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

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Abstract

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 around 40 μmol kg^{-1}. Only recently, the discovery of re-occurring mesoscale eddies with sometimes close to anoxic O_2 concentrations (< 1 μmol kg^{-1}) and located just below the mixed layer challenged our understanding of O_2 distribution and biogeochemical processes in this area.

Here, we present the first metagenomic dataset from a deoxygenated anticyclonic modewater eddy in the open waters of the ETNA. In the eddy, we observed a 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 increased carbon uptake rates up to three times as high as in surrounding waters. Carbon uptake below the euphotic zone correlated to the presence of a specific high-light ecotype of *Prochlorococcus*, which is usually underrepresented in the ETNA. Our combined data indicate that high primary production in the eddy fuels export production and the presence of a specific microbial community responsible for enhanced respiration at shallow depths, below the mixed layer base. Progressively decreasing O_2 concentrations in the eddy were found to promote transcription of the key gene for denitrification, *nirS*, in the O_2-depleted core waters. This process is usually absent from the open ETNA waters.

In the light of future ocean deoxygenation our results show exemplarily that even distinct events of anoxia have the potential to alter microbial community structures and with that critically impact 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 fuels, along with nutrient supply via Saharan dust deposition, one of the most productive areas in the ocean. Associated to this EBUS, a moderate oxygen minimum zone (OMZ) with lowest O_2 concentrations just below 40 µmol kg^{-1} is present at intermediate depths (Chavez and Messié, 2009; Jickells et al., 2005; Karstensen et al., 2008).

Over several years, O_2 time series records 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. The observation of distinct events of very low-O_2 concentrations (< 1 µmol kg^{-1}) at depth around 40 to 100 m over periods of more than one month challenged, however, 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 an origin of the water mass 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 when compared to the typical subtropical gyre values (Karstensen et al., 2008).

Mesoscale eddies are increasingly recognized as biogeochemical hot-spots of up to 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 related to dynamics of mesoscale eddies enable intense primary productivity (Lévy et al., 2001, 2012; 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).

O_2-depleted conditions are supposed to act as a critical switch for the marine microbial community, both with regard to functionality and diversity, as O_2 limitation 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 a heterotrophic community dominated by Flavobacteria, α- and γ- Proteobacteria which efficiently recycle organic matter (Buchan et al., 2014). Further, marine nitrogen (N) and carbon (C) cycling are significantly altered under low O_2 conditions (Vaquer-Sunyer and Duarte, 2008; Wright et al., 2012). A substantial N loss in low-O_2 eddies in the OMZ off Peru in the eastern tropical South Pacific (Altabet et al., 2012) along with enhanced nitrous oxide production (Arévalo-Martínez et al., 2015) has been described.

Classically, the N cycle in open ETNA ocean is assumed to be dominated by nitrification, and due to comparably high background O_2 concentrations ($\geq 40\,\mu\text{mol}\,\text{kg}^{-1}$) an N loss signal is not present (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 further deoxygenate and expand laterally 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 aimed at identifying 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. Ultimately, 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.

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) 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). 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 March 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; March 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 Meteor 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) 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 $\sigma_T = 26.4\,kg\,m^{-3}$, 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 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 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

The net O_2 respiration was calculated as follows:

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

where O_2 (S) denotes the lowest O_2 concentration detected on the shelf ($35.12 \pm 4.08\,\mu\text{mol kg}^{-1}$ at $\sigma_T = 26.3 \pm 0.15\,\text{kg m}^{-3}$, cruise M107, average of shelf stations between 18.10° N/16.59° W and 19.89° N/17.50° W). This region was chosen as it was identified (Schütte et al., 2015) 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 ($1.2\,\mu\text{mol kg}^{-1}$ at $\sigma_T = 26.35\,\text{kg m}^{-3}$).

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):

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

2.4 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 fluorometrically using a NanoDrop 2000 (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, 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 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).

2.4.1 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 V5V7 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 Nanodrop 1000 spectrophotometer (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.

2.4.2 Sequence analysis of 16S rDNA gene amplification

Sequence processing was performed using mothur software version 1.32.1 (Kozich et al., 2013). 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 V5V7.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.

2.5 Statistics

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 or-

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dered OTUs for which the cumulative mean percentage amounted to 99 % was retained in the filtered OTU table.

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, 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.

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" ($> 90\,\mu$M) and "low O_2" ($\leq 90\,\mu$M); 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.

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.

3-D 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).

For alpha diversity analysis, effective OTU richness (Shannon numbers equivalent, ^1D, (Jost, 2006, 2007)) was calculated from the filtered OTU table. ^1D 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 and 0.28 µM, respectively) on ^1D. 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 ^1D, 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.

2.6 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 ($> 18.2\,M\Omega\,cm^{-1}$, 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 as estimated from photosynthetically active radiation (PAR) sensor measurements from CTD profiles were stored at 12 °C in the dark. 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) 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, was characterized by extremely 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, while concentrations of less than 30 μmol kg^{-1}, which is significantly below average O_2 concentrations in that region, were observed in the eddy water column between 70 to 150 m depth (Figs. 2 and 3a). During the survey period (March 2014), O_2 concentrations in the core decreased (see Fiedler et al., 2015 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} at ~ 250 m depth were detected at CVOO (Fig. 1), which can be considered average O_2 concentrations for the open ETNA (Karstensen et al., 2008).

In the eddy, we observed nitrate and phosphate concentrations elevated in the low-O_2 core by a factor of ~ 2 relative to the background 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).

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). We thus 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, the community structure within the eddy appeared largely distinct from that in surrounding waters (Fig. 4). Phyla such as Bacteroidetes, Actinobacteria and Firmicutes were exclusively 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 (DeLong, 2009), decreased strongly in abundance within the eddy, while dominating CVOO samples. 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. Besides the dissimilarity regarding bacterial diversity, we also detected a substantial difference in archaeal community composition between eddy stations and CVOO (Fig. S1). 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 while absent from CVOO samples. The presence of methanogens in low-O_2 eddy core samples may indicate a 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: $F_{6,24} = 4.48$, $p < 0.001$). Changes in OTU composition mirrored the depth gradient (RDA "Depth": $F_{1,24} = 2.08$, $p \approx 0.03$; Fig. 5) and were thus strongly correlated to chemical (PO_4^{3-}, NO_3^-, SiO_2) and physical (T, S) properties (Fig. S3). The RDA model indicates a noticeable interaction effect of habitat ("Realm") and O_2 concentration (RDA "Realm:O_2": $F_{2,24} = 2.03$, $p \approx 0.02$), 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: $F_{7,23} = 5.37$, $p = 0.001$; GLS "Realm": $F_{2,23} = 16.26$, $p < 0.0001$), which 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 $0.19\,\mu mol\,kg^{-1}\,d^{-1}$ 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 $35.12 \pm 4.08\,\mu mol\,kg^{-1}$ compared to observed minimum O_2 concentrations of $1.2\,\mu mol\,kg^{-1}$ 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) calculated an increase in pCO_2 and dissolved inorganic carbon compared to coastal waters of $1.83\,\mu atm\,d^{-1}$ and $0.23\,\mu mol\,kg^{-1}\,d^{-1}$, respectively, both indicating enhanced remineralization and respiration. Although our dataset does not allow differentiating high-pCO_2 and low-O_2 effects on the microbial community, it supports the view of a general loss in diversity in direct or indirect response to factors related to deoxygenation and increasing pCO_2, such as the impact on nutrient stochiometry, as previously suggested (Bryant et al., 2012).

With regard to climate change-related ocean deoxygenation and consecutive shifts in nutrient stochiometry this would mean an overall loss of microbial diversity, probably along with a 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 coinciding 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 potentially fueled by increased nutrient availability from intermediate depths. We found a 3-fold increase of 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), 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. S4 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 only around 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 detectable 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 $p\mathrm{CO}_2$ levels, *Prochlorococcus* is predicted to substantially increase in abundance (Flombaum, 2013). Elevated $p\mathrm{CO}_2$ 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 total bacterial production (Bertillson et al., 2005).

Besides a direct impact of O_2, nutrient and $p\mathrm{CO}_2$, 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). Vice versa, *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., 2015; Morris et al., 2008). The close proximity of increased abundances of the HNLC-PCC maximum to the O_2 minimum in the eddy implies a beneficial relation between the HNLC-PCC and the eddy core water microbial community that largely consists of heterotrophic organisms.

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 ($> 90\,\mu mol\,kg^{-1}$) or low-O_2 conditions ($\leq 90\,\mu mol\,kg^{-1}$). 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 (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) (Tab. S3). 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.

Enhanced productivity and consecutive respiration and O_2 decrease may enable N loss processes in the open ETNA, which have previously not been described to occur in the ETNA waters in that region (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 (1164 µatm in the eddy core compared to 835 µatm in coastal waters; Fiedler et al., 2015) and a 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 also in the usually oxic open ETNA, a potential for N loss is present. As suggested by Kalvelage et al. (2013), feedback mechanisms between eutrophication, enhanced primary productivity and consecutive enhanced export production may promote denitrification in marine systems. This will ultimately lead to enhanced N loss, 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 of dinitrogen fixation (*nifH*, 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). The latter 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 exemplarily gene expression in a severely O_2-depleted anticyclonic modewater eddy in the open waters of the ETNA OMZ region and compared the eddy observations to background signals, represented by data from the ETNA open ocean CVOO time series site, and to data from 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. A similarity of the eddy core microbial community to the shelf microbial community, which may shape the distinct character of this O_2-depleted eddy progressively over time, was found.

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 specific organic C sources for the associated eddy-specific heterotrophic community. 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 and leads to consecutive 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), as such no large scale impact of the eddies are expected. However, an unexpected shift in elementary ratios or other anomalies, normally expect for regions with much lower minimal oxygen levels than the ETNA, may be detected and explained with 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.

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Author contributions. C. R. Löscher designed the study together with B. Fiedler, A. Körtzinger, H. Hauss and J. Karstensen; M. Philippi and C. R. Löscher validated the NGS primer sets for marine samples, performed the molecular analysis, processed the molecular data and analyzed hydrographic data. F. Schütte, J. Karstensen and A. Körtzinger designed the eddy tracking system. S. Künzel performed the high-throughput sequencing runs. M. A. Fischer and R. A. Schmitz modified the NGS-primers for archaeal community analysis. M. A. Fischer and S. C. Neulinger performed bioinformatic analysis of high-throughput datasets. A. Singh performed C-uptake measurements and data analysis, F. Schütte, J. Karstensen and A. Körtzinger designed the eddy detection and tracking system. B. Fiedler, F. Schütte, A. Körtzinger, H. Hauss and C. R. Löscher planned the sampling campaign and B. Fiedler performed hydrographical measurements and analyzed the data. S. C. Neulinger performed statistical analysis and modeling. C. R. Löscher wrote the manuscript with input from all co-authors.

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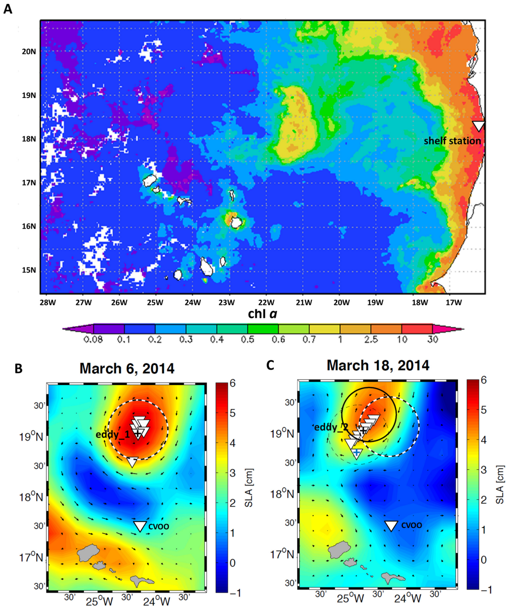

Figure 1. (A) MODIS-Aqua 4 km monthly mean chl *a* distribution in the ETNA (mg m^{-3}) in November 2013. Markedly increased chl *a* concentrations are associated with the low-oxygen ACME, located between 21° W and 22° W and 17.5° N and 19° N. Analyses and visualizations were produced with the Giovanni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

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Hidden biosphere in an oxygen-deficient Atlantic open ocean eddy

C. R. Löscher et al.

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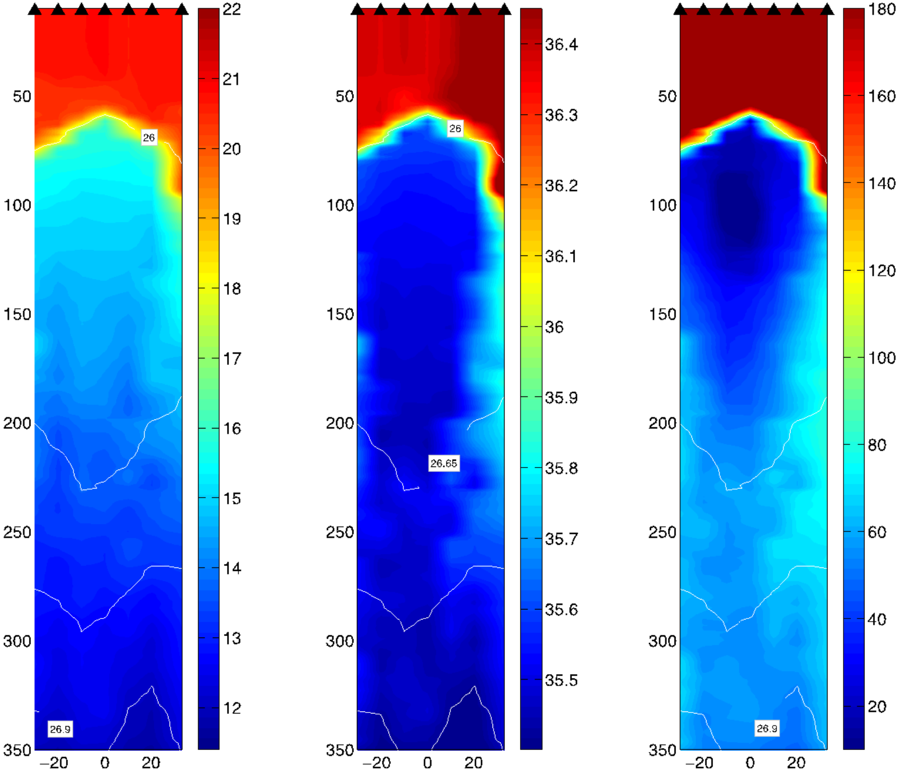

Figure 2. Temperature (left panel), salinity (middle panel) andO_2 concentration (right panel) measured during a section of RV *Meteor* Cruise M105 across the studied eddy. Minimum O_2 was 4.8 µmol kg^{-1} at $\sim$ 100 m water depth on that section; however, even lower O_2 was detected with a glider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

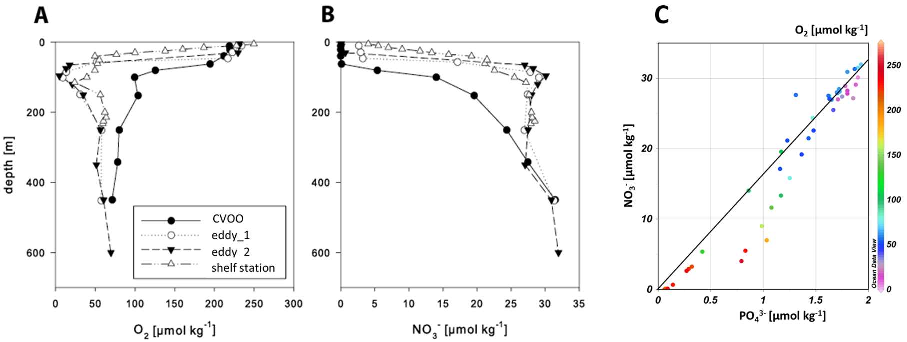

Figure 3. (A) O_2 and **(B)** nitrate concentrations measured at the open ocean station CVOO (black circles), in the first observation (eddy_1, open circles), second observation (eddy_2, black triangles) and on the Mauritanian shelf (open triangles). **(C)** Nitrate vs. phosphate concentration at the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

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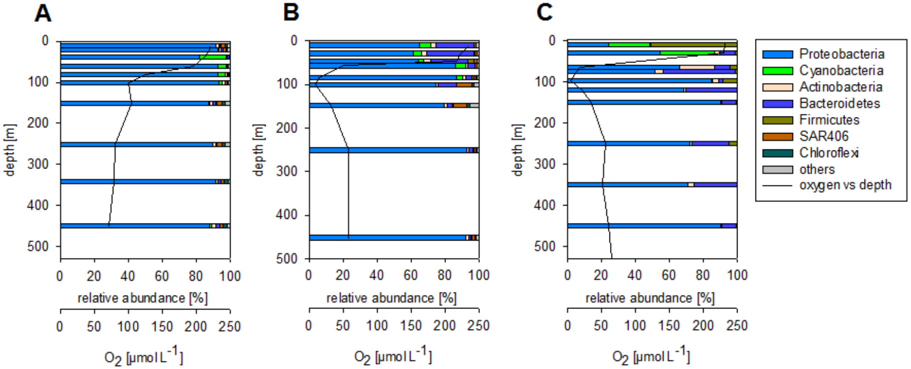

Figure 4. Distribution of bacterial phyla along vertical profiles of **(A)** CVOO, **(B)** first observation (eddy_1) and **(C)** second observation (eddy_2) is shown along with the O$_2$ gradient (black line). Datasets result from 16S rDNA amplicon sequencing (an overview on archaeal sequence distribution is given in the supplemental material).

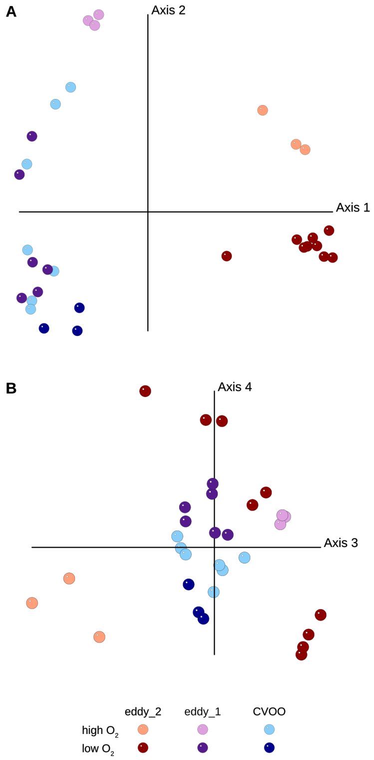

Figure 5. Redundancy analysis (RDA) of OTU distribution in samples from the first eddy observation (eddy_1), from the second eddy observation (eddy_2) and from CVOO based on 16S rDNA sequences. **(A)** First and second axis, **(B)** third and fourth axis of the RDA model, illustrating the interaction 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).

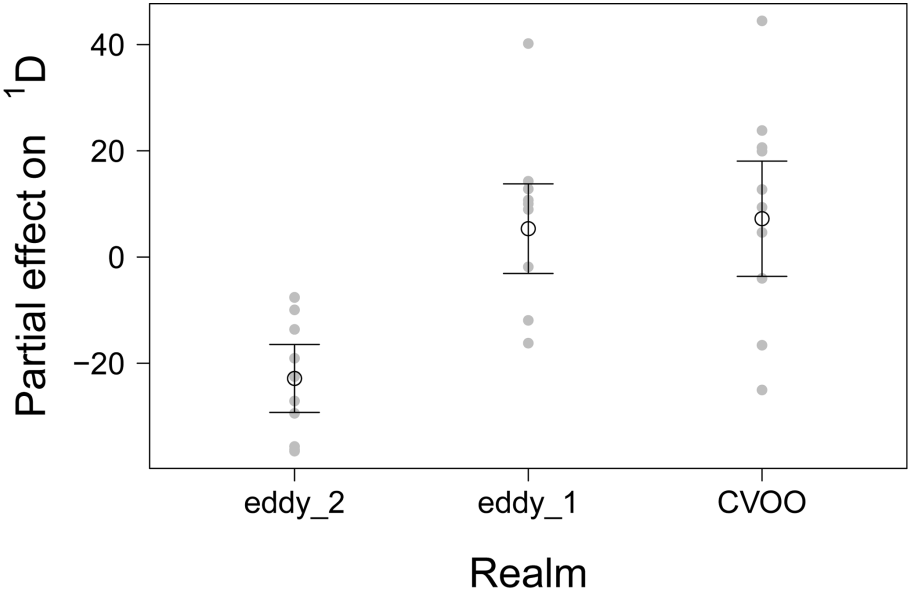

Figure 6. Alpha diversity analysis of eddy sampling stations (first observation (eddy_1), second observation (eddy_2)) and CVOO expressed as Shannon numbers equivalent (^{1}D). A strong and significant decrease in diversity is observed in the eddy. Partial response residuals (black symbols) were extracted 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.

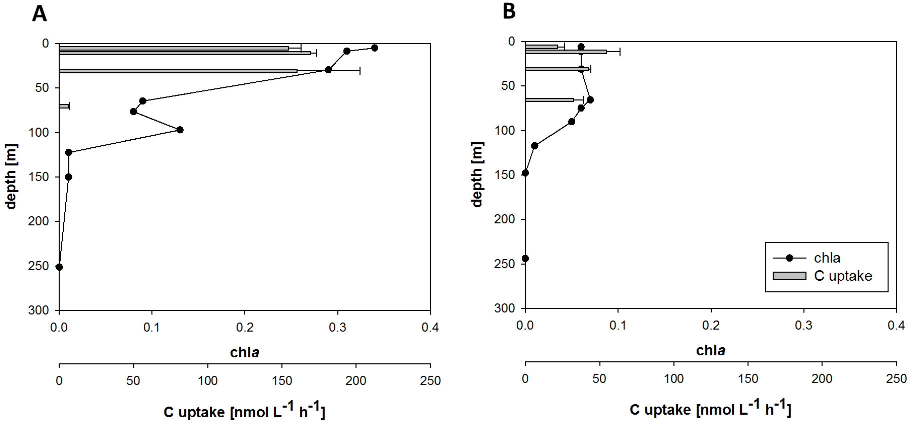

Figure 7. Remotely sensed chlorophyll *a* (chl *a*) distribution and carbon uptake rates **(A)** inside the eddy (eddy_2, second observation) and **(B)** at the eddy rim (location denoted in Fig. 1). Error bars indicate the standard deviation of three replicate samples for C uptake.

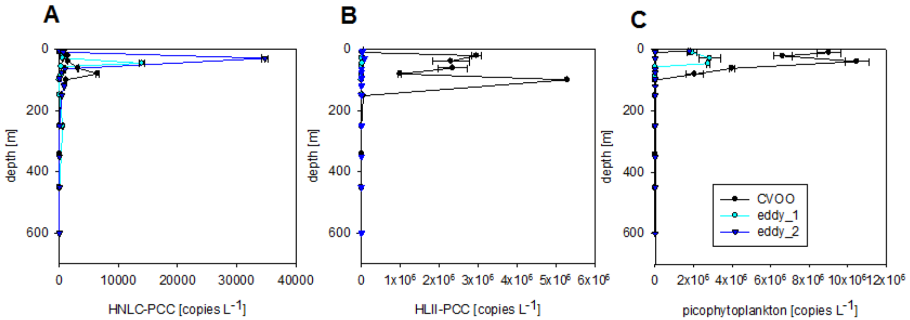

Figure 8. Vertical distribution of *Prochlorococcus* and *Synechococcus* ecotypes quantified by qPCR. While the HNLC-PCC **(A)** dominates the eddy water mass and increases from the first observation (eddy_1) to the second observation (eddy_2) it is nearly absent outside the eddy (CVOO). HLII-PCC **(B)** 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).

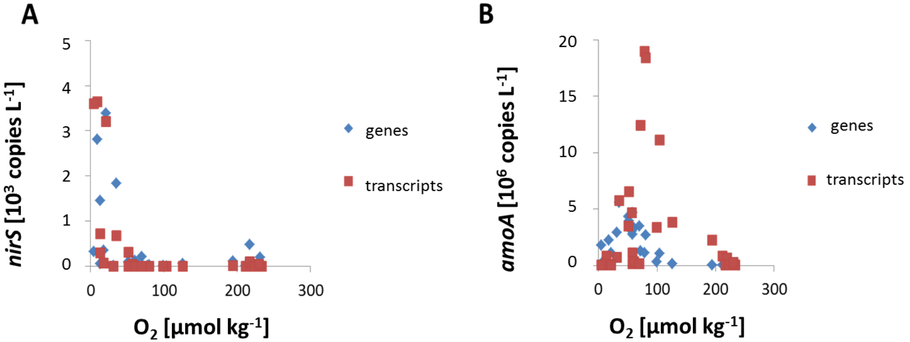

Figure 9. Gene and transcript abundance vs. O_2 concentrations of samples from the eddy observations (eddy_1 and eddy_2) and CVOO. **(A)** shows the key gene for denitrification, *nirS*, coding for the nitrite reductase, **(B)** shows archaeal *amoA* as key functional gene of ammonia oxidation, coding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