.) to the text.

RC12: p.14195, l. 12: With similar reasoning as in the comment above, it would make sense to compare the transcripts of the *nirS* gene to a housekeeping gene.

AC12: I do not agree on that. The potential for nitrite reduction is not depending on the overall abundance of bacteria or microbes or the biomass. It is more a question of the presence of certain organisms in an environment that enables the respective process. Absolute gene quantification is also from a methodological perspective preferable as it mitigates the risk of including a second PCR bias.

RC13: p. 14197, l. 22: space missing

AC13: Changed.

Response to Referee #2

The authors would like to thank Referee #2 for considering this manuscript being interesting and appropriate for publication in Biogeosciences. The manuscript largely improved by including the comments and suggestions provided by Referee #2. We addressed the comments as follows; modifications are highlighted in green in the main text:

General comments

Referee comment (RC) 1: The abstract says that "metagenomic" data are shown, but the molecular markers used in this study are for bacterial/archaeal diversity, and a few functional genes. "Meta" suggests that large portions of the community genes are evaluated, which is not the case. It would be more accurate to edit this phrase. Outside of the abstract, this term is not used, so the body of the text is appropriate in scope.

Author comment (AC)1: We removed the term from the abstract and replaced it with microbial community study.

RC2: Here the statement is made that enhanced primary productivity fuels enhanced export, but there is little to no primary evidence within the manuscript to support this statement. Is export flux greater within the eddy than in nearby regions, and if so, how is the time-space decoupling of productivity and flux resolved?

AC2: Within the framework of this special issue a paper (currently in typeset) has been submitted by Gerhard Fischer (MARUM; Bremen, Germany) and colleagues entitled: "Bathypelagic particle flux signatures from a suboxic eddy in the oligotrophic tropical North Atlantic: production, sedimentation and preservation". The paper discusses sediment trap data from a low-oxygen eddy observed in 2010 from that same area. The authors document a remarkable impact on all productivity related processes. They estimated a 3-fold higher productivity in the surface layer compared to surrounding waters. In particular they found a multiple times increase in the mass flux in the bathypelagic traps (2300 and 170 m above the seafloor) during the eddy passage. Furthermore, Fiedler et al. (to be submitted to this special issue as well) determined export flux derived from Carbon remineralization rates within the eddy and found a 3-4-fold enhanced export flux compared to background conditions in the open-ocean ETNA. We added these references and the related information to the text.

RC3: The cut-off of 90 umol/L oxygen concentration to differentiate 'realm' effects is not sufficiently supported. Is there evidence in the literature for such a cut-off, for example, are certain microorganisms known to respond differently across this threshold in relation to metabolism/productivity and therefore, it is an ecologically important distinction?

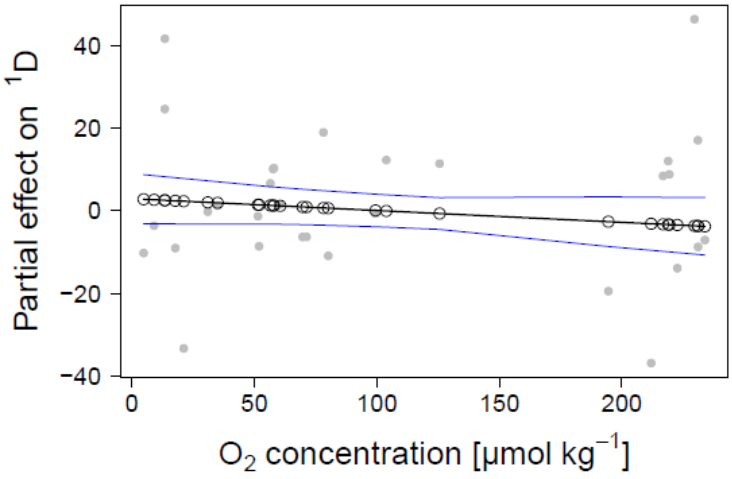
AC3: This range was chosen for two reasons: The first reason was to obtain sample groups of fairly equal size between stations for statistical reasons.

Indeed, there is evidence in the literature, that has not been explained sufficiently in our manuscript is that 90 µmol L^{-1} is the highest concentration of O_2 at which denitrification has been described to be active (Gao et al., 2010).We included this information and the reference into the methods section.

Gao, H., Schreiber, F., Collins, G., Jensen, M. M., Kostka, J. E., Lavik, G., de Beer, D., Zhou, H.-y., and Kuypers, M. M. M.: Aerobic denitrification in permeable Wadden Sea sediments, ISME J, 4, 417-426, 2010. http://www.nature.com/ismej/journal/v4/n3/abs/ismej2009127a.html

RC4: Relating again to the oxygen concentration cut-off – how do communities compare along the oxygen gradient? Does alpha diversity (or total OTU abundance) decrease with decreasing oxygen concentration?

AC4: As stated on p. 14187, l. 22-23, our analysis does not show a pronounced effect of O_2 on alpha diversity if other effects are controlled (see figure below): The black regression line through the fitted values (circles) is compatible with zero, as indicated by the blue 95% confidence interval.



RC5: Within the methods section, where volumes are given for reagents within assays, it would be more useful to provide the final concentration.

AC5: We agree and added the missing information to the text.

'Reactions were performed in technical duplicates in a final volume of 12.5 µL using 0.25 µL of each primer (10 pmol $µL^{-1}$), 3.25 µL nuclease-free water and 6.25 µL SYBR qPCR Supermix W/ROX (Life Technologies, Carlsbad, CA, USA) on a ViiA7 qPCR machine (Life Technologies, Carlsbad, CA, USA) according to established protocols (Ahlgren et al., 2006;West et al., 2011). TaqMan-based qPCRs were performed for picophytoplankton (*Prochlorococcus*/*Synechococcus*) and bacteria as previously described (Suzuki et al., 2001) in a final volume of 12.5 µL with primer/probe concentrations as shown elsewhere (Table 1, (West et al., 2011)), but with the addition of 0.5 µL BSA (20 mg mL^{-1})and 6.25 µL TaqMan Mix (Life Technologies, Carlsbad, CA, USA).'

RC6: On several occasions the phrase “of around” is used to mean “approximately.” While generally well written, the manuscript requires some additional editing for increased readability.

AC6: We changed this where adequate; the manuscript was also corrected by a native speaker, now (see also comments to referee 1).

Specific comments:

RC7: p. 14182 l. 18 – DNA and RNA were quantified fluorometrically using a Nanodrop. This instrument is a spectrophotometer.

AC7: This is true, we modified the sentence.

RC8: p. 14185 Statistics section – intent and readability would be improved for each subsection with an initial sentence about the statistical process and its purpose; especially for those less familiar with the exact procedures.

AC8: We addressed this by adding initial sentences to each section, which explain the purpose of the analysis. The section now reads:

‘2.5 Statistics

Low-abundance OTUs were removed to reduce noise and computation time. Statistical downstream analysis was performed in R v3.1.3 (R Core Team, 2015) with custom scripts (available from the authors on request). As OTUs of very low abundance only increase computation time without contributing useful information, they were removed from the data set as follows: After transformation of counts in the sample-by-OTU table to relative abundances (based on the total number of reads per sample), OTUs were ordered by decreasing mean percentage across samples. The set of ordered OTUs for which the cumulative mean percentage amounted to 99% was retained in the filtered OTU table.

Distribution of OTUs across samples was modeled by a set of environmental variables with minimal interdependence. The variance in OTU composition (i.e., the extent of change in OTU abundance across samples) explained by the measured environmental variables was explored by redundancy analysis (RDA) with Hellinger-transformed OTU counts (Langfeldt et al., 2014;Stratil et al., 2013;Stratil et al., 2014) using the R package vegan (Oksanen et al., 2013). In order to minimize collinearity of explanatory variables in the RDA model, a subset of the recorded environmental variables was chosen according to their variance inflation factor (VIF), employing vegan’s functions *rda* and *vif.cca*. Starting with an RDA model that contained all explanatory variables, the variable with the highest VIF was iteratively determined and removed from the model until all remaining explanatory variables had a VIF <2.5.

OTU distribution was subject to "Realm" depending on O_2 concentration. Model selection started with a full RDA model containing all main effects and possible interactions based on the set of

explanatory variables with minimal collinearity. This model was simplified by backward selection with function *ordistep*. The final RDA model exhibited a significant interaction effect "Realm:O_2" (see results section). For plotting and indicator analysis (see below), the continuous variable "O_2" was converted into a factor with two levels "high O_2" (>90 µM) and "low O_2" (≤90 µM); the threshold of 90 µM was chosen to obtain sample groups of fairly equal size between stations, which include low O_2 parts of the water column at all sampling stations in order to enable a comparison between the ETNA OMZ (outside the eddy) and the eddy OMZ.

We determined OTUs typical for a given combination of levels of factors "Realm" and "O_2". OTUs significantly correlated with any axis in the final RDA model were determined using the function *envfit* with 10^5 permutations, followed by Benjamini-Hochberg correction (false discovery rate, FDR) (Benjamini and Hochberg, 1995). In order to reduce the number of tests in this procedure, OTUs were pre-filtered according to their vector lengths calculated from corresponding RDA scores (scaling 1) by profile likelihood selection (Zhu and Ghodsi, 2006).

OTUs significant at an FDR of 5% were further subject to indicator analysis with function *multipatt* of the R package indicspecies v1.7.4 (De Cáceres and Legendre, 2009) with 10^5 permutations. Indicator OTUs – in analogy to indicator species *sensu* De Cáceres and Legendre (2009) – are OTUs that prevail in a certain sample group (here: a level of factor "Realm" within a chosen O_2 level) while being found only irregularly and at low abundance in other sample groups. In order to remove the effects of the covariate "Depth" in indicator analysis, Hellinger-transformed counts of significant OTUs were first subjected to a linear regression with "Depth"; residuals of this regression were then transformed to positive values by subtraction of their minimum and used as input for indicator analysis.

3D visualizations of the RDA model were produced in kinemage format (Richardson and Richardson, 1992) using the R package R2Kinemage developed by S.C.N., and displayed in KiNG v2.21 (Chen et al., 2009).

Diversity within samples was related to environmental variables by advanced linear regression. For alpha diversity analysis, effective OTU richness (Shannon numbers equivalent, ^{1}D, (Jost, 2006, 2007)) was calculated from the filtered OTU table. ^{1}D was fitted to the set of explanatory variables with minimal collinearity in a generalized least squares (GLS) model using function *gls* of the R package nlme v3.1-120 (Pinheiro et al., 2015). The variable "NO2" was square root-transformed to decrease the potential leverage effect of its two highest values (0.25 µM and 0.28 µM, respectively) on ^{1}D. Apart from main effect terms, the interaction term "Realm:O_2" was included into the GLS model for comparability with beta diversity analysis (see results section). The variance structure of the GLS model was chosen to account for both different variances per level of "Realm" and an overall decreasing variance by "Depth". The resulting model was validated following the recommendations of Zuur et al. (2009). While only the "Realm" effect was significant, the other terms were kept in the model to maintain a valid residual distribution. For visualization of the (partial) effect of only factor "Realm" on ^{1}D, partial response residuals were extracted from the full GLS model re-fitted without the "Realm" main effect. These partial response residuals were then modelled by the "Realm" main effect alone, using the same variance structure as for the full GLS model.'

RC9: p.14188 l. 13-15 What were the depths at which samples were collected that are considered below the euphotic zone?

AC9: We used the term 'euphotic zone' as the depth where photosynthetic available radiation (PAR) is <1% of its surface value. During this survey, this was the case below 60 m water depth, this information has been added to the text.

RC10: p. 14188 l. 16-17 How might carbon fixation measurements be affected if total volumes were not filtered for delta13C enrichment? Could primary productivity estimates be over-estimates?

AC10: Productivity could not be overestimated because the filtrate volume was taken into account for calculating primary productivity. Incubation bottles were also shaken periodically every ten minutes while doing filtration for homogenization of particulate matter.

RC11: p. 14192 l. 12-16 Are cell counts (microscopy and/or flow cytometry) available to conclude that the qPCR Prochloro/Synechococcus data are representative of relative differences in abundance of cyanobacteria and eukaryotes in- and outside of the eddy or within either chl max layer?

AC11: Unfortunately, cell counts are not available. Our approach was to compare the 16S rRNA amplicon dataset with qPCR data, which also includes two different methods. Quantification by qPCR has been performed against a standard dilution series, and then compared to the sequence abundance in the 16s rRNA pool to assure that the respective clusters are key organisms in the samples. As a related comment has been made by Referee 1 we also added a detailed table on cyanobacterial and eukaryotic phytoplankton distribution to the supplement.

RC12: p.14192 l. 17-28 Why might HL-adapted Prochlorococcus ecotypes be abundant below the euphotic zone? This seems counter-intuitive. Suggestions on why this might be would be interesting.

AC12: The detected ecotype is described to cluster among the HL Prochlorococcus strains. However, also in the Pacific it has been recovered from waters below the euphotic zone. It could therefore be thought that the 16S rRNA based classification of HL and LL strains may differ from the functional classification. A future approach to obtain information on that could include sampling for flow cytometry. A pigment and biomass analysis following cell sorting may lead to this information in future studies.

RC13: p. 14193 l. 12-13 This paper states that Prochlorococcus could contribute up to 40% of the DOC that could support bacterial production. As written, the statement suggests that Prochlorococcus is responsible for 40% of bacterial production.

AC13: Thanks for this comment, we corrected this statement, it now reads:

'This may be critical as *Prochlorococcus* is one of the most abundant photosynthetic organisms in the ocean and contributes up to ~40% of dissolved organic carbon for bacterial production (Bertillson et al., 2005).'

RC14: p. 14195 l. 20 I am surprised that nifH genes were not quantifiable from the eddy. nifH genes have been retrieved from this region. Assay detection limit? High inorganic dissolved nitrogen concentrations and N:P ratios close to Redfield do not exclude the possibility of diazotrophs and/or biological nitrogen fixation.

AC14: It is for sure true that inorganic N compounds do not exclude N_2 fixation in all cases. The argument was used to rather explain the absence of N_2 fixers. The detection limits were determined from no-template controls. Those were run in duplicate for each primer and probe set, and were undetectable after 45 cycles, thus setting the theoretical detection limit of our assay mixture to one *nifH* copy. In reality, the detection limit depends on the amount of seawater filtered per sample, elution volume after extraction, and the amount of sample loaded to the qPCR assay, too. We eluted in 100 µL of elution buffer, therefore, when using 2.5 µL of the eluate, a minimum of 40 copies would be needed for the detection of 1 copy. Based on a filtration volume of 2L seawater, the detection limit would be 20 copies L^{-1}. qPCR efficiencies were calculated using the formula $E = 10^{-1/slope} - 1$, and were between 95.3% and 96.8%.

Figures

1. no comments

2. For the oxygen concentrations, can the profile of discrete O_2 concentrations be shown?

AC: The discrete profiles are shown in figures 3 and 4.

3. Figures should be larger for easier readability.

AC: We will increase the size of figures; this has also been suggested by Referee 1.

4. Figures should be larger for readability. Greater transparency of the colored bars would make the trends easier to compare across panels. Figure legend reads 'oxygen versus depth,' but this line is oxygen concentration. Colors used for Proteobacteria and Bacteroidetes are very similar. Would be easier to discern with different colors.

AC: We will increase the size of this figure, too. Colors have been modified to make the difference between different phyla more obvious. The legend has been changed to O_2 [µmol kg^{-1}]

5. Dark purple and dark blue points are difficult to discern from one another.

AC: We modified the colors.

6. Check eddy axis labels. Is this correct with 'eddy_2' on the left?

AC: Yes, this is correct.

7. Transparency of bars needs to be greater so data points can be seen. Are these discrete or derived measurements of chl a? Legend edit is required and methods section should include description of chl a measurement methods. Chl a units are missing.

AC: These are discrete measurements, Chl a was measured from filter samples, the unit is µg L^{-1}. We modified the figure and the legend by adding this information; we further increased the transparency of the bars. A description of chla measurements has been added to the methods section:

'Sea water samples (0.5 – 1 L) for chlorophyll *a* (Chl *a*) analyses were filtered (200 mbar) on GF/F filters (25 mm, 0.7 µm; Whatman, Maidstone, UK). Filters were transferred to a plastic vial and 1 ml of MilliQ water was added. Filters were immediately frozen at -20°C and stored for at least 24 h. Afterwards, 9 ml acetone (100 %) was added to the vials and the fluorescence was measured with a Turner Trilogy fluorometer (Sunnyvale, CA, USA). Calibration took place using a Chl *a* standard dilution series (*Anacystis nidulans*, Walter CMP, Kiel, Germany). Chl *a* concentrations were determined as described by Parsons et al. (1984).'

8. Difficult to discern low concentration areas of the plots. Is the number zero copies, or "not detected?" What is the detection limit of the assays? Symbols are difficult to differentiate as plotted. Increase size of plots and/or data points. X axes' labels should be edited to be consistent in format.

AC: The size of the figure has been increased. Symbols sizes were increased and the colors were modified. The x axis of Fig. 8A was adjusted. The detection limits of the qPCR assays are mainly the same as described above for *nifH*, again determined from no-template controls. Those were run in duplicate for each primer and probe set and were undetectable after 45 cycles with a theoretical detection limit of 1 copy. As described, above, we eluted in 100µL buffer, therefore, when using 2.5µL of the eluate, a minimum of 40 copies would be needed for the detection of 1 copy. Based on a filtration volume of 2L seawater, the detection limit would be 20 copies L^{-1}.

9. One symbol could be 'open' so overlapping data can be more clearly seen. Again, what is the detection limit of the assay? Is the data point zero or 'not detected?'

AC: The data point zero means below detection limit of the assay (<20 copies L^{-1}), therefore it is between zero and 20 copies L^{-1}.

Hidden biosphere in an oxygen-deficient Atlantic open ocean eddy: Future implications of ocean deoxygenation on primary production in the eastern tropical North Atlantic

C. R. Löscher[1]*, M. A. Fischer[1†], S. C. Neulinger[1†], B. Fiedler[2], M. Philippi[1], F. Schütte[2], A. Singh[2#], H. Hauss[2], J. Karstensen[2], A. Körtzinger[2,3], S. Künzel[4], R. A. Schmitz[1]

[1] Institute for General Microbiology, Kiel, Germany

[2] GEOMAR, Helmholtz Centre for Ocean Research Kiel, Kiel University, Germany

Now at: Physical Research Laboratory, Geosciences Division, Ahmedabad, India 380 009

[3] Christian-Albrechts-Universität zu Kiel, Kiel, Germany

[4] Max Planck Institute for Evolutionary Biology, Plön, Germany

* Correspondence should be addressed to cloescher@geomar.de

† Equal contribution.

The eastern tropical North Atlantic (ETNA) is characterized by a highly productive coastal upwelling system and a moderate oxygen minimum zone with lowest open ocean oxygen (O_2) concentrations of approximately 40 µmol kg^{-1}. The recent discovery of re-occurring mesoscale eddies with close to anoxic O_2 concentrations (<1 µmol kg^{-1}) located just below the mixed layer has challenged our understanding of O_2 distribution and biogeochemical processes in this area.

Here, we present the first microbial community study from a deoxygenated anticyclonic modewater eddy in the open waters of the ETNA. In the eddy, we observed significantly lower bacterial diversity compared to surrounding waters, along with a significant community shift. We detected enhanced primary productivity in the surface layer of the eddy indicated by elevated chlorophyll concentrations and carbon uptake rates of up to three times as high as in surrounding waters. Carbon uptake rates below the euphotic zone correlated to the presence of a specific high-light ecotype of *Prochlorococcus*, which is usually underrepresented in the ETNA. Our data indicate that high primary production in the eddy fuels export production and supports enhanced respiration in a specific microbial community at shallow depths, below the mixed layer base. The O_2-depleted core waters eddy promoted transcription of the key gene for denitrification, *nirS*. This process is usually absent from the open ETNA waters.

In light of future projected ocean deoxygenation, our results show that even distinct events of anoxia have the potential to alter microbial community structure with critical impacts on primary productivity and biogeochemical processes of oceanic water bodies.

1 Introduction

The eastern tropical North Atlantic (ETNA) region is influenced by an eastern boundary upwelling system (EBUS) off northwest Africa, which along with nutrient supply via Saharan dust deposition, fuels one of the most productive ocean regions in the world. A moderate oxygen minimum zone (OMZ) is associated with this EBUS, with lowest oxygen (O_2) concentrations just below 40 μmol kg^{-1} present at intermediate depths (Chavez and Messié, 2009;Jickells et al., 2005;Karstensen et al., 2008). O_2 records over several years from the Cape Verde Ocean Observatory (CVOO) mooring (located at 17° 35'N, 24° 15'W, Fig. 1) confirmed the well-ventilated character of the ETNA. However, the observation of distinct events of very low-O_2 concentrations (<1 μmol kg^{-1}) at depths around 40 to 100 m over periods of more than one month challenged our understanding of the biogeochemistry in that area (Karstensen et al., 2015a). The meridional current structure observed during these low-O_2 events revealed the passage of anticyclonic modewater eddies (ACME) crossing the CVOO mooring (Karstensen et al., 2015a). The ocean is filled with eddies (Chelton et al., 2011) but only a few of them have the dynamical and biogeochemical boundary conditions that support formation of a low-O_2 core. Anomalous low salinity within the ETNA low-O_2 eddies suggested the water mass originated from the EBUS off Mauritania, which was confirmed by analyzing sea-level anomaly data. In combination with other data from the upwelling region, Karstensen et al. (2015a) showed that O_2 concentrations decreased over a period of a few months during westward propagation of the eddies into the open north Atlantic Ocean. Respiration in these eddies was estimated to be about three to five times higher than typical subtropical gyre values (Karstensen et al., 2008).

Mesoscale eddies are increasingly recognized as biogeochemical hot-spots of basin-wide relevance for the world's oceans (Altabet et al., 2012;Baird et al., 2011;Chelton et al., 2011;McGillicuddy et al., 2007;Oschlies and Garcon, 1998;Stramma et al., 2013). Upward nutrient supply to the euphotic zone through mesoscale eddy dynamics enables intense primary productivity (Lévy et al., 2012;Lévy et al., 2001;McGillicuddy et al., 2007). Classically, primary producers in the ETNA open waters area are dominated by a range of diatom clades, flagellates and cyanobacteria (Franz et al., 2012), but so far no specific information on the primary producers in productive ETNA eddies has been reported. As a result of enhanced primary production in the surface, increased organic matter export flux below the euphotic zone is expected, which in turn supports increased respiration at intermediate depths. Indeed, particle maxima a few meters above the O_2 minimum have been reported based on autonomous observations of O_2-depleted eddies in the ETNA (Karstensen et al., 2015a) indicating enhanced organic matter export and provide environments of enhanced remineralization (Ganesh et al., 2014). Observations from a low-O_2 eddy from the ETNA revealed a remarkable impact on all productivity-related processes in that particular system (Fischer et al., 2015). Estimated productivity was three-fold higher in the surface layer compared to surrounding waters along with a multiple times increase in

mass flux in bathypelagic during the eddy passage. Furthermore, Fiedler et al. (in prep. for this special issue) determined export flux derived from carbon remineralization rates within the eddy and found a 3-4-fold enhanced export flux compared to background conditions in the open-ocean ETNA.

O_2-depleted conditions are supposed to act as a critical switch for the marine microbial community, both with regard to functionality and diversity. O_2 begins to limit oxidative pathways and reductive pathways are induced (Stewart et al., 2011;Ulloa et al., 2012;Wright et al., 2012). A loss in microbial diversity related to vertical O_2 gradients has previously been described for the Pacific Ocean (Beman and Carolan, 2013;Bryant et al., 2012), but to date no comparable data are available from the ETNA. O_2-loss related microbial community shifts and modified functionality are supposed to favor heterotrophic communities dominated by Flavobacteria, α- and γ- Proteobacteria, which efficiently recycle organic matter (Buchan et al., 2014). Furthermore, marine nitrogen (N) and carbon (C) cycling are significantly altered under low O_2 conditions (Vaquer-Sunyer and Duarte, 2008;Wright et al., 2012). Substantial N loss (Altabet et al., 2012) along with enhanced nitrous oxide production (Arévalo-Martínez et al., 2015) has been described in low-O_2 eddies in the OMZ off Peru in the eastern tropical South Pacific.

Classically, the N cycle in the open ETNA is assumed to be dominated by nitrification. An N loss signal is not present due to comparably high background O_2 concentrations (≥40 µmol kg^{-1},(Löscher et al., 2012;Ryabenko et al., 2012). However, any drop in O_2 concentration in the water column, as potentially induced by the low-O_2 eddies, could potentially activate anammox and/or denitrification. During recent decades, the ETNA OMZ has been expanding both in terms of vertical extent and intensity and is predicted to expand further in the future (Stramma et al., 2008) with unknown consequences for the ecology and biogeochemistry of that system. Thus, it is critical to understand the biogeochemical response to changing O_2 concentrations in that region.

In this study, we investigated differences in microbial community structure in an O_2-depleted eddy, surrounding ETNA open waters, and upwelled waters on the Mauritanian shelf. This was achieved using a combined high-throughput 16S rDNA amplicon sequencing/qPCR approach along with carbon uptake rate measurements and hydrochemical observations. This study aimed to understand the microbial community response to O_2 depleted conditions with regard to primary production and remineralization in these poorly-described anomalies, to improve understanding of the sensitivity of the ETNA biogeochemistry to future ocean deoxygenation.

2 Material and Methods

2.1 Data collection

Remotely sensed sea level anomalies (SLA), in combination with temperature and salinity data measured by Argo floats (an overview is presented by Körtzinger et al., (2015) in preparation for this issue) were used for general eddy identification and tracking in this area. After identification of a low-O_2 eddy candidate that was propagating towards CVOO, a pre-survey was started using autonomous gliders (see Karstensen et al. (2015b), in preparation for this issue). Once the glider data had confirmed the low O_2 concentration in the candidate eddy, a ship-based survey was started. First, we performed a survey with the Cape Verdean RV Islandia on Mar 6, 2014 (samples from this survey are further referred to as eddy_1), followed by a second survey with the German RV Meteor (cruise M105; Mar 19, 2014; samples from this survey are further referred to as eddy_2). Moreover, the background signal (i.e. waters outside the eddy) was measured, in order to compare the eddy with the typical open ocean ETNA environment. For this purpose, we used metagenomic samples from the CVOO time series monitoring site (collected on 03/19/2014 during cruise M105). Samples from the Mauritanian shelf collected during R/VMeteor Cruise M107 (station 675, 18.22°N/ 16.56°W, collected on 06/24/2014) represent data from the eddy formation area. Station 675 was chosen according to its location within the area that Schütte et al. (2015, in preparation, this issue) identified as the region of eddy formation and further because of the observed low O_2 concentrations of 33.9 µmol kg^{-1} at 115 m depth (which corresponds to a potential density of σ_T= 26.4 kg m^{-3}, thus similar to the core density of minimal O_2 concentrations in the eddy).

In addition to metagenomic sampling, carbon uptake measurements were performed during the R/V Meteor M105 survey at two stations: no. 186 (profile 10, 19.3°N, 24.77°W) and no. 190 (profile 15, 18.67°N, 24.87°W, see Fig. 1C, blue crosses).

2.2 Water sampling and Hydrographic parameters

Discrete samples for salinity, dissolved O_2 and nutrients on all surveys were taken from a CTD rosette equipped with Niskin-bottles. The CTD data were calibrated against salinity samples and CTD oxygen probe data (SBE 43 Clark electrode sensor) were calibrated against O_2 concentrations, determined following the Winkler method using 50 or 100 mL samples. Salinity and nutrient concentrations were determined as described in Grasshoff et al. (1999). The CTD on R/V Meteor was equipped with double sensors for conductivity, temperature, and oxygen. Calibration followed standard procedures (GO-SHIP Manual; (Hood et al., 2010)).

2.3 Oxygen respiration

In order to estimate the net O_2 consumption as a potential driver for microbiological community shifts a simple calculation was performed as follows:

(1) $\Delta O_2 = O_2(S) - O_2(E)$

where O_2(S) denotes the lowest O_2 concentration detected on the shelf (36.69 ± 6.91 µmol kg^{-1} at σ_T= 26.3 ± 0.15 kg m^{-3}, cruise M107, average of shelf stations between 18.10°N/ 16.59°W and 18.25°N/ 16.45°W). This region was chosen as it was identified (Schütte et al. (2015), in preparation, this issue) to be the area were the eddy most likely originated. O_2(E) denotes the lowest O_2 concentration measured in the eddy core at the same potential density (4.8 µmol kg^{-1} at σ_T= 26.35 kg m^{-3} during M105).

The daily O_2 loss rate (ΔO_{2d}) was calculated as follows, assuming a lifetime of 180 days of the eddy (Schütte et al. (2015), in preparation, this issue):

(2) $\Delta O_{2d} = \Delta O_2/180$

2.4 Chlorophyll a measurements

Sea water samples (0.5 – 1 L) for chlorophyll *a* (Chl *a*) analyses were filtered (200 mbar) on GF/F filters (25 mm, 0.7 µm; Whatman, Maidstone, UK). Filters were transferred to a plastic vial and 1 mL of MilliQ water was added. Filters were immediately frozen at -20°C and stored for at least 24 h. Afterwards, 9 mL acetone (100 %) was added to the vials and the fluorescence was measured with a Turner Trilogy fluorometer (Sunnyvale, CA, USA). Calibration took place using a Chl *a* standard dilution series (*Anacystis nidulans*, Walter CMP, Kiel, Germany). Chl *a* concentrations were determined as described by Parsons et al. (1984).

2.5 Molecular Methods

Seawater samples were taken from the Niskin-Bottles at selected CTD casts. For nucleic acid purification 2 L seawater was rapidly filtered (exact filtration volumes and times were recorded continuously) through 0.2 µm polyethersulfone membrane filters (Millipore, Billerica, MA, USA). The filters were immediately frozen and stored at −80°C until further analysis. Nucleic acids were purified using the Qiagen DNA/RNA AllPrep Kit (Qiagen, Hilden, Germany) with modifications as previously described (Löscher et al., 2012).

Extracts of DNA and RNA were quantified using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). To remove DNA from RNA extracts, a DNase I treatment (Invitrogen, Carlsbad, CA) was performed; purity of RNA was checked by PCR amplification before random reverse transcription with the Quanti Tect® Reverse Transcription Kit (Qiagen, Hilden, Germany). HNLC, HLII and other *Prochlorococcus* ecotypes were qPCR-amplified using primers and PCR conditions as previously described (Ahlgren et al., 2006). Reactions were performed in technical duplicates in a final volume of 12.5 µL using 0.25 µL of each primer (10 pmol μL^{-1}), 3.25 µL nuclease-free water and 6.25 µL SYBR qPCR Supermix W/ROX (Life Technologies, Carlsbad, CA,

USA) on a ViiA7 qPCR machine (Life Technologies, Carlsbad, CA, USA) according to established protocols (Ahlgren et al., 2006;West et al., 2011). TaqMan-based qPCRs were performed for picophytoplankton (*Prochlorococcus*/*Synechococcus*) and bacteria as previously described (Suzuki et al., 2001) in a final volume of 12.5 µL with primer/probe concentrations as shown elsewhere (Table 1, (West et al., 2011)), but with the addition of 0.5 µL BSA (20 mg mL^{-1}) and 6.25 µL TaqMan Mix (Life Technologies, Carlsbad, CA, USA). Dilution series of plasmids containing the target gene were used as standards as described (Lam et al., 2007;Löscher et al., 2012). Nitrogen cycle key functional genes *amoA*, *nirS*, *hzo* and *nifH* were amplified and quantified from DNA and cDNA following established protocols (Lam et al., 2007;Langlois et al., 2008;Löscher et al., 2014;Löscher et al., 2012). Detection limits of qPCR assays were determined from no-template controls, which were run in duplicate for each primer (and probe) set, and were undetectable after 45 cycles, thus setting the theoretical detection limit of our assay mixtures to one gene copy. However, detection limits additionally depend on the amount of filtered seawater per sample, elution volume after extraction, and the amount of sample loaded to the qPCR assay. Based on a filtration volume of 2L seawater, a detection limit of 20 copies L^{-1} has been determined. qPCR efficiencies were calculated using the formula $E = 10^{-1/slope} - 1$, and were between 95.3% and 96.8%.

2.5.1 PCR amplification of bacterial and archaeal 16S rDNA for Illumina MiSeq amplicon sequencing

For the analysis of the bacterial community, hypervariable regions V1 and V2 of the 16S rDNA was amplified from genomic DNA using the primer set 27 forward (Frank et al., 2007) and 338 reverse (Fierer et al., 2008). Beside the target-specific region the primer sequence contained a linker sequence, an 8-base barcode and the Illumina specific region P5 (forward primer) or P7 (reverse primer), respectively, as recently described (Kozich et al. 2013). The PCR reaction mixture consisted of 13.6 µL DEPC H_2O (Roth, Karlsruhe, Germany), 0.4 µL of 10 mM dNTPs (Thermo Fisher Scientific), 4 µL 5x HF-buffer (Thermo Fisher Scientific, Waltham, MA, USA), 0.8 µL primers (5 µM, Eurofins, Ebersberg, Germany), 0.2 µL Phusion high fidelity polymerase (2 U μL^{-1}, Thermo Fisher Scientific, Waltham, MA, USA) and 1 µL genomic DNA with a concentration between 10 and 100 ng μL^{-1}. Negative controls consisted of the reaction mixture as described above without the addition of DNA. PCR reaction conditions started with an initial denaturation step for 5 min at 95°C followed by 30 cycles of 15 s denaturation at 95°C, 30 s primer annealing at 52°C and 30 s elongation at 72°C and a final elongation at 72°C for 5 min.

For analysis of the archaeal community, hypervariable regions V5-V7 of the 16S rDNA were amplified from genomic DNA using the primer set 787 forward and 1059 reverse (Yu et al., 2005) with 8-base barcode and Illumina specific adapters. Reaction mixture, PCR protocol and purification

were identical to the amplification of bacterial community DNA amplification, the only difference was the annealing temperature (58°C). Amplification was checked for correct size and band intensity on a 2.5% agarose gel. Amplicons were purified using the MinElute Gel Extraction Kit (Qiagen, Hildesheim, Germany and quantified on a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Waltham, MA, USA). Pooled purified amplicons were prepared and sequenced according to the manufacturer's protocol on a MiSeq Instrument using the MiSeq reagent Kit V3 chemistry (Illumina, San Diego, CA, USA). Sequences were submitted to NCBI Sequence Read Archive under accession number PRJNA288724.

2.5.2 Sequence analysis of 16S rDNA gene amplification

Sequence processing was performed using mothur software version 1.32.1 (Kozich et al., 2013;Schloss et al., 2009). 4,054,723 bacterial sequence read pairs could be concatenated to contiguous sequences (contigs) using the command *make.contig*. Contigs containing ambiguous bases, homopolymers longer than 8 bases or contigs longer than 552 bases were deleted from the dataset. Redundant sequences were clustered using the command *unique.seqs*, which led to 645,444 unique sequences. Sequences were consecutively aligned with *align.seqs* against a modified version of the SILVA database release 102 (Pruesse et al., 2007) containing only the hypervariable regions V1 and V2. The alignment was optimized by removing sequences not aligning in the correct region with *screen.seqs*, and by the removal of gap-only columns using *filter.seqs*. The optimized alignment contained 636,701 sequences of lengths between 255 and 412 bases. Rare sequences with up to 3 positional differences compared to larger sequence clusters were merged with the latter by the *pre.cluster* command. Chimeric sequences were removed with the implemented software UCHIME (Edgar et al., 2011) using the command *chimera.uchime*, followed by *remove.seqs*.

Taxonomic classification of the remaining sequences was done using the Wang approach based on a modified version of the Greengenes database (DeSantis et al., 2006) with a bootstrap threshold of 80%. Sequences of archaea, chloroplasts and mitochondria were removed with *remove.lineage*. Operational taxonomic units (OTUs) were formed by average neighbor clustering using the *cluster.split* command, parallelizing the cluster procedure by splitting the dataset at the taxonomic order level. A sample-by-OTU table was generated with *make.shared* at the 97 % sequence similarity level. The resulting table contained 15,509 OTUs. OTUs were classified taxonomically using the modified Greengenes database mentioned above and the command *classify.otu*.

Archaeal sequences showed lower quality in the reverse read, which lead to multiple ambiguous bases in the contigs formed. For this reason only the forward read starting from base 36 was used for analysis. Sequence analysis was performed as described above for bacterial 16S sequences, except that the alignment (*align.seqs*) was accomplished using the SILVA archaeal reference release 102 (Pruesse

et al., 2007) fitted for hypervariable regions V5-V7.Classification (*classify.seqs* and *classify.otu*) was conducted using the RDP database file release 10 (Cole et al., 2014;Wang et al., 2007). Results and additional information on the archaeal community structure are listed in the supplemental material.
An overview of the sequencing output is given in table S1.

2.6 Statistics

Low-abundance OTUs were removed to reduce noise and computation time. Statistical downstream analysis was performed in R v3.1.3 (R Core Team, 2015) with custom scripts (available from the authors on request). As OTUs of very low abundance only increase computation time without contributing useful information, they were removed from the data set as follows: After transformation of counts in the sample-by-OTU table to relative abundances (based on the total number of reads per sample), OTUs were ordered by decreasing mean percentage across samples. The set of ordered OTUs for which the cumulative mean percentage amounted to 99% was retained in the filtered OTU table.
Distribution of OTUs across samples was modeled by a set of environmental variables (Table S2) with minimal interdependence. The variance in OTU composition (i.e., the extent of change in OTU abundance across samples) explained by the measured environmental variables was explored by redundancy analysis (RDA) with Hellinger-transformed OTU counts (Langfeldt et al., 2014;Stratil et al., 2013;Stratil et al., 2014) using the R package vegan (Oksanen et al., 2013). In order to minimize collinearity of explanatory variables in the RDA model, a subset of the recorded environmental variables was chosen according to their variance inflation factor (VIF), employing vegan's functions *rda* and *vif.cca*. Starting with an RDA model that contained all explanatory variables, the variable with the highest VIF was iteratively determined and removed from the model until all remaining explanatory variables had a VIF <2.5.

OTU distribution was subject to "Realm" depending on O_2 concentration. Model selection started with a full RDA model containing all main effects and possible interactions based on the set of explanatory variables with minimal collinearity. This model was simplified by backward selection with function *ordistep*. The final RDA model exhibited a significant interaction effect "Realm:O_2" (see results section). For plotting and indicator analysis (see below), the continuous variable "O_2" was converted into a factor with two levels "high O_2" (>90 µmol L^{-1}) and "low O_2" (≤90 µmol L^{-1}); the threshold of 90 µmol L^{-1} was chosen for two reasons: (1) to obtain sample groups of fairly equal size between stations, which include low O_2 parts of the water column at all sampling stations in order to enable a comparison between the ETNA OMZ (outside the eddy) and the eddy OMZ. (2) 90 µmol L^{-1} has previously described the highest concentration of O_2 at which denitrification has been detected to be active (Gao et al., 2010). The presence of *nirS* transcripts (see section 3.4) indicated a potential importance for denitrifiers in the eddy, therefore the theoretical upper limit of 90 µmol L^{-1} was chosen.

We determined OTUs typical for a given combination of levels of factors "Realm" and "O_2". OTUs significantly correlated with any axis in the final RDA model were determined using the function *envfit* with 10^5 permutations, followed by Benjamini-Hochberg correction (false discovery rate, FDR) (Benjamini and Hochberg, 1995). In order to reduce the number of tests in this procedure, OTUs were pre-filtered according to their vector lengths calculated from corresponding RDA scores (scaling 1) by profile likelihood selection (Zhu and Ghodsi, 2006).

OTUs significant at an FDR of 5% were further subject to indicator analysis with function *multipatt* of the R package indicspecies v1.7.4 (De Cáceres and Legendre, 2009) with 10^5 permutations. Indicator OTUs – in analogy to indicator species *sensu* De Cáceres and Legendre (2009) – are OTUs that prevail in a certain sample group (here: a level of factor "Realm" within a chosen O_2 level) while being found only irregularly and at low abundance in other sample groups. In order to remove the effects of the covariate "Depth" in indicator analysis, Hellinger-transformed counts of significant OTUs were first subjected to a linear regression with "Depth"; residuals of this regression were then transformed to positive values by subtraction of their minimum and used as input for indicator analysis.
3D visualizations of the RDA model were produced in kinemage format (Richardson and Richardson, 1992) using the R package R2Kinemage developed by S.C.N., and displayed in KiNG v2.21 (Chen et al., 2009).

Diversity within samples was related to environmental variables by advanced linear regression. For alpha diversity analysis, effective OTU richness (Shannon numbers equivalent, ^{1}D, (Jost, 2006, 2007)) was calculated from the filtered OTU table. ^{1}D was fitted to the set of explanatory variables with minimal collinearity in a generalized least squares (GLS) model using function *gls* of the R package nlme v3.1-120 (Pinheiro et al., 2015). The variable "NO2" was square root-transformed to decrease the potential leverage effect of its two highest values (0.25 µmol L^{-1} and 0.28 µmol L^{-1}, respectively) on ^{1}D. Apart from main effect terms, the interaction term "Realm:O_2" was included into the GLS model for comparability with beta diversity analysis (see results section). The variance structure of the GLS model was chosen to account for both different variances per level of "Realm" and an overall decreasing variance by "Depth". The resulting model was validated following the recommendations of Zuur et al. (2009). While only the "Realm" effect was significant, the other terms were kept in the model to maintain a valid residual distribution. For visualization of the (partial) effect of only factor "Realm" on ^{1}D, partial response residuals were extracted from the full GLS model re-fitted without the "Realm" main effect. These partial response residuals were then modelled by the "Realm" main effect alone, using the same variance structure as for the full GLS model.

2.7 Carbon fixation rate measurements

Seawater incubations were performed in triplicate at two stations, one inside the eddy (station 10, M105 cruise) and one in ETNA open waters (station 15, M105 cruise, both stations indicated in Fig. 1C). Seawater was sampled from a CTD system and directly filled into 2.8 L polycarbonate bottles (Nalgene, Thermo Fisher Scientific, Waltham, MA, USA). For carbon fixation measurements, $NaH^{13}CO_3$ (Cambridge Isotope Laboratories, MA, USA) was dissolved in sterile deionized water (>18.2 MΩ cm^{-1}, MilliQ, Merck-Millipore, Darmstadt, Germany; 5 g/294 mL). A volume of 1 ml (2.8 L bottles) was added to the incubations with a syringe (~4.4 atom % final). After amendment, bottles were stored on deck in a seawater-cooled Plexiglas incubator covered with light foils (blue-lagoon, Lee filters, Andover, Hampshire, UK) that mimic light intensities at corresponding sampling depths (5/10/30/70 m). Samples from below the euphotic zone were stored at 12°C in the dark. The depth of the euphotic zone was estimated from photosynthetically active radiation (PAR) sensor measurements from CTD profiles as the depth where PAR is <1% of the surface value. This corresponded to 60 m water depth during this survey. After 24 h of incubation, 1.5–2.8 L of seawater were filtered onto pre-combusted (450°C, 5 h) 25 mm diameter GF/F filters (Whatman, Maidstone, UK) under gentle vacuum (-200 mbar). Filtrations were stopped after 1 h since high particle load of surface water led to a clogging of the filters. Filters were oven dried (50°C) for 24 h and stored over desiccant until analysis. Environmental samples of 2.8 L untreated seawater were filtered and prepared in the same way to serve as blank values. For isotope analysis, GF/F filters were acidified over fuming HCl overnight in a desiccator. Filters were then oven-dried for 2 h at 50°C and pelletized in tin cups. Samples were analysed for particulate organic carbon and nitrogen (POC and PON) and isotopic composition using a CHN analyser coupled to an isotope ratio mass spectrometer.

3 Results and Discussion

3.1 Hydrography of low-O_2 eddy reveals similarities to shelf waters

As the detailed properties of the investigated eddy are described in Schütte et al. (2015, in preparation for this issue) only the main characteristics are mentioned here:

The surveyed low-O_2 eddy belongs to the group of the anticyclonic modewater eddies (ACME) (Karstensen et al. 2015a). It has been reported that ACME promote intense primary production in surface and mixed layer waters (Mahadevan, 2014) fueled by nutrient supply to the euphotic zone. The surveyed eddy had a diameter of about 100 km and was characterized by highly elevated mixed layer chlorophyll a (chl*a*) concentrations, a positive SLA signature (Fig. 1) and a low O_2/ low salinity core (Fig. 2). The O_2-depleted core, with concentrations of less than 5 µmol kg^{-1}, was centered rather deep for an ACME at ~100 m depth. Concentrations of less than 30 µmol kg^{-1} were observed in the eddy water column between 70 to 150 m depth (Fig. 2, 3A), which is significantly below average O_2 concentrations in that region,. O_2 concentrations in the core decreased over the survey period (March 2014), (see Fiedler et al. (2015) in this issue, for a detailed description of O_2 properties). During the

metagenomic sampling of the background signal ("no eddy") on the shelf (Meteor M107 cruise station 675, 18.22°N/ 16.56°W, Fig. 1), O_2 concentrations of 33.9 µmol kg^{-1} were observed at 115 m depth, which corresponds to the potential density layer of the low O_2 core in the eddy. The open ocean background minimum O_2 concentrations of about 70 µmol kg^{-1} were detected at ~250 m depth at CVOO (Fig. 1). This can be considered average O_2 concentrations for the open ETNA (Karstensen et al., 2008).

In the low-O_2 eddy core, we observed nitrate and phosphate concentrations around twice as high as background concentrations at CVOO at the same depth (Fig. 3). However, N:P ratios below the mixed layer were close to Redfield stoichiometry (16.15 ± 0.63, Fig. 3) and thus comparable to surrounding waters. Nitrate concentrations in the O_2-min core (~100 m depth) were similar to concentrations on the Mauritanian shelf at 100 m depth (Fig. 3) and most likely generated by very efficient local remineralization of nitrate from the sinking material (Karstensen et al. 2015b, in preparation for this issue).

3.2 Loss of phylogenetic diversity in low-O_2 eddy waters

A critical issue regarding climate change induced pressures on ocean ecosystems is to understand the effects of ocean acidification and deoxygenation on microbial communities as major drivers of the ocean's biogeochemistry (Riebesell and Gattuso, 2015). Thus, we investigated phylogenetic diversity of the microbial community with a 16S rDNA amplicon sequencing approach of bacteria and archaea inside and outside the eddy.

Although the bacterial community was dominated by Proteobacteria in all samples, there were distinct differences between the community structures inside compared to outside the eddy (Fig. 4). Increased abundances of the uncultivated SUP05 clade (up to 20% of proteobacterial sequences), have been recovered from eddy samples compared to surrounding waters (Fig S1, Table S3). This clade is known to occur frequently in O_2 depleted environments (Swan et al., 2011). Phyla such as Bacteroidetes, Actinobacteria and Firmicutes were only present in the eddy and increased in relative abundance over time. Those phyla were also detected in potential source waters on the shelf (Fig. S2). Interestingly, the family of Pelagibacteraceae, which belong to the ubiquitous SAR11 clade (Giovannoni et al., 1990), were strongly decreased in the eddy (to ~1% of all reads), compared to CVOO samples (~65% of all reads). SAR11 was previously described as being sensitive to decreasing O_2 concentrations (Forth et al., 2014), which may explain the absence of this classically highly abundant group from the eddy. In addition to the dissimilarity in bacterial diversity, we also detected a substantial difference in archaeal community composition between eddy stations and CVOO (Fig. S3). This was most obvious in samples from the eddy_2 station, where Methanomicrobia dominated the archaeal community in the O_2-depleted parts of the water column but was absent in CVOO samples. The presence of methanogens in the low-O_2 eddy core samples may indicate potential for methanogenesis. Although

the eddy has not been shown to become fully anoxic, methanogenesis tolerates O_2 concentrations at low ranges (Angel et al., 2011).

Redundancy analysis (RDA) confirmed that the distribution of bacterial OTUs strongly differed between the two eddy stations and CVOO samples (Fig. 6A; RDA model: $F_{6,24}$ = 4.48, p<.001). Changes in OTU composition mirrored the depth gradient (RDA "Depth": $F_{1,24}$ = 2.08, p≈ .03; Fig. 5) and were thus strongly correlated to chemical (PO_4^{3-}, NO_3^-, SiO_2) and physical (T, S) properties (Fig. S4). The RDA model indicates a noticeable interaction effect of habitat ("Realm") and O_2 concentration (RDA "Realm:O_2": $F_{2,24}$ = 2.03, p≈ .02), meaning that the "Realm" effect on bacterial community structure depends on the O_2 level and vice versa. An overview of the parameters included in the RDA model is given in table S2. O_2 and nutrient availability can thus be considered the major determining variables for the composition of the microbial community.

Our results show further a significant decrease in bacterial alpha diversity in the eddy relative to CVOO (Fig. 6). The community in eddy_2 samples was also markedly less diverse compared to those of the other realms (Fig. 6; generalized least squares (GLS) model: $F_{7,23}$ = 5.37, p = .001; GLS "Realm": $F_{2,23}$ = 16.26, p<.0001). This may be attributed to an aging effect of the eddy, and corresponds to progressive O_2 loss and consecutive changes in the eddy biogeochemistry. We calculated an overall O_2 loss of 0.18 µmol kg^{-1} d^{-1} at 100 m depth by respiration, when comparing the eddy core water to the potential origin waters on the shelf, assuming a lifetime of 180 days for the eddy (average O_2 concentrations on the shelf from Meteor M107 were 36.69 ± 6.91 µmol kg^{-1} compared to observed minimum O_2 concentrations of 4.8 µmol kg^{-1} in the eddy core). These results are comparable to previous estimates on low O_2-eddies in that region (Karstensen et al., 2015a). Likewise, Fiedler et al. (2015) also observed a significant increase in pCO_2 and dissolved inorganic carbon compared to coastal waters, indicating enhanced remineralization and respiration. Although our dataset does not allow differentiation between high-pCO_2 and low-O_2 effects on the microbial community, it supports the view of a general loss in diversity. This may be attributed to a direct or indirect response to factors related to deoxygenation and increasing pCO_2, such as the impact on nutrient stoichiometry, as previously suggested (Bryant et al., 2012).

Hence, climate change-related ocean deoxygenation and consequent shifts in nutrient stoichiometry may mean an overall loss of microbial diversity, with potential for substantial loss in the spectrum of metabolic functions in the future ocean.

3.3 Specific *Prochlorococcus* clade contributes to primary production in the eddy

The detected ACME was characterized by shoaling of the mixed layer depth in the center of the eddy. This coincided with a pronounced surface chl*a* maximum as observed by ocean color based and remotely sensed chl*a* estimates (Fig. 1a, Fig. 7), which was slightly deeper (~50-70 m water depth) outside the eddy. In accordance with increased chl*a* concentrations, enhanced carbon uptake was observed via direct rate measurements of $H^{13}CO_3^-$ uptake which was potentially fueled by increased

nutrient availability from intermediate depths. We found a 3-fold increase in depth-integrated carbon uptake rate in the chl*a* maximum of the eddy (178.3 ± 30.8 m mol C m^{-2} d^{-1}) compared to surrounding waters (59.4 ± 1.2 mmol C m^{-2} d^{-1}).

While the upper chl*a* maximum in the eddy may likely be ascribed to eukaryotic primary producers such as diatoms and flagellates that are widely distributed and abundant in that region (Franz et al., 2012), confirmed by increased abundances of plastids in surface samples of our amplicon dataset (Table S3). A secondary chl*a* maximum dominated by cyanobacteria was detected in the eddy at about 100 m water depth, coinciding with the O_2 minimum.

The quantitative analysis of cyanobacterial primary producers by 16S rDNA-qPCR further revealed dominance of a specific clade of *Prochlorococcus* in the secondary chl*a* maximum (Fig. S5 depicts phylogenetic relations of detected *Prochlorococcus* clades). This ecotype has so far not been identified in the ETNA and is only known from high nutrient low chlorophyll (HNLC) regions of the eastern tropical Pacific Ocean (West et al., 2011). Its described adaptation to high nutrient conditions such as present in this O_2-depleted ACME points towards a selective advantage for this clade. Gene abundance of this ecotype—for convenience further referred to as HNLC-PCC (results of an ecotypespecific16S rDNA based qPCR)— showed a strong correlation with chlorophyll (R^2= 0.95, n=22) below the euphotic zone within the eddy. This correlation was not present outside the eddy, where HNLC-PCC abundance was approximately one third compared to the second eddy observation (Fig. 8). The *Prochlorococcus* community in surrounding waters was, however, dominated by another high-light ecotype of *Prochlorococcus* (further referred to as HL-PCC (West et al., 2011)). Contrary to HNLC-PCC, HL-PCC was not detected inside the eddy. The difference between the CVOO, eddy_1 and eddy_2 observations points towards a community shift of *Prochlorococcus* related clades depending on specific characteristics of the eddy (O_2, nutrient availability) with the potential to alter primary productivity in that region. Under increasing pCO_2 levels, *Prochlorococcus* is predicted to substantially increase in abundance (Flombaum, 2013). Elevated pCO_2 levels in the eddy core water may therefore—apart from favorable elevated nutrient concentrations—explain the additional selective advantage of specific *Prochlorococcus* clades, in this case of HNLC-PCC. This may be critical as *Prochlorococcus* is one of the most abundant photosynthetic organisms in the ocean and contributes to ~40% of dissolved organic carbon supporting bacterial production (Bertillson et al., 2005).

Besides a direct impact of O_2, nutrients and pCO_2, increased abundances of *Prochlorococcus* in the eddy may be explained from an interaction effect in the microbial community present in the eddy. *Prochlorococcus* is supposed to play a major role in sustaining heterotrophs with organic carbon compounds such as glycine and serine, thus favoring their growth (Biller et al., 2015;Carini et al., 2013). Conversely, *Prochlorococcus* benefits from the presence of heterotrophs as they diminish the concentration of reactive oxygen species in their immediate surroundings, which is not feasible for *Prochlorococcus* due to the lack of catalase and peroxidase genes (Berube et al., 2014;Morris et al., 2008). The close proximity of increased abundances of the HNLC-PCC maximum to the O_2 minimum

in the eddy may thus point towards a beneficial relationship between the HNLC-PCC and the heterotroph-dominated, eddy core water microbial community.

3.4 Increased primary productivity promotes a specific heterotrophic microbial community in underlying waters

We analyzed species indicative for the eddy and CVOO for either high-O_2 conditions (>90 µmol kg^{-1}) or low-O_2 conditions (≤90 µmol kg^{-1}). Indicator OTUs for high O_2 in the eddy were mostly associated with different clades of Proteobacteria, whereas Pelagibacteraceae dominated at CVOO in accordance with several studies describing those organisms as ubiquitous in open-ocean oxic waters (Morris et al., 2002;Rappé et al., 2002);(Poretsky et al., 2009;DeLong, 2009;Brown et al., 2014). High-O_2 samples of all three sampling stations were dominated - as most parts of the ocean - by indicator OTUs belonging to the Proteobacteria. The *Prochlorococcus* clade HNLC-PCC targeted by qPCR could be recovered in the 16S rDNA amplicon sequences, as well.

For low-O_2 conditions, indicator species present in the eddy were mostly affiliated to the Cytophaga-Flavobacteria-Bacteroides (CFB) group (Glöckner et al., 1999) (Table S4). Members of Bacteroidetes and Proteobacteria (*Gramella, Leeuwenhoekiella marinoflava*, unclassified Comamonadaceae species) were found to be indicative for the low-O_2 realm. *Gramella*-like organisms are usually a quantitatively important fraction of the heterotrophic marine bacterioplankton, often attached to marine snow but also found free-living in nutrient-rich microenvironments (Buchan et al., 2014). Frequently associated with extensive phytoplankton blooms (Buchan et al., 2014), their ability to degrade high molecular weight compounds in both the dissolved and particulate fraction of the marine organic matter pool points towards a specific role in respiration processes and the marine C cycle (as described for '*Gramella forsetii*' KT0803, Bauer et al. (2006). Karstensen et al. (2015a) described a particle maximum associated to the low-O_2 core of those eddies which likely harbors this specific heterotrophic community. Further, in the core of the ACME presented here, the integrated abundance (upper 600 m) of large aggregates was five times higher than in surrounding waters (Hauss et al., 2015).

Enhanced productivity and consecutive respiration and O_2 decrease may enable N loss processes to occur in the open ETNA, which have previously not been described for the ETNA waters (Löscher et al., 2015;Löscher et al., 2012;Ryabenko et al., 2012). qPCR results of key gene distribution (*amoA* for nitrification as sum of bacterial and archaeal nitrifiers, *nirS* as key gene for denitrification) in that area show a decrease of *amoA* in the eddy, while *nirS* shows higher abundances inside the eddy with ~3000 copies L^{-1} at depth of the O_2 minimum (compared to ~100 copies L^{-1} outside the eddy). Besides a direct sensitivity of nitrifiers to anoxic conditions, the decrease in *amoA* gene abundance (determined by qPCR) towards the O_2 minimum in the eddy may result from an effect of elevated pCO_2 (see Fiedler et al. (2015), this issue) and the corresponding drop in pH on ammonia due to a shift in the

ammonia/ammonium equilibrium. The latter has previously been described to alter the efficiency of nitrification (Beman et al., 2011). Further, *nirS* transcripts as quantified by qPCR were detected in abundances up to 3600 transcripts L^{-1} in the eddy O_2 minimum, while no transcripts were detected outside the eddy (Fig. 9).

The presence and expression of *nirS* supports the view that potential for N loss is also present in the usually oxic open ETNA. This is in line with another study on nitrous oxide (N_2O) production from the same eddy (Grundle et al., submitted), where the authors observed massively increased N_2O concentrations in the oxygen deficient eddy core waters in connection with denitrification. Observations from e.g. the eastern tropical Pacific Ocean demonstrated previously that mesoscale eddies are drifting hotspots of N loss (Altabet et al., 2012). This might be explained by feedback mechanisms between eutrophication, enhanced primary productivity and consecutive enhanced export production, which may promote denitrification in those systems as suggested by Kalvelage et al. (2013). Our results strongly suggest that N loss is possible in eddy systems of that region, thus altering one major biogeochemical cycle with unknown consequences for the ETNA biogeochemistry.

In case of the described eddy, we neither detected key genes for anammox (*hzo*, Schmid et al. (2008)) nor significant abundances of the key genes for dinitrogen fixation. The latter has been investigated by screening for the functional key gene, *nifH*, which has been tested for classical diazotrophs as *Trichodesmium*, UCYN-A, UCYN-B, UCYN-C, gamma proteobacterial diazotrophs and DDAs; all of which were not quantifiable by qPCR. This may be explained by the high availability of inorganic N sources, as well as the prevalence of N:P close to the Redfield ratio of 16:1 as mentioned above.

Although N_2 fixation does not appear to play a role in the low-oxic core waters or adjacent surface waters of the eddy, it may occur as a result of increasing N loss and resulting excess P as previously discussed for other O_2 depleted marine habitats (Deutsch et al., 2007;Fernandez et al., 2011;Löscher et al., 2014;Ulloa et al., 2012).

4 Conclusions

We investigated the microbial community structure and gene expression in a severely O_2-depleted anticyclonic modewater eddy in the open waters of the ETNA OMZ region. This was then compared the eddy observations to background signals from the ETNA open ocean CVOO time series site and the Mauritanian upwelling region, where the eddy was likely formed.

A significant difference between microbial communities outside and inside the eddy along with an overall loss in bacterial diversity in the low-O_2 core of the eddy was observed. Similarity was found between the microbial community in the eddy core and on the shelf. This unique microbial community may shape the specific character of this O_2-depleted eddy progressively over time.

We observed enhanced primary production in the eddy, presumably due to an increased nutrient supply related to the eddy dynamics (Karstensen et al. 2015b). We found a specific HNLC ecotype of

Prochlorococcus, which may play a role in mediating inorganic C to certain organic C sources for the associated heterotrophic community present in the eddy. Importantly, we found the first indication for N loss processes in the ETNA region. Low-O_2 eddies in that region thus represent an isolated ecosystem in the open ocean, forced by strongly elevated biological productivity, which travels with the eddy. This leads to consequent enhanced respiration and further deoxygenation in its core waters.

At one stage the low-O_2 eddies will lose coherence and the extreme signatures will be released into and mixed with the surrounding waters (Karstensen et al. 2015a). The ACME formation frequency for the ETNA (12°-22°N and 15°-26°W) has been estimated to be about 2 to 3 yr^{-1} (Schütte et al. 2015, in preparation for this issue), hence no large scale impact of the eddies are expected. However, an unexpected shift in elemental ratios or other anomalies, normally expected for regions with much lower minimal oxygen levels than the ETNA, may be detected and explained by the dispersal of low-O_2 eddies. Another factor to consider is the impact of deoxygenation of the ETNA (Stramma et al., 2008) as it may result in even lower O_2 conditions to be created in the low-O_2 eddies. With regard to the distinct character of the low-O_2 eddies and the critical shift in microbial diversity and biogeochemistry that occur over relatively short times, this study contributes to understand and evaluate the far-reaching effects of future and past ocean deoxygenation.

Author contribution

C.R.L. designed the study together with B.F., A.K., H.H. and J.K.; M.P. and C.R.L. validated the NGS primer sets for marine samples, performed the molecular analysis, processed the molecular data and analyzed hydrographic data. S.K. performed the high-throughput sequencing runs. M.A.F. and S.C.N. performed bioinformatic analysis of high-throughput datasets. A.S. performed C-uptake measurements and data analysis, F.S., J.K. and A.K. designed the eddy detection and tracking system. B.F., F.S., A.K., H.H. and C.R.L. planned the sampling campaign and B.F. performed hydrographical measurements and analyzed the data. S.C.N. performed statistical analysis and modeling. C.R.L. wrote the manuscript with input from all co-authors.

Acknowledgements

We thank the authorities of Cape Verde and Mauritania for the permission to work in their territorial waters. We acknowledge the support of the captains and crews of R/V Islandia and R/V Meteor as well as the chief scientist of M105, M. Visbeck, for his spontaneous support. Moreover, we acknowledge I. Monteiro and M. Lohmann for performing oxygen and nutrient measurements, D. Grundle for sampling on RV Islandia, C. Hoffman for technical assistance and the captains, crews and chief scientists of Meteor M107, S. Sommer and M. Dengler, for providing hydrographical data. We acknowledge H. Bange, G. Krahmann and R. Kiko for helpful discussion of the results. G. Petrick, flying to Cape Verde to repair the liquid N_2 generator was priceless- we thank you a lot for this

personal effort! We thank T. Treude for editing this manuscript. Further, we thank J. M. Beman and two anonymous reviewers for helpful discussion of the manuscript and A. Paul for language editing. Financial support for this study was provided by a grant from the cluster of excellence 'The Future Ocean' to C.R.L., A.K. and H.H.. Authors C.R.L., S.C.N., H.H. and M.P. were funded by the DFG Collaborative Research Centre754 (www.sfb754.de). M.A.F. was funded and S.C.N. was co-funded by the BMBF project BioPara funded to R.A.S (grant no. 03SF0421B), A.S. was funded by the cluster of excellence 'The Future Ocean', B.F. was funded by the BMBF project SOPRAN (grant no. 03F0662A).

References

Ahlgren, N., Rocap, G., and Chisholm, S.: Measurement of Prochlorococcus ecotypes using real-time polymerase chain reaction reveals different abundances of genotypes with similar light physiologies, Environmental Microbiology, 8, 441–454, 2006.

Altabet, M. A., Ryabenko, E., Stramma, L., Wallace, D. W. R., Frank, M., Grasse, P., and Lavik, G.: An eddy-stimulated hotspot for fixed nitrogen-loss from the Peru oxygen minimum zone, Biogeosciences, 9, 4897–4908, 2012.

Angel, R., Matthies, D., and Conrad, R.: Activation of Methanogenesis in Arid Biological Soil Crusts Despite the Presence of Oxygen, PlosOne, 6, doi:10.1371/journal.pone.0020453, 2011.

Arévalo-Martínez, D. L., Kock, A., Löscher, C. R., Schmitz R. A., and Bange, H. W.: Influence of mesoscale eddies on the distribution of nitrous oxide in the eastern tropical South Pacific, Biogeosciences Discuss., 12, 9243-9273, doi:10.5194/bgd-12-9243-2015, 2015.

Baird, M. E., Suthers, I. M., Griffin, D. A., Hollings, B., Pattiaratchi, C., Everett, J. D., Roughan, M., Oubelkheir, K., and Doblin, M.: The effect of surface flooding on the physicalâ€“biogeochemical dynamics of a warm-core eddy off southeast Australia, Deep Sea Research Part II: Topical Studies in Oceanography, 58, 592-605, 2011.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Würdemann, C. A., Quast, C., Kuhl, H., Knaust, F., Woebken, D., Bischof, K., Mussmann, M., Choudhuri, J. V., Meyer, F., Reinhardt, R., Amann, R. I., and Glöckner, F. O.: Whole genome analysis of the marine Bacteroidetes'Gramella forsetii' reveals adaptations to degradation of polymeric organic matter, Environ Microbiol. , 8, 2201-2213, 2006.

Beman, J. M., Chow, C. E., King, A. L., Feng, Y. Y., Fuhrman, J. A., Andersson, A., Bates, N. R., Popp, B. N., and Hutchins, D. A.: Global declines in oceanic nitrification rates as a consequence of ocean acidification, Proc. Natl. Acad. Sci. U. S. A., 108, 208-213, 10.1073/pnas.1011053108, 2011.

Beman, J. M., and Carolan, M. T.: Deoxygenation alters bacterial diversity and community composition in the ocean's largest oxygen minimum zone, Nature Communications, 4, doi:10.1038/ncomms3705, 2013.

Benjamini, Y., and Hochberg, Y.: Controlling the false discovery rate: A practical and powerful approach to multiple testing, Journal of the Royal Statistical Society: Series B (Statistical Methodology), 57, 289-300, 1995.

Bertillson, S., Berglund, O., Pullin, M. J., and Chisholm, S. W.: Release of dissolved organic matter by Prochlorococcus, ie et milieu - Life and environment 55, 225-231, 2005.

Berube, P. M., Biller, S. J., Kent, A. G., Berta-Thompson, J. W., Roggensack, S. E., Roache-Johnson, K. H., Ackerman, M., Moore, L. R., Meisel, J. D., Sher, D., Thompson, L. R., Campbell, L., Martiny A., and Chisholm, S. W.: Physiology and evolution of nitrate acquisition in Prochlorococcus, ISME J, http://dx.doi.org/10.1038/ismej.2014.211, 2014.

Biller, S. J., Berube, P. M., Lindell, D., and Chisholm, S. W.: Prochlorococcus: the structure and function of collective diversity, Nat Rev Micro, 13, 13-27, 2015.

Brown, M. V., Ostrowski, M., Grzymski, J. J., and Lauro, F. M.: A trait based perspective on the biogeography of common and abundant marine bacterioplankton clades, Marine Genomics, 15, 17-28, 2014.

Bryant, J. A., Stewart, F. J., Eppley, J. M., and DeLong, E. F.: Microbial community phylogenetic and trait diversity declines with depth in a marine oxygen minimum zone, Ecology, 93, 1659-1673, 2012.

Buchan, A., LeCleir, G. R., Gulvik, C. A., and González, J. M.: Master recyclers: features and functions of bacteria associated with phytoplankton blooms, Nat Rev Microbiol. , 12, 686-698, doi: 10.1038/nrmicro3326, 2014.

Carini, P., Steindler, L., Beszteri, S., and Giovannoni, S. J.: Nutrient requirements for growth of the extreme oligotroph 'Candidatus Pelagibacter ubique' HTCC1062 on a defined medium, ISME J, 7, 592–602, 2013.

Chavez, F. P., and Messié, M.: A comparison of Eastern Boundary Upwelling Ecosystems, Progress in Oceanography, 83, 80-96, 2009.

Chelton, D. B., Gaube, P., Schlax, M. G., Early, J. J., and Samelson, R. M.: The Influence of Nonlinear Mesoscale Eddies on Near-Surface Oceanic Chlorophyll, Science, 334, 328–332, 2011.

Chen, V. B., Davis, I. W., and Richardson, D. C.: KING (Kinemage, Next Generation): A versatile interactive molecular and scientific visualization program, Prot Sci, 18, 2403-2409, 10.1002/pro.250, 2009.

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., Brown, C. T., Porras-Alfaro, A., Kuske, C. R., and Tiedje, J. M.: Ribosomal Database Project: data and tools for high throughput rRNA analysis, Nucleic Acids Res., 42, 633-642, doi: 10.1093/nar/gkt1244, 2014.

De Cáceres, M., and Legendre, P.: Associations between species and groups of sites: indices and statistical inference, Ecology, 90, 3566-3574, 10.1890/08-1823.1, 2009.

DeLong, E. F.: The microbial ocean from genomes to biomes, Nature, 459, 200-206, 10.1038/nature08059, 2009.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G. L.: Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB, Applied and environmental microbiology, 72, 5069–5072, 10.1128/aem.03006-05, 2006.

Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., and Dunne, J. P.: Spatial coupling of nitrogen inputs and losses in the ocean, Nature, 445, 163-167, 10.1038/nature05392, 2007.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R.: UCHIME improves sensitivity and speed of chimera detection, Bioinformatics 27, d2194–2200, 10.1093/bioinformatics/btr381, 2011.

Fernandez, C., Farias, L., and Ulloa, O.: Nitrogen Fixation in Denitrified Marine Waters, Plos One, 6, 9, e20539,10.1371/journal.pone.0020539, 2011.

Fiedler, B., Grundle, D., Hauss, H., Krahmann, G., Schütte, F., Monteiro, I., Silva, P., Vieira, N., and Körtzinger, A.: Biogeochemistry of oxygen depleted mesoscale Eddies in the open eastern tropical North Atlantic In prep for Biogeosciences Discussion, 2015.

Fierer, N., Hamady, M., Lauber, C. L., and Knight, R.: The influence of sex, handedness, and washing on the diversity of hand surface bacteria, Proc. Natl. Acad. Sci. U. S. A., 105, 17994–17999, 10.1073/pnas.0807920105, 2008.

Fischer, G., Karstensen, J., Romero, O., Baumann, K.-H., Donner, B., Hefter, J., Mollenhauer, G., Iversen, M., Fiedler, B., Monteiro, I., and Körtzinger, A.: Bathypelagic particle flux signatures from a suboxic eddy in the oligotrophic tropical North Atlantic: production, sedimentation and preservation, Biogeosciences Discuss., 12, 18253-18313, 10.5194/bgd-12-18253-2015, 2015.

Flombaum, P., et al. : Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus, Proc. Natl. Acad. Sci. USA., 110, 9824–9829, 2013.

Forth, M., Liljebladh, B., Stigebrandt, A., Hall, P. O. J., and Treusch, A. H.: Effects of ecological engineered oxygenation on the bacterial community structure in an anoxic fjord in western Sweden, ISME Journal, 9, DOI: 10.1038/ismej.2014.172 2014.

Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., and Pace, N. R.: Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, Proc. Natl. Acad. Sci. U. S. A., 104, 13780–13785, 10.1073/pnas.0706625104, 2007.

Franz, J. M. S., Hauss, H., Sommer, U., Dittmar, T., and Riebesell, U.: Production, partitioning and stoichiometry of organic matter under variable nutrient supply during mesocosm experiments in the tropical Pacific and Atlantic Ocean, Biogeosciences, 9, 4629-4643, 2012.

Ganesh, S., Parris, D. J., DeLong, E. F., and Stewart, F. J.: Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone, ISME J, 8, 187-211, 2014.

Gao, H., Schreiber, F., Collins, G., Jensen, M. M., Kostka, J. E., Lavik, G., de Beer, D., Zhou, H.-Y., and Kuypers, M. M. M.: Aerobic denitrification in permeable Wadden Sea sediments, ISME J, 4, 417-426, 2010.

Giovannoni, S. J., Britschgi, T. B., Moyer, C. L., and Field, K. G.: Genetic diversity in Sargasso Sea bacterioplankton, Nature, 345, 60–63, doi:10.1038/345060a0, 1990.

Glöckner, F. O., Fuchs, B. M., and Amann, R.: Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization, Environ Microbiol., 65, 3721–3726, 1999.

Grasshoff, G., Kremling, K., Erhardt, M. : Methods of seawater analysis, 3 ed., Wiley VCH, Weinheim, 1999.

Grundle, D. S., Löscher, C. R., Krahmann, G., Altabet, M. A., Bange, H. W., Karstensen, J., Körtzinger, A., and Fiedler, B.: Extreme N_2O activity in an oxygenated ocean, submitted.

Hauss, H., Christiansen, S., Schütte, F., Kiko, R., Edvam Lima, M., Rodrigues, E., Karstensen, J., Löscher, C. R., Körtzinger, A., and Fiedler, B.: Dead zone or oasis in the open ocean? Zooplankton distribution and migration in low-oxygen modewater eddies, Biogeosciences Discuss., 12, 18315-18344, doi:10.5194/bgd-12-18315-2015, 2015.

Hood, E. M., Sabine, C. L., and Sloyan, B. M.: The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines, 2010.

Jickells, T. D., An, Z. S., Andersen, K. K., Baker, A. R., Bergametti, G., Brooks, N., Cao, J. J., Boyd, P. W., Duce, R. A., Hunter, K. A., Kawahata, H., Kubilay, N., laRoche, J., Liss, P. S., Mahowald, N., Prospero, J. M., Ridgwell, A. J., Tegen, I., and Torres, R.: Global iron connections between desert dust, ocean biogeochemistry, and climate, Science, 308, 67-71, 2005.

Jost, L.: Entropy and diversity, Oikos, 113, 363-375, DOI 10.1111/j.2006.0030-1299.14714.x, 2006.

Jost, L.: Partitioning diversity into independent alpha and beta components, Ecology, 88, 2427-2439, 10.1890/06-1736.1, 2007.

Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Artega, L., Löscher, C. R., Oschlies, A., Paulmier, A., Stramma, L., and Kuypers, M. M. M.: Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone, Nature Geosci, 6, 228-234, 2013.

Karstensen, J., Stramma, L., and Visbeck, M.: Oxygen minimum zones in the eastern tropical Atlantic and Pacific oceans, Progress in Oceanography, 77, 331-350, 10.1016/j.pocean.2007.05.009, 2008.

Karstensen, J., Fiedler, B., Schütte, F., Brandt, P., Körtzinger, A., Fischer, G., Zantopp, R., Hahn, J., Visbeck, M., and Wallace, D.: Open ocean dead-zone in the tropical North Atlantic Ocean, Biogeosciences Discussion, 12, 2597-2605, doi:10.5194/bg-12-2597-2015, 2015a.

Karstensen, J., Schütte, F., Pietri, A., Krahmann, G., Fiedler, B., Körtzinger, A., Löscher, C. R., Grundle, D., and Hauss, H.: Anatomy of open ocean dead-zones based on high-resolution multidisciplinary glider data, In prep for Biogeosciences Discussion, 2015b.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D.: Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform, Appl. Environ. Microbiol., 79, 5112–5120, 10.1128/aem.01043-13, 2013.

Lam, P., Jensen, M. M., Lavik, G., McGinnis, D. F., Muller, B., Schubert, C. J., Amann, R., Thamdrup, B., and Kuypers, M. M. M.: Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea, Proc. Natl. Acad. Sci. U. S. A., 104, 7104-7109, 10.1073/pnas.0611081104, 2007.

Langfeldt, D., Neulinger, S. C., Heuer, W., Staufenbiel, I., Künzel, S., Baines, J. F., Eberhard, J., and Schmitz, R. A.: Composition of microbial oral biofilms during maturation in young healthy adults, PlosOne, 9, e87449, 10.1371/journal.pone.0087449, 2014.

Langlois, R. J., Hummer, D., and LaRoche, J.: Abundances and distributions of the dominant nifH phylotypes in the Northern Atlantic Ocean, Applied and Environmental Microbiology, 74, 1922-1931, 10.1128/aem.01720-07, 2008.

Lévy, M., Klein, P., and Treguier, A.-M.: Impacts of sub-mesoscale physics on phytoplankton production and subduction, J. Mar. Res., 59, 535-565, doi: 10.1357/002224001762842181, 2001.

Lévy, M., Ferrari, R., Franks, P. J. S., Martin, A. P., and Riviere, P.: Bringing physics to life at the submesoscale, Geophysical Research Letters, 39, 10.1029/2012gl052756, 2012.

Löscher, C. R., Kock, A., Könneke, M., LaRoche, J., Bange, H. W., and Schmitz, R. A.: Production of oceanic nitrous oxide by ammonia-oxidizing archaea, Biogeosciences 9, 2419-2429, 2012.

Löscher, C. R., Großkopf, T., Desai, F., Gill, D., Schunck, H., Croot, P., Schlosser, C., Neulinger, S. C., Lavik, G., Kuypers, M. M. M., LaRoche, J., and Schmitz, R. A.: Facets of diazotrophy in the oxygen minimum zone off Peru, ISME J, 8, 2180-2192, doi: 10.1038/ismej.2014.71, 2014.

Löscher, C. R., Bange, H. W., Schmitz R. A., Callbeck, C. M., Engel, A., Hauss, H., Kanzow, T., Kiko, R., Lavik, G., Loginova, A., Melzner, F., Neulinger, S. C., Pahlow, M., Riebesell, U., Schunck, H., Thomsen, S., and Wagner, H.: Water column biogeochemistry of oxygen minimum zones in the eastern tropical North Atlantic and eastern tropical South Pacific Oceans Biogeosciences Discussion, 12, 4495-4556, doi:10.5194/bgd-12-4495-2015, 2015.

Mahadevan: Ocean science: Eddy effects on biogeochemistry, Nature Geoscience, 506, 168-169, doi:10.1038/nature13048, 2014.

McGillicuddy, D. J., Anderson, L. A., Bates, N. R., Bibby, T., Buesseler, K. O., Carlson, C. A., Davis, C. S., Ewart, C., Falkowski, P. G., Goldthwait, S. A., Hansell, D. A., Jenkins, W. J., Johnson, R., Kosnyrev, V. K., Ledwell, J. R., Li, Q. P., Siegel, D. A., and Steinberg, D. K.: Eddy/wind interactions stimulate extraordinary mid-ocean plankton blooms, Science, 316, 1021-1026, 10.1126/science.1136256, 2007.

Morris, J. J., Kirkegaard, R., Szul, M. J., Johnson, Z. I., and Zinser, E. R.: Facilitation of robust growth of Prochlorococcus colonies and dilute liquid cultures by 'helper' heterotrophic bacteria, Appl. Environ. Microbiol., 74, 4530–4534, 2008.

Morris, R. M., Rappé, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J.: SAR11 clade dominates ocean surface bacterioplankton communities, Nature 420, 806-810, doi:10.1038/nature01240, 2002.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., and Minchin, P. R.: vegan: Community ecology package. R package version 2.0-6. , 2013.

Oschlies, A., and Garcon, V.: Eddy-induced enhancement of primary production in a model of the North Atlantic Ocean, Nature, 394, 266–269, 1998.

Pinheiro, J. C., Bates, D. M., DebRoy, S., Sarkar, D., and R Core Team: nlme: Linear and nonlinear mixed effects models, R package version 3.1-120 ed., 2015.

Poretsky, R. S., Hewson, I., Sun, S. L., Allen, A. E., Zehr, J. P., and Moran, M. A.: Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre, Environmental Microbiology, 11, 1358-1375, 10.1111/j.1462-2920.2008.01863.x, 2009.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glöckner, F. O.: SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB, Nucleic acids research, 35, 7188–7196, 10.1093/nar/gkm864, 2007.

R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2015.

Rappé, M. S., Connon, S. A., Vergin, K. L., and Giovannoni, S. J.: Cultivation of the ubiquitous SAR11 marine bacterioplankton clade, Nature, 418, 630-633 doi:10.1038/nature00917, 2002.

Richardson, D. C., and Richardson, J. S.: The kinemage: A tool for scientific communication, Protein Science, 1, 3-9, 10.1002/pro.5560010102, 1992.

Riebesell, U., and Gattuso, J.-P.: Lessons learned from ocean acidification research, Nature Climate Change, 5, 2015.

Ryabenko, E., Kock, A., Bange, H. W., Altabet, M. A., and Wallace, D. W. R.: Contrasting biogeochemistry of nitrogen in the Atlantic and Pacific oxygen minimum zones, Biogeosciences 9, 203-215, 2012.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., and al., e.: Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities, Applied and Environmental Microbiology, 75, 7537–7541, doi: 10.1128/aem.01541-09 2009.

Schmid, M. C., Hooper, A. B., Klotz, M. G., Woebken, D., Lam, P., Kuypers, M. M. M., Pommerening-Roeser, A., op den Camp, H. J. M., and Jetten, M. S. M.: Environmental detection of octahaem cytochrome c hydroxylamine/hydrazine oxidoreductase genes of aerobic and anaerobic ammonium-oxidizing bacteria, Environmental Microbiology, 10, 3140-3149, 10.1111/j.1462-2920.2008.01732.x, 2008.

Schütte, F., Karstensen, J., Krahmann, G., Fiedler, B., Brandt, P., Visbeck, M., and Körtzinger, A.: Characterization of “dead-zone eddies” in the tropical North Atlantic Ocean, in prep for Biogeosciences Discussion, 2015.

Stewart, F. J., Ulloa, O., and DeLong, E. F.: Microbial metatranscriptomics in a permanent marine oxygen minimum zone, Environ Microbiol, 14, 23-40, 10.1111/j.1462-2920.2010.02400.x, 2011.

Stramma, L., Johnson, G. C., Sprintall, J., and Mohrholz, V.: Expanding oxygen-minimum zones in the tropical oceans, Science, 320, 655-658, 10.1126/science.1153847, 2008.

Stramma, L., Bange, H. W., Czeschel, R., Lorenzo, A., and Frank, M.: On the role of mesoscale eddies for the biological productivity and biogeochemistry in the eastern tropical Pacific Ocean off Peru, Biogeosciences, 10, 7293-7306, doi:10.5194/bg-10-7293-2013, 2013.

Stratil, S. B., Neulinger, S. C., Knecht, H., Friedrichs, A. K., and Wahl, M.: Temperature-driven shifts in the epibiotic bacterial community composition of the brown macroalga Fucus vesiculosus. , MicrobiologyOpen, 10.1002/mbo1003.1079, 2013.

Stratil, S. B., Neulinger, S. C., Knecht, H., Friedrichs, A. K., and Wahl, M.: Salinity affects compositional traits of epibacterial communities on the brown macroalga *Fucus vesiculosus*, FEMS Microbiology Ecology, 88, 272–279, 10.1111/1574-6941.12292, 2014.

Suzuki, M., Preston, C., Chavez, F., and DeLong, E.: Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay, Aquatic Microbial Ecology, 24, 117–127, 2001.

Swan, B. K., Martinez-Garcia, M., Preston, C. M., Sczyrba, A., Woyke, T., Lamy, D., Reinthaler, T., Poulton, N. J., Masland, E. D. P., Gomez, M. L., Sieracki, M. E., DeLong, E. F., Herndl, G. J., and Stepanauskas, R.: Potential for chemolithoautotrophy among ubiquitous bacteria lineages in the dark ocean, Science, 333, 1296–1300, 10.1126/science.1203690, 2011.

Ulloa, O., Canfield, D. E., DeLong, E. F., Letelier, R. M., and Stewart, F. J.: Microbial oceanography of anoxic oxygen minimum zones, Proc. Natl. Acad. Sci. U. S. A., 109, 15996-16003, 10.1073/pnas.1205009109, 2012.

Vaquer-Sunyer, R., and Duarte, C. M.: Thresholds of hypoxia for marine biodiversity, Proc. Natl. Acad. Sci. USA. , 105, 15452–15457, 2008.

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R.: Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy, Appl. Environ. Microbiol. , 73, 5261-5267 doi: 10.1128/AEM.00062-07, 2007.

West, N. J., Lebaron, P., Strutton, P. G., and Suzuki, M. T.: A novel clade of Prochlorococcus found in high nutrient low chlorophyll waters in the South and Equatorial Pacific Ocean, ISME J, 5, 933-944, 2011.

Wright, J. J., Konwar, K. M., and Hallam, S. J.: Microbial ecology of expanding oxygen minimum zones, Nat. Rev. Microbiol., 10, 381-394, 10.1038/nrmicro2778, 2012.

Yu, Y., Lee, C., Kim, J., and Hwang, S.: Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction, 89, 670–679, 10.1002/bit.20347, 2005.

Zhu, M., and Ghodsi, A.: Automatic dimensionality selection from the scree plot via the use of profile likelihood, Computational Statistics & Data Analysis, 51, 918-930, 10.1016/j.csda.2005.09.010, 2006.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M.: Mixed effects models and extensions in ecology with R, Statistics for Biology and Health, edited by: Gail, M., Krickeberg, K., Samet, J. M., Tsiatis, A., and Wong, W., Springer, 2009.

Figures

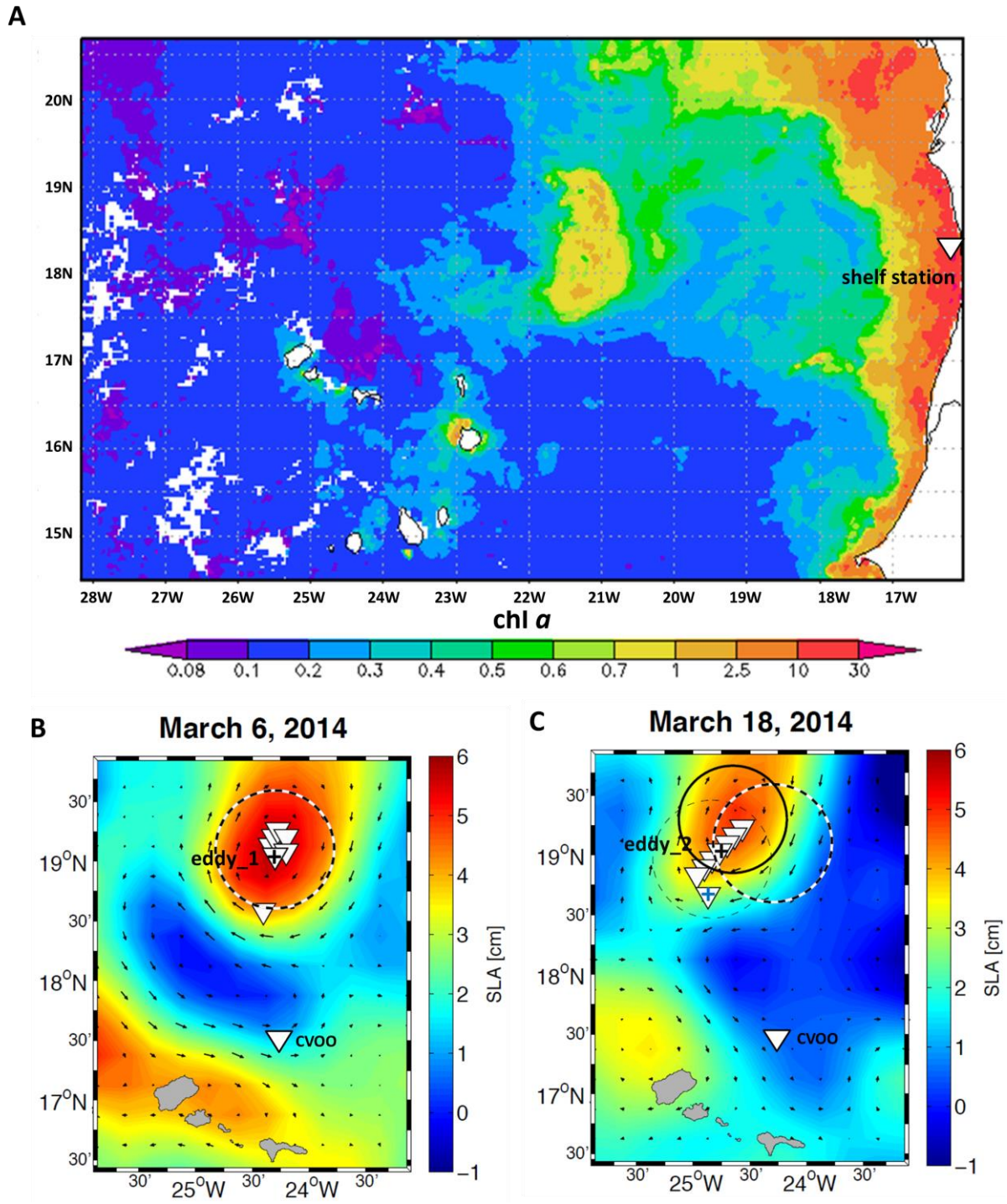


Figure 1: (A) MODIS-Aqua 4-km monthly mean chl*a* distribution in the ETNA (mg m^{-3}) in November 2013. Markedly increased chl*a* concentrations are associated with the low-oxygen ACME, located between 21°W and 22°W and 17.5°N and 19°N. Analyses and visualizations were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC.

Eddy location indicated by sea level anomaly (SLA) during the time of the two surveys: (B) First eddy observation; + denotes the eddy_1 station, (C) Second eddy observation + denotes the eddy_2 station, an additional station was sampled at the eddy rim for C uptake measurements, indicated by the blue +

White triangle marks the sampling station for the potential source water of the eddy. The dashed circles indicate the location of the eddy during the RV Islandia survey, the black circle indicates the eddy location during the RV Meteor survey, and the dashed black line indicates the direction of eddy propagation. Sampling stations are shown with white triangles.

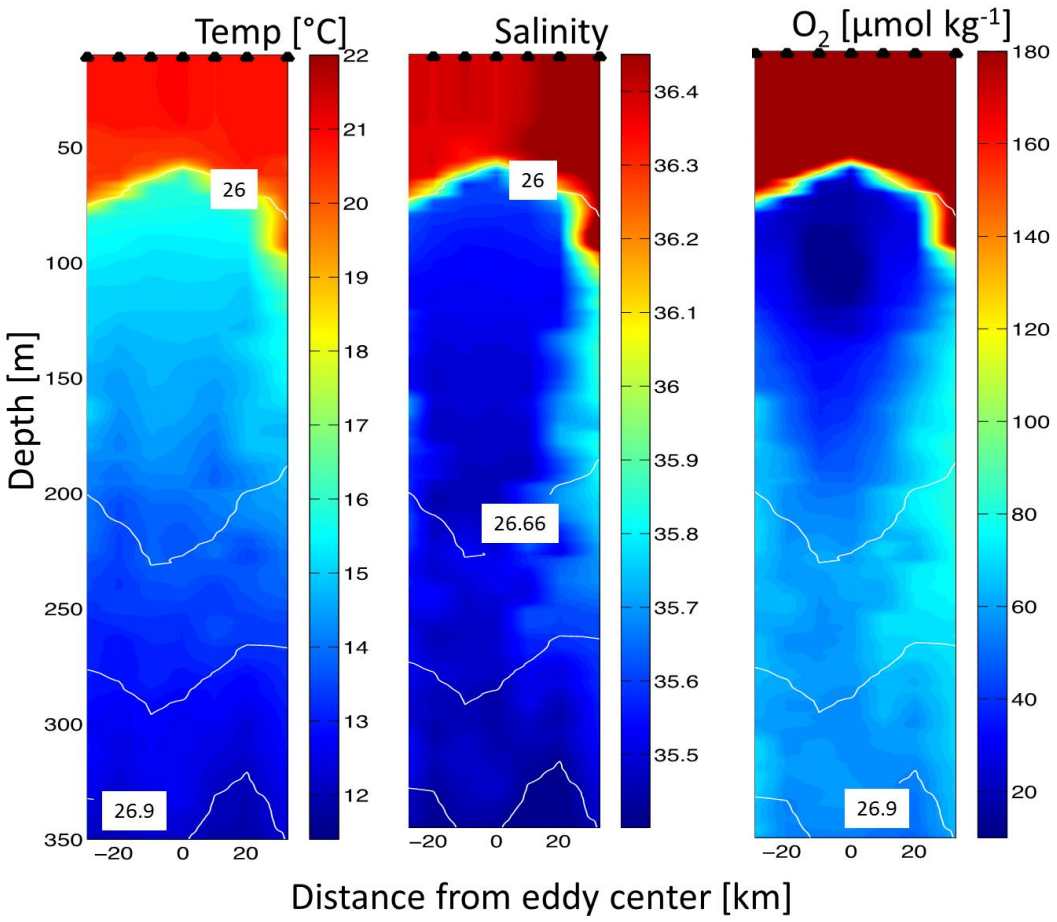


Figure 2: Temperature (left panel), salinity (middle panel) andO_2 concentration (right panel) measured during a section of RV Meteor Cruise M105 across the studied eddy. Minimum O_2 was 4.8 µmol kg^{-1} at ~100 m water depth on that section; however, even lower $O_2$was detected with a glider (1.2µmol kg^{-1}). Isopycnals are indicated by white lines.

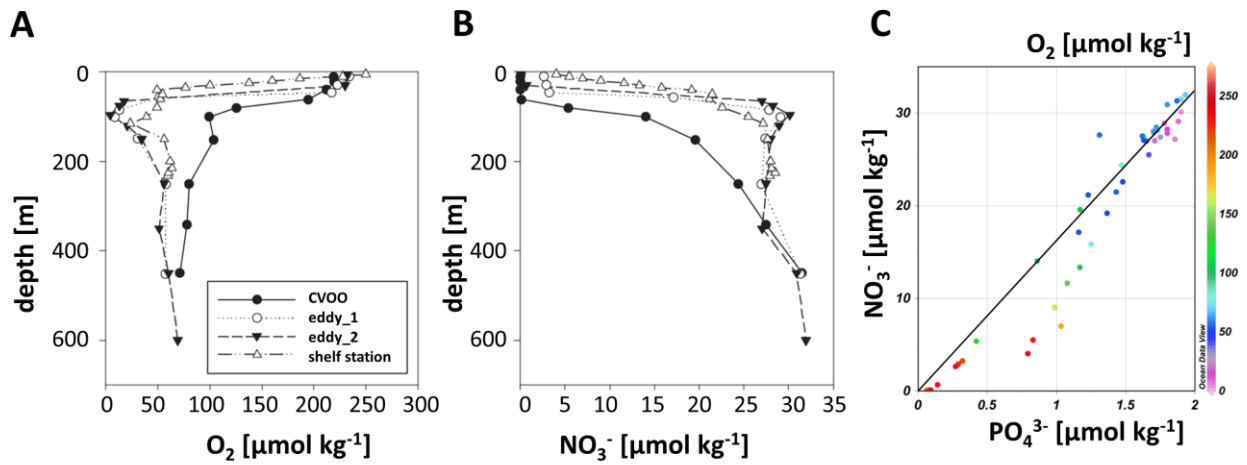


Figure 3: (A) O_2 and (B) nitrate (C) nitrite concentrations measured at the open ocean station CVOO (black circles), in the first observation (eddy_1, open circles), second observation (eddy_2, black triangles) and on the Mauritanian shelf (open triangles). (D) Nitrate vs. phosphate concentration at the 4 sampling stations. The color code denotes the O_2 concentration and the black line indicates the Redfield ratio of N:P = 16:1.

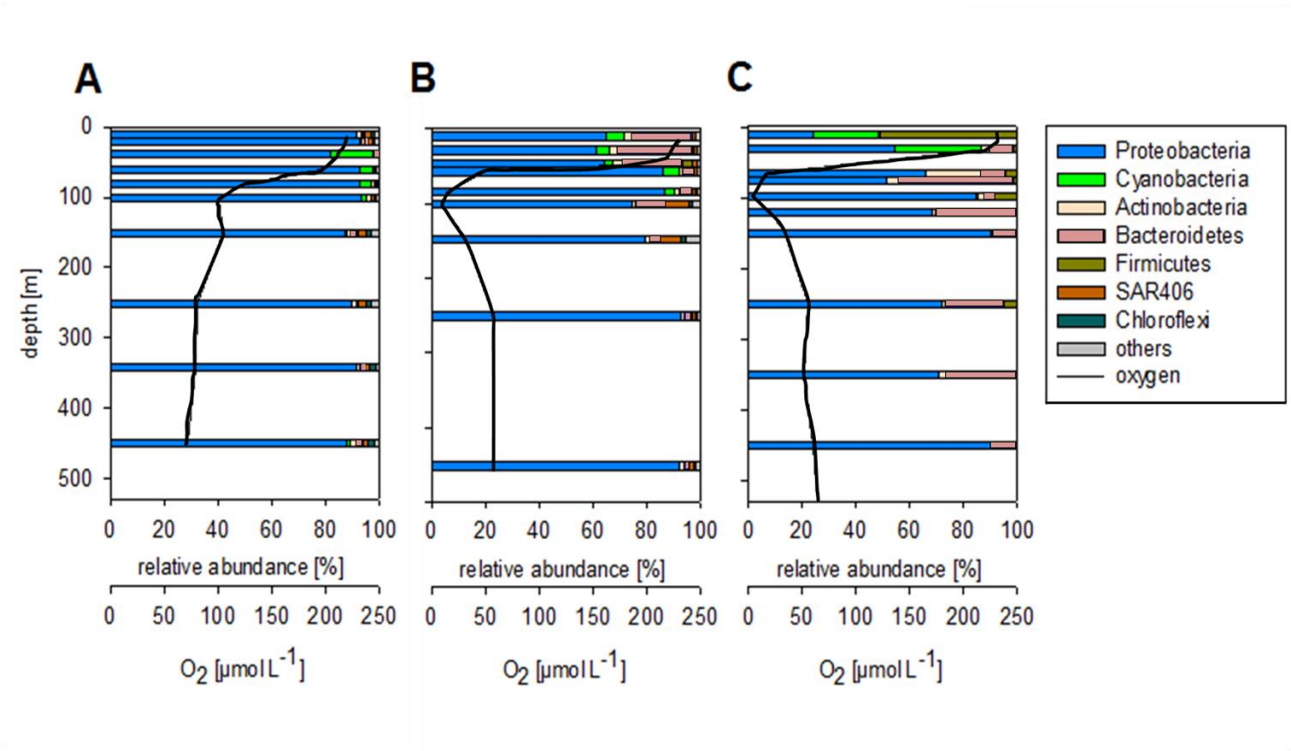


Figure 4: Distribution of bacterial phyla along vertical profiles of (A) CVOO, (B) first observation (eddy_1) and (C) second observation (eddy_2) is shown along with the O_2 gradient (black line). Datasets result from 16S rDNA amplicon sequencing (an overview on archaeal sequence distribution is given in the supplemental material).

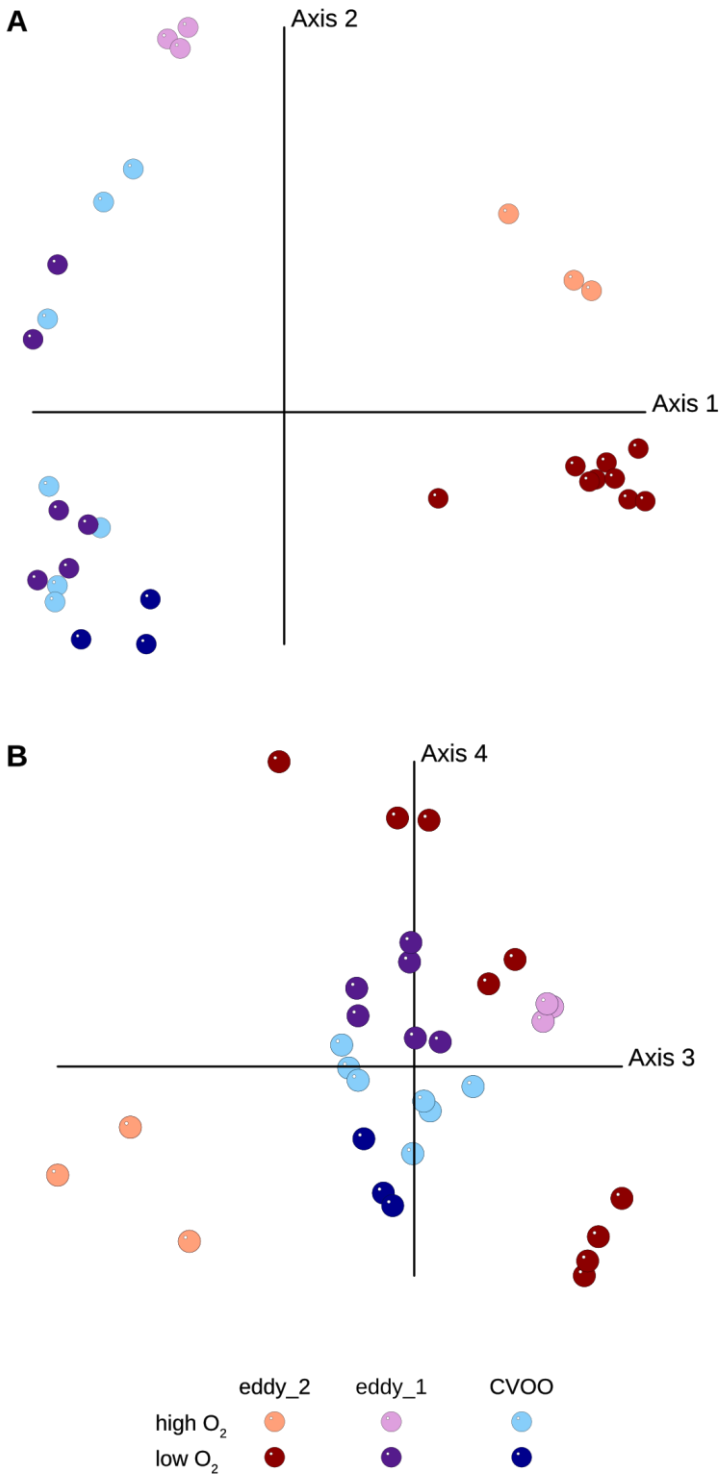


Figure 5: Redundancy analysis (RDA) of OTU distribution in samples from the first eddy observation (eddy_1), from the second eddy observation (eddy_2) and from CVOO based on 16S rDNA sequences. (A) First and second axis, (B) third and fourth axis of the RDA model, illustrating the interaction effect of factor "Realm" and O_2 concentration. For plotting, the continuous variable "O_2" was converted into a factor with two levels "high O_2" (>90 µM) and "low O_2" (≤90 µM).

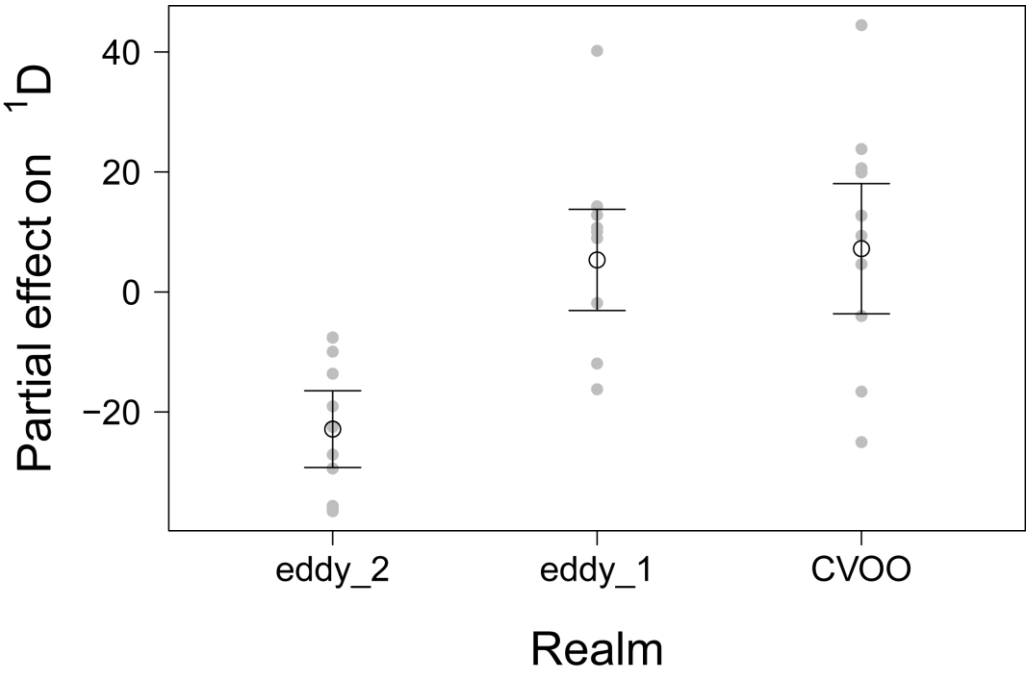


Figure 6: Alpha diversity analysis of eddy sampling stations (first observation (eddy_1), second observation (eddy_2)) and CVOO expressed as Shannon numbers equivalent (^{1}D). A strong and significant decrease in diversity is observed in the eddy. Partial response residuals (black symbols) were extracted from full GLS model re-fitted without the "Realm" main effect. Predicted values for partial residualsmodelled by the "Realm" main effect alone (and thus adjusted for differences in O_2 concentration) are shown as blue symbols. Error bars represent 95% confidence interval for fitted values.

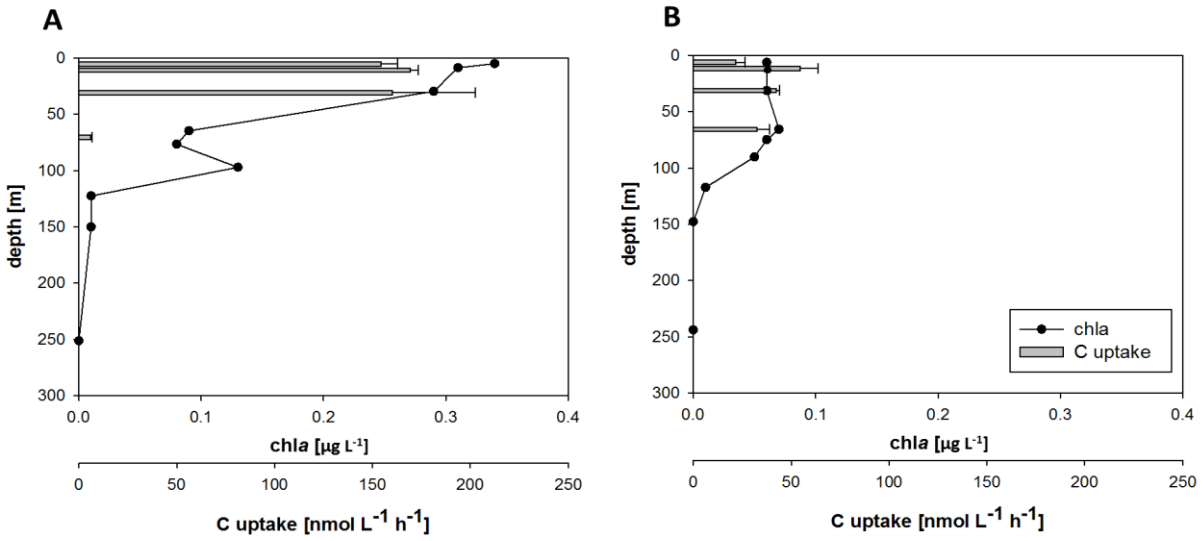


Figure 7: Chlorophyll *a* (chl*a*, µg L^{-1}) distribution as determined from discrete measurements and carbon uptake rates (A) inside the eddy (eddy_2, second observation) and (B) at the eddy rim (location denoted in Fig. 1). Error bars indicate the standard deviation of three replicate samples for C uptake.

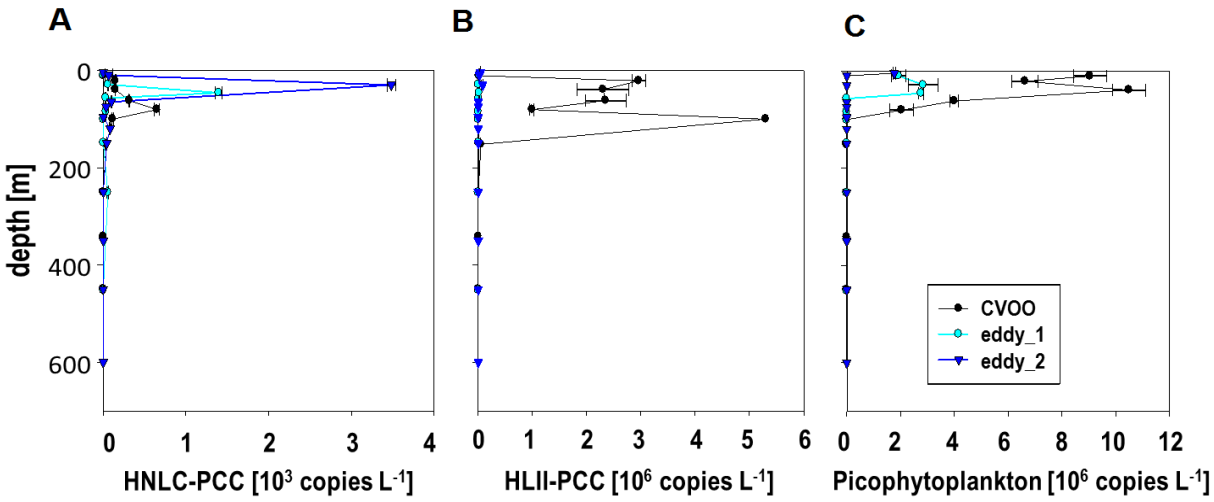


Figure 8: Vertical distribution of *Prochlorococcus* and *Synechococcus* ecotypes quantified by qPCR. While the HNLC-PCC (A) dominates the eddy water mass and increases from the first observation (eddy_1) to the second observation (eddy_2) it is nearly absent outside the eddy (CVOO). HLII-PCC (B) occurs in highest abundances outside the eddy, while being close to the detection limit inside the eddy. (C) shows the distribution of pico-phytoplankton as detected with a general primer-probe system (Suzuki et al., 2001).

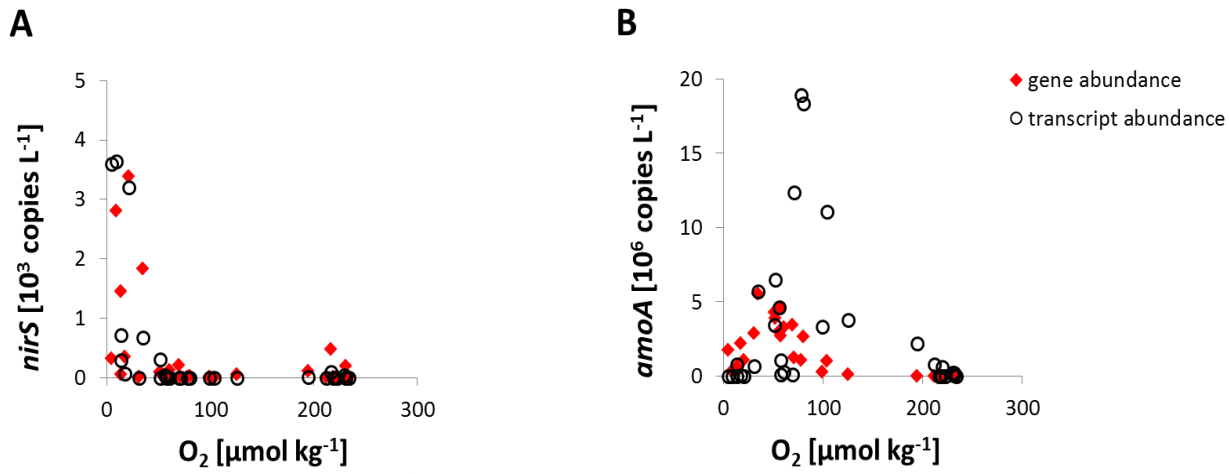


Figure 9: Gene and transcript abundance vs. O_2 concentrations of samples from the eddy observations (eddy_1 and eddy_2) and CVOO. (A) shows the key gene for denitrification, *nirS*, coding for the nitrite reductase, (B) shows archaeal *amoA* as key functional gene of ammonia oxidation, coding for the ammonia monooxygenase. Gene abundances are denoted in red, transcript abundances are indicated by black circles.