

1 Dear editor,

2 Thanks for the thorough review and editing of our manuscript! We are happy about your decision to  
3 accept our manuscript for publication and addressed all issues from your side by following your  
4 suggestions.

5  
6 Best regards

7  
8 Carolin Löscher

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10 -----

11 The following issues were included into the manuscript according to the editor's suggestions:

- 12
- 13 Line 52: Delete "conventionally"
  - 14 Line 60: Delete "the" before "recent"
  - 15 Line 63: Change "denitrified" to "denitrifying"
  - 16 Line 64: Change "A biogeochemical" to "The biogeochemical"
  - 17 Line 72: Delete "on" after "impact"
  - 18 Line 104: Add "old" after "5 months"
  - 19 Line 117: Change "at Redfield conditions" to "as per the Redfield stoichiometry"
  - 20 Line 162: Change "lead" to "led"?
  - 21 Line 164: Change "blank values" to "blanks"
  - 22 Line 171: Change "supplemental" to "supplementary"
- 23
- 24 Line 261: Change "B" to "eddy B"
- 25
- 26 Line 266: Change "allow" to "allows"
  - 27 Line 276: Change "in Fong et al" to "by Fong et al"
  - 28 Line 323: Delete "however" ("Although" and "however" do not go together)
  - 29 Line 341: Change "N2 fixation was not increased" to "elevated N2 fixation was not observed"

30

31 For the following issues, we rephrased the sentences/paragraphs:

32 This paragraph was restructured:

33 Line 226: "possibly due to nutrient subduction via organic matter export out of the anticyclonic eddy" . .  
34 not clear to me.

35 "These signals of enhanced ongoing N loss weakened over time as eddy A aged (Stramma et al., 2013).  
36 This may possibly be due to nutrient subduction via organic matter export out of the anticyclonic eddy  
(Omand et al., 2015). An extensive characterization of N loss signals in this eddy revealed a complete

37 consumption of  $\text{NO}_3^-$  (Bourbonnais et al., 2015). Most intense N loss signals were observed near the core  
38 of eddy A...'

39

40 Line 258: What do you mean by "upper isopycnal"?

41 Upper isopycnal refers to the upper 'border' of this eddy, as defined by density. To clarify, we added the  
42 approximate depth range and the reference Stramma et al., 2013 to it, as Stramma and colleagues  
43 describe the density distribution in those eddies in detail. The sentence was changed as follows:

44 In eddy B, the maximum in carbon fixation was exclusively present above the upper isopycnal (between  
45 100 and 200 m, Stramma et al., 2013), and thus not in direct contact with the  $\text{N}_2$  fixation zone.

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49 **N<sub>2</sub> fixation in eddies of the eastern tropical South Pacific Ocean**

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52 W. Bange<sup>1,8</sup>, R. Czeschel<sup>1</sup>, C. Hoffmann<sup>2</sup>, R. Schmitz<sup>2</sup>

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72 Short title: N<sub>2</sub> fixation in ETSP eddies

73

74 **Abstract**

75 Mesoscale eddies play a major role in controlling ocean biogeochemistry. By impacting nutrient  
76 availability and water column ventilation, they are of critical importance for oceanic primary production.  
77 In the eastern tropical South Pacific Ocean off Peru, where a large and persistent oxygen deficient zone is  
78 present, mesoscale processes have been reported to occur frequently. However, investigations on their  
79 biological activity are mostly based on model simulations, and direct measurements of carbon and  
80 dinitrogen ( $N_2$ ) fixation are scarce.

81 We examined an open ocean cyclonic eddy and two anticyclonic mode water eddies: a coastal one and an  
82 open ocean one in the waters off Peru along a section at  $16^\circ S$  in austral summer 2012. Molecular data and  
83 bioassay incubations point towards a difference between the active diazotrophic communities present in  
84 the cyclonic eddy and the anticyclonic mode water eddies.

85 In the cyclonic eddy, highest rates of  $N_2$  fixation were measured in surface waters but no  $N_2$  fixation  
86 signal was detected at intermediate water depths. In contrast, both anticyclonic mode water eddies showed  
87 pronounced maxima in  $N_2$  fixation below the euphotic zone as evidenced by rate measurements and  
88 geochemical data.  $N_2$  fixation and carbon (C) fixation were higher in the young coastal mode water eddy  
89 compared to the older offshore mode water eddy. A co-occurrence between  $N_2$  fixation and biogenic  $N_2$ ,  
90 an indicator for N loss, indicated a link between N loss and  $N_2$  fixation in the mode water eddies, which  
91 was not observed for the cyclonic eddy. The comparison of two consecutive surveys of the coastal mode  
92 water eddy in November 2012 and December 2012 revealed also a reduction of  $N_2$  and C fixation at  
93 intermediate depths along with a reduction in chlorophyll by half, mirroring an aging effect in this eddy.  
94 Our data indicate an important role for anticyclonic mode water eddies in stimulating  $N_2$  fixation and thus  
95 supplying N offshore.

## 1 Introduction

Reactive nitrogen (N) limits primary production in large parts of the ocean (Codispoti, 1989). Biological dinitrogen (N<sub>2</sub>) fixation is an important external input of N, representing more than 60-80% of the new N provided to the Ocean (Codispoti, 2007;Duce et al., 2008), and can partially relieve N limitation. For decades, N<sub>2</sub> fixation was thought to occur mainly in nutrient-depleted surface waters such as found in the subtropical gyres (Sohm et al., 2011). However, some recent modeling studies have suggested a close spatial link between fixed N loss, i.e. N<sub>2</sub> production via anammox and/or denitrification, occurring in oxygen deficient zones (ODZs), and N<sub>2</sub> fixation taking place in the adjacent surface ocean with the consequence that the potential habitat of N<sub>2</sub> fixing organisms is larger than previously thought (Deutsch et al., 2007). Furthermore, as both processes are favored under oxygen depleted conditions and as some organisms responsible for these processes do not need light, their coupling in ODZ waters would damp excursions in the oceanic N inventory and promote stability of the global N budget.

In recent years, efforts have been placed on investigating N<sub>2</sub> fixation in the eastern tropical South Pacific (ETSP) (Dekaezemacker et al., 2013;Fernandez et al., 2015;Löscher et al., 2014) and the results of those field studies have significantly advanced our understanding of diazotrophy in low-O<sub>2</sub> regions of the ocean. They indeed confirmed the frequent occurrence of N<sub>2</sub> fixation in denitrifying waters and below the euphotic zone. The biogeochemical significance of non-cyanobacterial diazotrophs (i.e., microbes capable of N<sub>2</sub> fixation) has been described, and their enormous potential to fix N<sub>2</sub> in the ETSP seems also to depend on organic matter supply (Fernandez et al., 2015).

In addition to its remarkable biological activities, the physically dynamic character of the ETSP in the upwelling system off Peru favors mesoscale activities (Chelton et al., 2011). Compared to other upwelling regions (e.g. off California, Benguela) enhanced frequency of eddies has been reported for this region (Chaigneau et al., 2009). Mesoscale eddies are physical structures with horizontal scales of less than 100 km and timescales of around one month. These features can transport physical and chemical properties from the coast towards the open ocean (Klein and Lapeyre, 2009) and impact the ocean by modulating nutrient availability (Fong et al., 2008;Altabet et al., 2012). Cyclonic and mode water eddies can inject nutrients to the euphotic zone through vertical displacement of isopycnal surfaces, which increases surface primary production (McGillicuddy et al., 2007). Overall, investigations on the impact of mesoscale eddies on N<sub>2</sub> fixation are scarce. Fong et al. (2008) reported a stimulation of N<sub>2</sub> fixation in a mode water eddy of the oligotrophic North Pacific. Another study showed increased abundances of *Trichodesmium* in mesoscale eddies of the Western South and North Atlantic associated with strong temporal variations (Olson et al., 2015).

In the ODZ off Peru, mesoscale eddies have previously been identified as N loss hotspots (Altabet et al., 2012;Bourbonnais et al., 2015), but to date no detailed surveys on their relevance for N<sub>2</sub> fixation in this region are available. The spatial connection between N loss and N<sub>2</sub> fixation that has been proposed

for this region (Fernandez et al., 2011) may, however, indicate a potential for N<sub>2</sub> fixation associated to eddies in the ODZ off Peru.

The major goal of this study was to advance our understanding of eddy-related N<sub>2</sub> fixation by surveying one cyclonic and two anticyclonic mode water eddies along a 16.45°S transect during the R/V Meteor cruises M90 and M91 in November-December 2012. During the survey of these three eddies, we measured both N<sub>2</sub> fixation rates and abundances of *nifH*, a key functional molecular marker gene. Additionally, N<sub>2</sub> fixation was compared to N loss signals in the water column to investigate their coupling in the eddy systems. One particular eddy was surveyed twice (in November 2012 and December 2012), allowing monitoring the temporal development of N<sub>2</sub> fixation and primary production in an aging eddy.

## 2 Material and Methods

### 2.1 Sampling description and biogeochemical parameters

Selection of sampling stations and identification of eddy cores and edges were based on sea level height anomaly data from Aviso (<http://aviso.altimetry.fr>) and followed the criteria defined by Stramma et al. (2013). Briefly, the eddies were tracked during the R/V Meteor cruises M90 and M91 in November-December 2012. Three eddies were detected in area extending from the Peruvian coast to ~84°W and from 15°S to 18°S (Fig. 1, Stramma et al., 2013). Two eddies (further referred to as eddy A centered at about 16°S, 76°W and eddy B centered in the open ocean at about 17°S, 83°W) were mode water eddies and one was cyclonic (further referred to as eddy C, centered in the open ocean at 16°S, 80°W, Fig. 1). The age of the eddy was determined by Stramma et al. (2013) bases on satellite monitoring of sea level height anomaly data. At the time of the survey, the near-coastal eddy A was about 2 months old (3 months during the second survey), while the open-ocean eddy B was 5 months **old** and the cyclonic open-ocean eddy C was 2 months old.

Samples for salinity, O<sub>2</sub> concentrations and nutrients (nitrate, NO<sub>3</sub><sup>-</sup>; nitrite, NO<sub>2</sub><sup>-</sup>; phosphate, PO<sub>4</sub><sup>3-</sup> and ammonium, NH<sub>4</sub><sup>+</sup>) were taken from a 24-Niskin- bottle rosette equipped with a conductivity-temperature-depth (CTD) sensor or from a pump-CTD (Friedrich et al., 1988). O<sub>2</sub> concentrations were determined using a Seabird sensor, calibrated to the Winkler method (precision of 0.45 μmol L<sup>-1</sup>; the lower detection limit was 2 μmol L<sup>-1</sup>; (Stramma et al., 2013)). Nutrient concentrations were determined as previously described (Grasshoff, 1999) using a QuAatro auto-analyzer (SEAL Analytical GmbH, Germany; precision for NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> were ± 0.1 μmol L<sup>-1</sup>, ± 0.1 μmol L<sup>-1</sup>, ± 0.02 μmol L<sup>-1</sup>, respectively). Excess PO<sub>4</sub><sup>3-</sup>, P\* (i.e., the anomaly in P relative to expected stoichiometry with N) was calculated from dissolved inorganic nitrogen (DIN= NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>) and PO<sub>4</sub><sup>3-</sup> measurements according to Deutsch et al. (2007):

$$P^* = \text{PO}_4^{3-} - \text{DIN} / r_{16:1},$$

where  $r_{16:1}$  is the ratio of nitrate to phosphate **as per the Redfield stoichiometry**. Positive  $P^*$  has been thought to stimulate  $\text{N}_2$  fixation

## 2.2 $\text{N}_2/\text{Ar}$ , Biogenic $\text{N}_2$ measurements

High precision measurements of  $\text{N}_2/\text{Ar}$  were made on septum sealed samples using on-line gas extraction system coupled to a multicollector continuous flow-IRMS as described in Charoenpong et al. (2014).  $\text{N}_2$  excess ( $[\text{N}_2]_{\text{excess}}$ ), i.e. the observed  $[\text{N}_2]$  minus the equilibrium  $[\text{N}_2]$  at in-situ temperature and salinity, was calculated based on the  $\text{N}_2/\text{Ar}$  ratio with daily calibration against seawater standards equilibrated with air at fixed temperatures (5°C, 15°C and 25°C). Precision (standard deviation) for duplicate measurements was generally better than  $\pm 0.7 \mu\text{mol L}^{-1}$  for  $[\text{N}_2]_{\text{excess}}$ .

We calculated biogenic  $[\text{N}_2]$  ( $[\text{N}_2]_{\text{biogenic}}$ ), the  $[\text{N}_2]$  produced by denitrification or anammox, by subtracting the  $[\text{N}_2]_{\text{excess}}$  at a background station ( $[\text{N}_2]_{\text{excess\_bkgd}}$ ) unaffected by N loss ( $[\text{O}_2] > 10 \mu\text{mol L}^{-1}$ ) located north of the ODZ (1.67°N, 85.83°W, M90 cruise) from the observed  $[\text{N}_2]_{\text{excess}}$  at corresponding  $\sigma_\theta$  (as described in Bourbonnais et al., 2015):

$$[\text{N}_2]_{\text{excess\_bkgd}} (\mu\text{mol L}^{-1}) = 1 \times 10^{-9} e^{0.84\sigma_\theta}$$

This corrects for non-local biological N loss as well as physically-produced deviations in equilibrium  $\text{N}_2/\text{Ar}$  (Hamme and Emerson, 2002).

## 2.3 $\text{N}_2/\text{C}$ -fixation rate measurements

Sample seawater was taken from the Niskin bottles or from the pump-CTD and filled into 4.5 L polycarbonate bottles (Nalgene, Thermo Fisher, Waltham, Massachusetts, USA) capped with Teflon-coated butyl rubber septum. Incubations were performed as previously described (Grosskopf et al., 2012) with the method developed by Mohr et al. (2010). In contrast to the traditionally used bubble addition method (Montoya et al., 1996),  $^{15}\text{N}_2$  gas (Cambridge Isotopes, Lot no.: I-16727) was dissolved in degassed water from the same sampling depth in order to guarantee a high dissolution and a stable enrichment in  $^{15}\text{N}_2$ . Each incubation bottle was supplemented with 100 mL of  $^{15}\text{N}_2$ -enriched seawater containing defined amounts of 98%  $^{15}\text{N}_2$  gas in order to reach final and constant  $^{15}\text{N}_2$  enrichment of  $2.4 \pm 0.144$  atom%. A recent study reported a slight potential contamination of  $^{15}\text{N}_2$  gas with  $0.024 \pm 0.006 \mu\text{moles } ^{15}\text{N-NO}_3^-/\text{NO}_2^-$  and  $0.014 \pm 0.004 \mu\text{moles } ^{15}\text{N-NH}_4^+$  per mole  $^{15}\text{N}_2$  (Dabundo et al., 2014). According to Dabundo et al. (2014), however, low concentrations of

contaminants in Cambridge- $^{15}\text{N}_2$  gas do not significantly inflate  $\text{N}_2$  fixation rates such as those presented here. In addition, we examined the  $^{15}\text{N}_2$  gas used in our incubations following the hypobromide oxidation method (Warembourg, 1993) and no contamination has been detected. For each bottle, the initial enrichment of  $^{15}\text{N}_2$  has been determined and considered for the calculation of the rates.

For carbon fixation measurements,  $\text{NaH}^{13}\text{CO}_3$  (98 atom%  $^{13}\text{C}$ , Sigma-Aldrich, St. Louis, Mo, USA) was dissolved in sterile MilliQ water (1g/ 50 mL). 1 ml was added to the incubations with a syringe (~3.5 atom% final in 4.5 L bottles). In order to investigate the contribution of heterotrophic vs. autotrophic diazotrophs to  $\text{N}_2$  fixation, glucose addition experiments were performed with  $^{13}\text{C}$ -labelled glucose (Sigma-Aldrich, St. Louis, Mo, USA), dissolved in MilliQ water ( $1.44 \text{ g L}^{-1}$ ), and the concentrated solution was added through the septum with a syringe to yield a final concentration of  $2 \mu\text{mol L}^{-1}$  glucose. Bottles from surface water were kept in a seawater-cooled on-deck Plexiglas incubators covered with blue light foil (blue-lagoon, Lee filters, Andover, Hampshire, UK) that mimics the ambient irradiance at around 10 m depth. Samples from the ODZ were stored at  $12^\circ\text{C}$  in the dark. After 24 hours of incubation, 0.7 – 2.5 L of seawater were filtered onto pre-combusted ( $450^\circ\text{C}$ , 5 hours) 25 mm diameter GF/F filters (Whatman, GE healthcare, Chalfont St Giles, UK) under gentle vacuum (-200 mbar). The filtrations were stopped after one hour since high particle load in surface waters often led to a clogging of the filters. Filters were oven dried ( $50^\circ\text{C}$ ) for 24 hours and stored over desiccant until analysis. Environmental samples of 2 L untreated seawater were filtered and prepared in the same way to serve as blanks for natural abundance. For isotope analysis, GF/F filters were acidified over fuming HCl overnight in a desiccator to remove inorganic C. Filters were then oven-dried for 2 hours at  $50^\circ\text{C}$  and pelletized in tin cups. Samples for particulate organic carbon and nitrogen (POC and PON) and isotopic composition were analyzed on an Elemental Analyzer Flash EA 1112 series (Thermo Fisher, Waltham, Massachusetts, USA) coupled to a mass spectrometer (Finnigan Delta Plus XP, Thermo Fisher, Waltham, Massachusetts, USA). Measurements were calibrated using reference gases between each sample and caffeine every 6 samples. A table of  $\text{N}_2$  and C fixation rate measurements is given in the supplementary material.

Possible correlations between environmental parameters and  $\text{N}_2$  fixation rates were explored by principal component analysis (PCA) based on 58 cases. Computations were performed in PAST version 3.07 (Hammer et al., 2001). Metadatasets for M90 and M91 were deposited at PANGAEA (doi:10.1594/PANGAEA.830245, doi:10.1594/PANGAEA.857751, doi:10.1594/PANGAEA.817193, doi:10.1594/PANGAEA.817174).

## 2.4 Molecular methods



For molecular analysis, nucleic acid samples were collected by filtering up to 1 L of seawater (exact volumes were recorded and the filtration time was shorter than 20 min) onto polycarbonate membrane filters with a pore size of 0.2  $\mu\text{m}$  (Millipore, Darmstadt, Germany). Immediately after collection, samples were flash frozen in liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$  until extraction. Nucleic acids were extracted using DNA/RNA AllPrep Kit (Qiagen, Hildesheim, Germany) with minor changes in the protocol (Löscher et al., 2014).

For cDNA library construction, residual DNA was removed from the purified RNA by a DNase I treatment (Life Technologies, Carlsbad, CA, USA). The extracted RNA was gene specifically reverse transcribed to cDNA using the Superscript III First Strand synthesis Kit (Life Technologies, Carlsbad, CA, USA) following the manufacturers' protocol and *nifH* cluster specific no-template qPCRs were performed to assure the purity of RNA. Quantitative PCRs were performed with cDNA as described, before (Löscher et al., 2014); however, a ViiA7 qPCR system (Life Technologies, Carlsbad, CA, USA) was used and the reaction volume was reduced to 12.5  $\mu\text{l}$ . The detection limit of the qPCRs was deducted from non-template controls. No amplification was detected after 45 cycles, setting the theoretical detection limit to one copy  $\text{L}^{-1}$ . As the detection limit depends on the sample and elution volumes, we calculated a detection limit of 40 copies  $\text{L}^{-1}$ . For *nifH* transcript diversity analysis, a PCR based amplification of the *nifH* gene was performed followed by Topo TA cloning and sequencing using established protocols (Lam et al., 2007; Langlois et al., 2005). Sequences were submitted to GenBank (accession numbers: KX090448-KX090515).

Phylogenetic analysis of *nifH* transcripts was conducted using a Muscle alignment on a 321 bp fragment with the Mega 6.0 package (Tamura et al., 2013), sequence differences were set at a minimum of 5%, neighbor joining trees were constructed as previously described (Löscher et al., 2014).

### 3 Results and Discussion

The investigated eddies originated from the shelf-slope region of the Peru margin (however, the exact origin of eddy B could not be determined). While eddy A remained close to the coast during the two months period, eddies B and C propagated further offshore. Key hydrographical properties are specified below but an extensive description can be found in Stramma et al. (2013).

**Eddy A**, a nearshore mode water eddy, showed a pronounced  $\text{O}_2$  minimum from 100 m downwards with lowest concentrations close to the detection limit of the Winkler method ( $\sim 2\text{ }\mu\text{mol O}_2\text{ kg}^{-1}$ ). The influence of the coastal upwelling was visible from the lifting of the upper isopycnal towards the shore (Arévalo-Martínez et al., 2015). Below the oxycline at  $\sim 100$  m depth, nutrient concentrations were generally higher in the ODZ relative to surface waters. While  $\text{PO}_4^{3-}$  concentrations were not

considerably different in the eddy compared to surrounding waters,  $\text{NO}_3^-$  concentrations showed a pronounced decrease in the ODZ of eddy A compared to surrounding waters (see figure S1 for individual sections through eddies A, B and C). This decrease correlated with an increase in  $\text{NO}_2^-$  concentrations at the same depth ( $r^2 = 0.76$ ,  $n = 52$  below the oxycline). A comparison to biogenic  $\text{N}_2$  as indicator for active or past N loss processes showed a maximum along with the  $\text{NO}_2^-$  maximum thus supporting the view of ongoing N loss in eddy A (Fig. 2, see Bourbonnais et al. (2015) for details on N loss processes in eddy A). As a result of this N loss, we observed large values for excess  $\text{P}^*$ , which is classically considered to promote  $\text{N}_2$  fixation in surface waters (Karl et al., 2002).

Eddy A, which was estimated to exist for two months at the time of the first survey, was sampled again one month later; the first observation is further referred to as “eddy A1”, the second survey is referred to as “eddy A2”. Since the first observation, a decrease in  $\text{O}_2$  (table 1) and  $\text{NO}_3^-$  (Arévalo-Martínez et al., 2015; Stramma et al., 2013) has been observed indicating ongoing respiration and N loss. These signals of enhanced ongoing N loss weakened over time as eddy A aged (Stramma et al., 2013). This may possibly be due to nutrient subduction via organic matter export out of the anticyclonic eddy (Omand et al., 2015). An extensive characterization of N loss signals in this eddy revealed a complete consumption of  $\text{NO}_3^-$  (Bourbonnais et al., 2015). Most intense N loss signals were observed near the core of eddy A, where the ODZ is in direct contact with the euphotic zone via uplifting of isopycnals (Bourbonnais et al., 2015) thus supporting the impact of freshly produced organic matter on N loss (Babbin et al., 2014).

**Eddy B**, an offshore mode water eddy, was characterized by slightly deeper oxycline and nutriclines compared to eddy A (at ~200 m water depth). Although less pronounced than in eddy A,  $\text{NO}_3^-$  concentrations decreased within the ODZ in the eddy along with an increase in  $\text{NO}_2^-$  and biogenic  $\text{N}_2$  between 200 and 300 m depth, again indicating N loss.  $\text{P}^*$  was slightly higher in the  $\text{O}_2$  depleted core waters, however, to a lesser extent and slightly deeper compared to eddy A. Stramma et al. (2013) observed weaker signals for N loss in eddy B, which were also mirrored by lower  $\text{N}_2\text{O}$  production (Arévalo-Martínez et al., 2015). This weakening may result from less organic matter export into the core of the eddy (Fig. 2).

**Eddy C**, was the investigated offshore cyclonic eddy (Fig. 2) (Stramma et al., 2013).  $\text{NO}_3^-$  did not show the same pronounced decrease in the core of the eddy as detected in eddies A and B, but  $\text{NO}_2^-$  and biogenic  $\text{N}_2$  were found slightly enriched between 200 and 300 m water depth, possibly from the onset of N loss at this location or a left-over signal from enhanced N loss within the coastal upwelling as previously described for this region (Kalvelage et al., 2013), and confirmed by the excess of P compared to N in its core waters. A coastal origin of eddy C has been described; however, compared to the mode water eddies A and B, eddy C moved westward without staying in the shelf/slope region (Stramma et al., 2013), which may be one reason for the lower N loss signals.

### 3.2 Patterns of N<sub>2</sub> and C fixation in the three eddies

N<sub>2</sub> fixation was strongly associated with intermediate waters of the mode water eddies A and B, while the cyclonic eddy C showed maximum rates of N<sub>2</sub> fixation in surface waters but no detectable N<sub>2</sub> fixation in the O<sub>2</sub> depleted core waters.

In eddy A1, intense N<sub>2</sub> fixation was detected between 200 and 350 m water depth in the eddy center with maximum rates of 4.4 nmol N L<sup>-1</sup> d<sup>-1</sup> at 250 m depth (Fig. 3). At the same depth, carbon fixation was the highest reaching 0.51 μmol C L<sup>-1</sup> d<sup>-1</sup>, coinciding with elevated N<sub>2</sub> fixation. High carbon fixation associated to the center of eddy A extended, however, deeper down to 400 m. High carbon fixation in the absence of light (compare chl a data in Stramma et al., 2013) is likely attributed to dark carbon fixation as previously described to take place in this and other OMZs (Schunck et al., 2013, Taylor et al., 2001). Similarly, eddy B showed high rates of N<sub>2</sub> fixation in its center with maxima of 1.89 nmol N L<sup>-1</sup> d<sup>-1</sup> at 350 m depth. In eddy B, the maximum in carbon fixation was exclusively present above the upper isopycnal (between 100 and 200 m, Stramma et al., 2013), and thus not in direct contact with the N<sub>2</sub> fixation zone. However, a smaller peak could be observed at ~380 m depth. Maximum N<sub>2</sub> fixation rates in eddy C (0.51- 1.48 nmol N L<sup>-1</sup> d<sup>-1</sup>) were detected in surface waters (Table S1). Carbon fixation in eddy C was lower compared to eddy A and eddy B, however also mostly present towards the rim, while close to the detection limit in the center.

Compared to previous studies in this area, N<sub>2</sub> fixation rates for eddies A and B are generally 1-2 orders of magnitude higher (e.g. DeKaemacker et al. (2013): 0.01-0.88 nmol N L<sup>-1</sup> d<sup>-1</sup>; Löscher et al. (2014): 0.01- 0.4 nmol N L<sup>-1</sup> d<sup>-1</sup>); here, it must be noted that we used the improved method by Mohr et al. (2010) which allows us to present here the first quantitative rates of N<sub>2</sub> fixation in this area, while previous studies may have underestimated N<sub>2</sub> fixation rates (Grosskopf et al., 2012). An aging effect was mirrored by a decrease in N<sub>2</sub> and C fixation below 200m in the center of Eddy A when comparing the measurements from eddy A1 sampled in November 2012 with eddy A2 surveyed a month later in December 2012. C fixation rates increased towards the eddy edge. This may be attributed to biological consumption or export of nutrients needed for biological activities within the eddy that are still available due to lower consumption or diffusion through the rim.

Observations of higher N<sub>2</sub> fixation rates in accordance with our dataset suggest an overall stimulation of N<sub>2</sub> fixation associated with anticyclonic mode water eddies (Fong et al., 2008). N<sub>2</sub> fixation rates of 8.6 nmol N L<sup>-1</sup> d<sup>-1</sup> have been measured in surface waters of an eddy in the oligotrophic North Pacific Ocean (Fong et al., 2008). N<sub>2</sub> fixation was only measured in surface water samples (5 m depth) by Fong et al. (2008), thus a direct comparison with N<sub>2</sub> fixation within the O<sub>2</sub>-depleted eddy core waters, as measured in this study, is not possible.

The occurrence of enhanced N<sub>2</sub> fixation associated with intermediate water depths is in accordance with our previous study from that region, where we detected a variety of non-cyanobacterial diazotrophs compared to relatively minor numbers of cyanobacterial diazotrophs related to

*Crocospaera* (Löscher et al., 2014). In order to characterize the expression of the key functional marker gene for N<sub>2</sub> fixation, *nifH*, we conducted a phylogenetic study on *nifH* diversity in the transcript pool. Similar to the previous study, most of the detected *nifH* transcripts were affiliated to non-cyanobacterial diazotrophs (P1-P8) with some cyanobacterial *Crocospaera*-related (UCYN-B) *nifH* sequences present at much lesser extent (Fig. 4, table S2). Quantification of *nifH* transcripts related to the detected clusters showed maximum abundances associated with the maxima in N<sub>2</sub> fixation in eddy A, B and C (Fig. 4). A potential for heterotrophic N<sub>2</sub> fixation was deduced from glucose fertilization experiments with water samples from the cores of eddies A and B. Here, glucose addition greatly enhanced N<sub>2</sub> fixation from  $0.86 \pm 0.1 \text{ nmol N L}^{-1} \text{ d}^{-1}$  to  $39.19 \pm 4.31 \text{ nmol N L}^{-1} \text{ d}^{-1}$  at 100 m depth in eddy A and from  $0.251 \pm 0.03 \text{ nmol N L}^{-1} \text{ d}^{-1}$  to  $62.18 \pm 1.9 \text{ nmol N L}^{-1} \text{ d}^{-1}$  at 125 m depth in eddy B, respectively. However, no increase in N<sub>2</sub> fixation by glucose addition could be achieved in eddy C (100 m, Fig. 5), which may result from different diazotrophic communities (i.e. cyanobacterial UCYN-B *nifH* sequences present). Therefore, the availability of reduced carbon compounds may essentially control N<sub>2</sub> fixation in modewater eddies. Assuming that organic matter export is limiting for N loss as previously suggested (Babbin et al., 2014; Ogawa et al., 2001; Bianchi et al., 2014) and that deep water N<sub>2</sub> fixation is a non-cyanobacterial (i.e., heterotrophic) process as shown by the diversity of the diazotrophs and the stimulation of N<sub>2</sub> fixation rates after glucose addition, the interplay between both may be even closer as previously thought.

### 3.3 Co-occurrence of N<sub>2</sub> fixation and N loss in mode water eddies

Largely consistent with the distribution of NO<sub>2</sub><sup>-</sup>, biogenic N<sub>2</sub> showed pronounced maxima below the mixed layer depth in eddies A and B, and a less pronounced maximum in eddy C. A similar distribution has been determined for P\* (Fig. 2). The consistency of those parameters indicates either ongoing N loss or its left-over signal as already reported for the upwelling off Peru (Kalvelage et al., 2013). In an earlier study from that region (i.e., Löscher et al., 2014), we found a close spatial coupling between N loss, or a relic signal as suggested by Kalvelage et al. (2013), and N<sub>2</sub> fixation for the same upwelling region off Peru. The strongest signals for both N<sub>2</sub> fixation and N loss were tightly linked to a coastal sulphidic plume (Schunck et al., 2013). The westward propagation of mesoscale eddies implies that properties of the waters which were “trapped” within its center at the time of formation are transported offshore (Chelton et al., 2007). Enhanced N<sub>2</sub> and C fixation rates, as well as high N deficit, as depicted by P\*, and biogenic N<sub>2</sub> concentrations in eddy B indeed suggest that this coupling can be transported far offshore.

A coupling between N loss and N<sub>2</sub> fixation is indicated by (i) the concurrent deepening of the maxima in N loss (i.e. maximum in biogenic N<sub>2</sub>) and N<sub>2</sub> fixation from the coast to the open ocean, (ii) the concurrent decrease in both biogenic N<sub>2</sub> concentrations and N<sub>2</sub> fixation rates over time and (iii) the co-occurrence between NO<sub>2</sub><sup>-</sup> (either resulting from NO<sub>3</sub><sup>-</sup> reduction or from remineralization of organic matter through NH<sub>4</sub><sup>+</sup> oxidation) and N<sub>2</sub> fixation rates, and between biogenic N<sub>2</sub> and N<sub>2</sub> fixation rates

(Fig. 2, 3). We observed lower biogenic  $N_2$  signals and  $N_2$  fixation in eddy B compared to eddy A1 with the maximum in  $N_2$  fixation located deeper (~350 m) in the water column, compared to eddy A1 (~250 m). While we detected enhanced carbon fixation in eddy A at the same depth as  $N_2$  fixation, this coupling was far less pronounced in eddy B. Still  $P^*$  was lower in eddy B compared to eddy A, which points towards a normalization of N:P ratio *via*  $N_2$  fixation.

Although, our results provide evidence for a coupling of  $N_2$  fixation and N loss, statistical analysis of our dataset did not confirm N loss as exclusive control on  $N_2$  fixation, but displays a dependence of  $N_2$  fixation on  $O_2$  and temperature, as well (Fig. 6). The dependency on  $O_2$  would explain the difference of  $N_2$  fixation between the mode-water eddies A and B, and the cyclonic eddy C, which was in its ODZ slightly less anoxic. Several studies suggested primary production to be limited by Fe availability in the upwelling system off Peru (Bruland et al., 2005; Hutchins and al., 2002; Messie and Chavez, 2015). Baker et al. (2015) report in their study from the same cruise series excess Fe supply (with respect to N supply) via atmospheric deposition to the southern part of this region (~15-16°S). As previous studies identified iron (Fe) to generally (co-)limit  $N_2$  fixation (Mills et al., 2004; Moore and Doney, 2007; Moore et al., 2009), atmospheric Fe sources may promote surface water  $N_2$  fixation, which may explain enhanced  $N_2$  fixation in surface waters of the eddies. Other studies emphasize the comparably higher importance of the benthic Fe source in these waters (Chever et al., 2015; Scholz et al., 2014), which may be particularly important in eddies A and B due to their longer residence time at the coast. Enhanced  $N_2$  fixation in the mode-water eddies A and B may, besides a coupling to N loss, be additionally promoted by Fe availability possibly from benthic sources.

#### 4. Conclusions

We conducted the first detailed survey of  $N_2$  fixation in three eddies off the coast of Peru in the ETSP. Our results demonstrated enhanced  $N_2$  fixation rates connected to two anticyclonic mode water eddies off Peru, while elevated  $N_2$  fixation was not observed in the cyclonic eddy.  $N_2$  fixation rates were highest in the ODZ of the two anticyclonic mode water eddies. This is in agreement with recent findings, which demonstrated that  $N_2$  fixation is not only present in oligotrophic surface waters but also widely distributed throughout the water column.  $N_2$  fixation co-occurred with N loss processes, which in combination with low  $O_2$  concentrations may largely explain the presence of  $N_2$  fixation in ODZ waters. Taken together, our results point towards an important role for eddies in supplying fixed N compounds to the open ocean via enhanced  $N_2$  fixation. Although our data do not allow quantification of the overall impact of eddies on  $N_2$  fixation in the ETSP off Peru, they clearly underscore the importance of high-resolution surveys for understanding the biogeochemistry of N cycle processes in eddies.

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## Tables

Table 1. Vertically integrated biogeochemical parameters of the three eddies in the ETSP during the M90 and M91 cruises. N<sub>2</sub> and C fixation rates as well as O<sub>2</sub> concentrations are expressed as integrated concentrations/abundances over the upper 500 m of the water column (data taken from the eddy centers). Chl *a* concentrations are taken from Stramma et al. (2013) and represent maximum concentrations in the subsurface maximum.

	A (M90)	A (M91)	B (M90)	C (M90)
N <sub>2</sub> fixation ( $\mu\text{mol N m}^{-2} \text{d}^{-1}$ )	628.7	490.8	245.0	150.6
C fixation ( $\text{mmol C m}^{-2} \text{d}^{-1}$ )	64.4	3.9	42.8	6.7
O <sub>2</sub> ( $\text{mol m}^{-2}$ )	37.5	27.6	37.7	45.2
chl <i>a</i> max. ( $\mu\text{g L}^{-1}$ )	6.1	2.5	2.5	2.8

## Figures

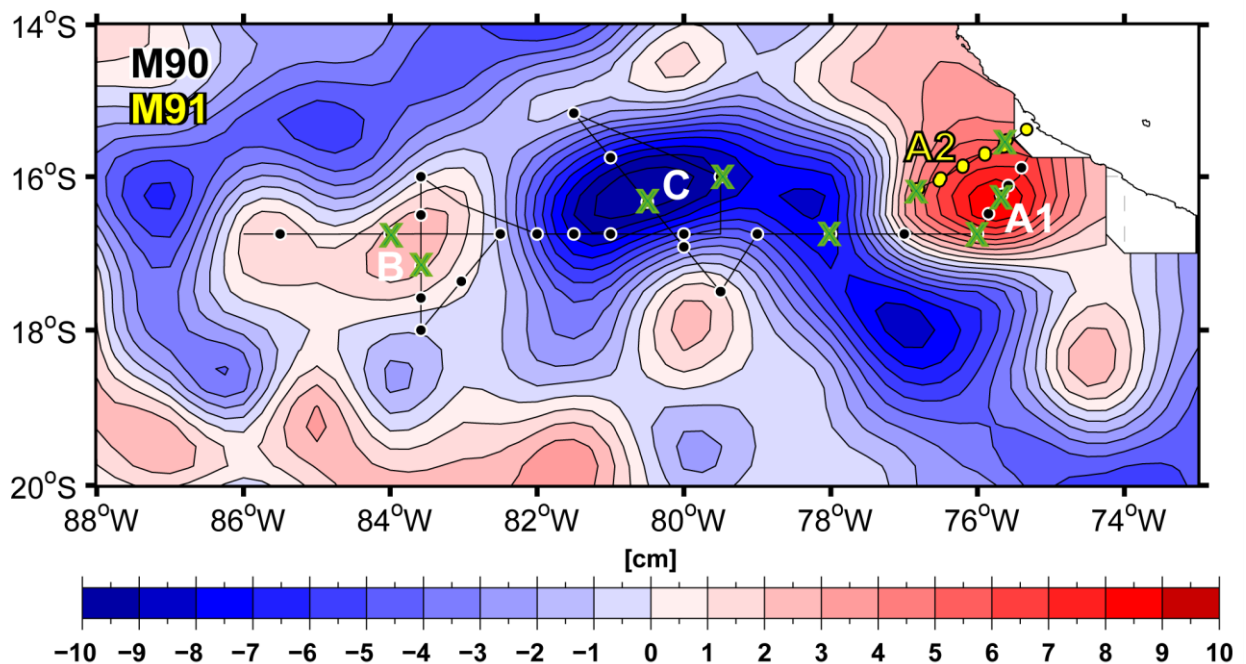


Figure 1: Distribution of Aviso satellite-derived sea surface height anomaly (SSHA) distribution as described by Stramma et al. (2013) on 21 November 2012. Eddies are labelled in white (A and B denote the coastal and the open ocean mode water eddies, respectively; C denotes the cyclonic eddy). The cruise track from the M90 cruise is shown in black, CTD-bottle stations are indicated with black dots, green crosses denote stations sampled for N<sub>2</sub> fixation. The cross section through the aged coastal mode water eddy during the consecutive cruise M91 is denoted with yellow dots (A2).

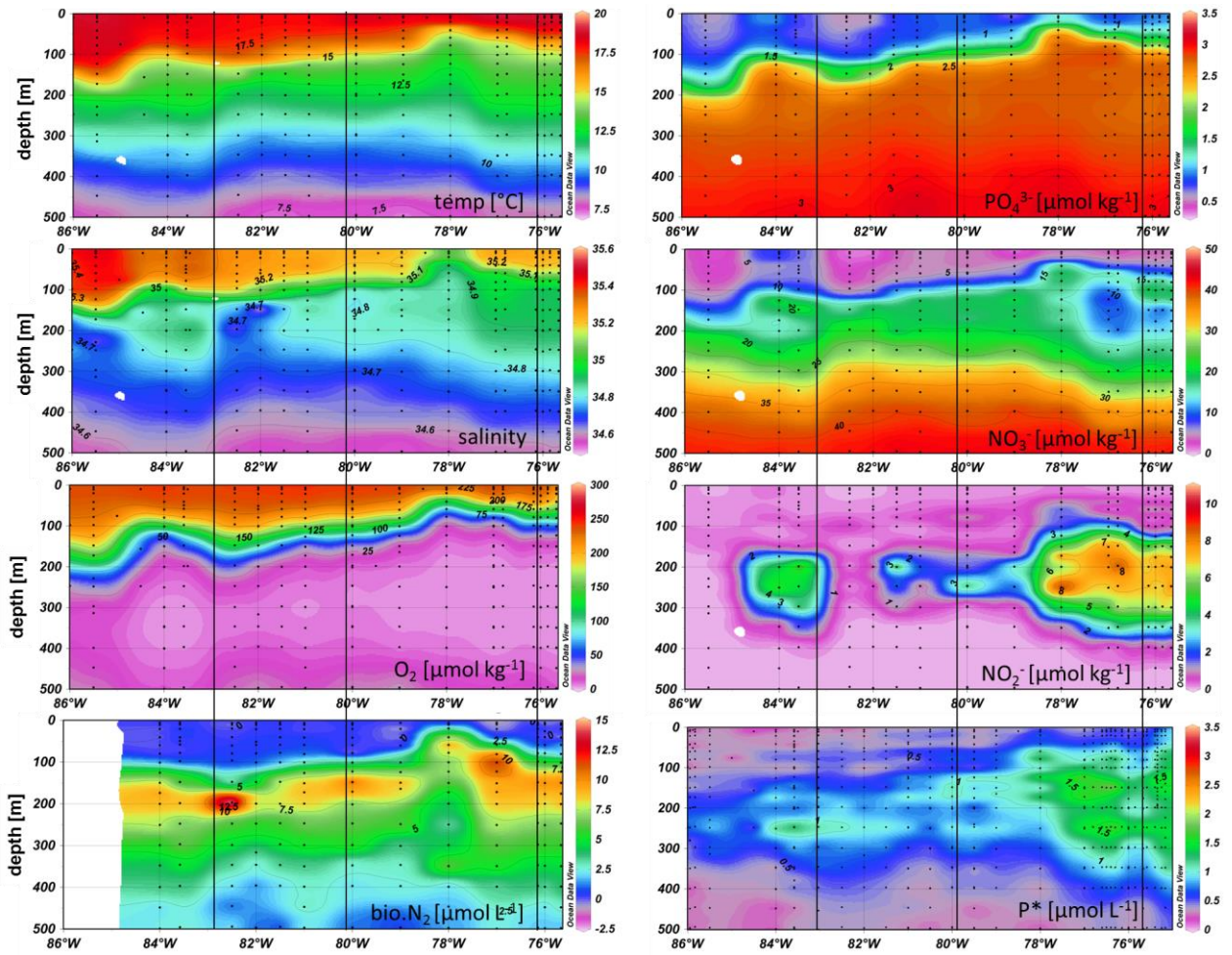


Figure 2: Temperature, salinity and oxygen, phosphate, nitrate, nitrite, biogenic N<sub>2</sub> and P\* for eddies A, B and C along a cross section at 16°45'S during the M90 cruise are shown. The black lines indicate the eddy centers at ~76°W (eddy A), ~80.1 °W (eddy C) and ~83.3°W (eddy B).

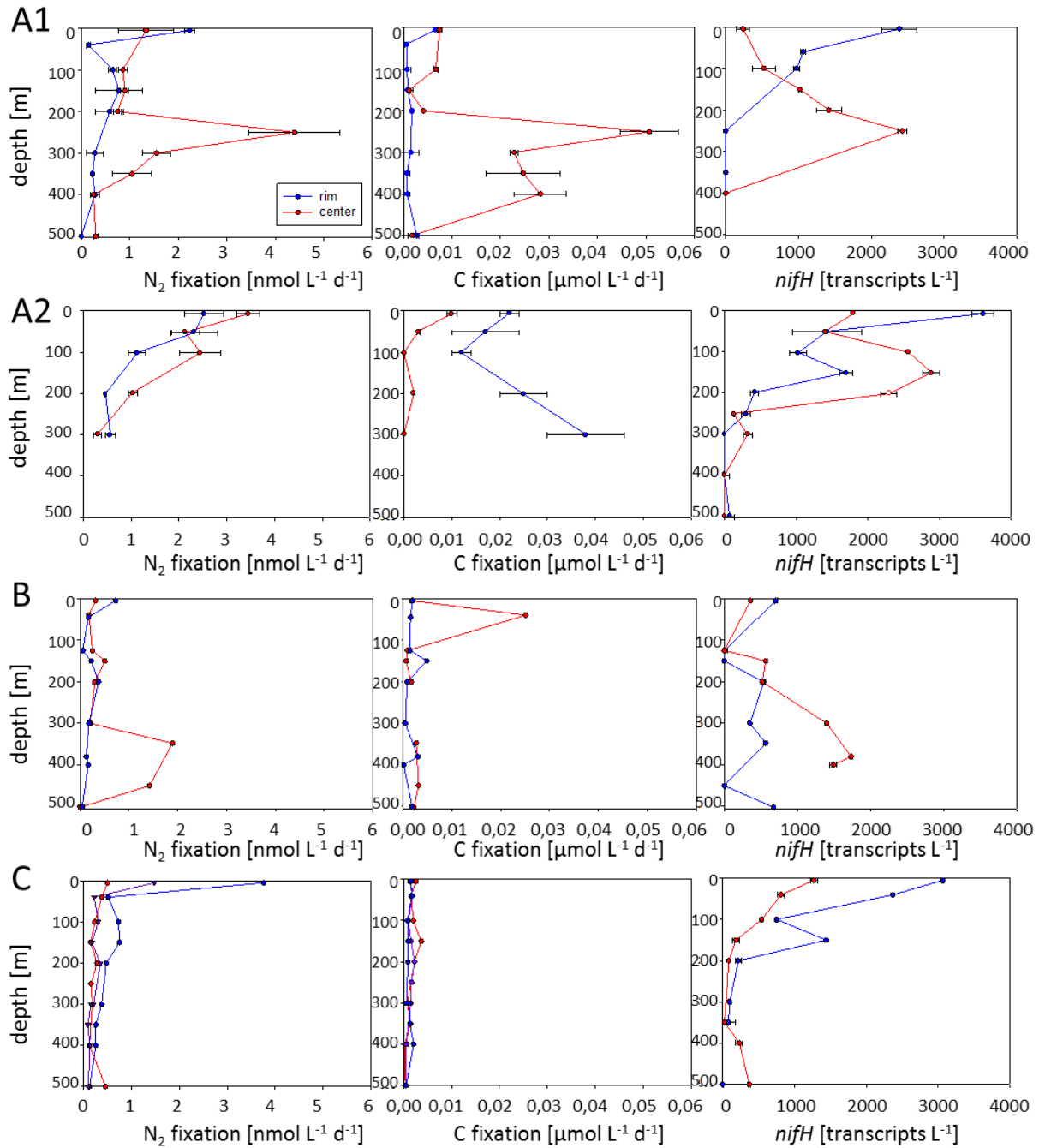


Figure 3: Vertical distributions of N<sub>2</sub> and C fixation rates and *nifH* transcript abundance (sum of detected clusters P2, P7 and *Crocospaera*-like diazotrophs as quantified by qPCR) in eddy A1 (M90), eddy A2 (M91), eddy B and eddy C.

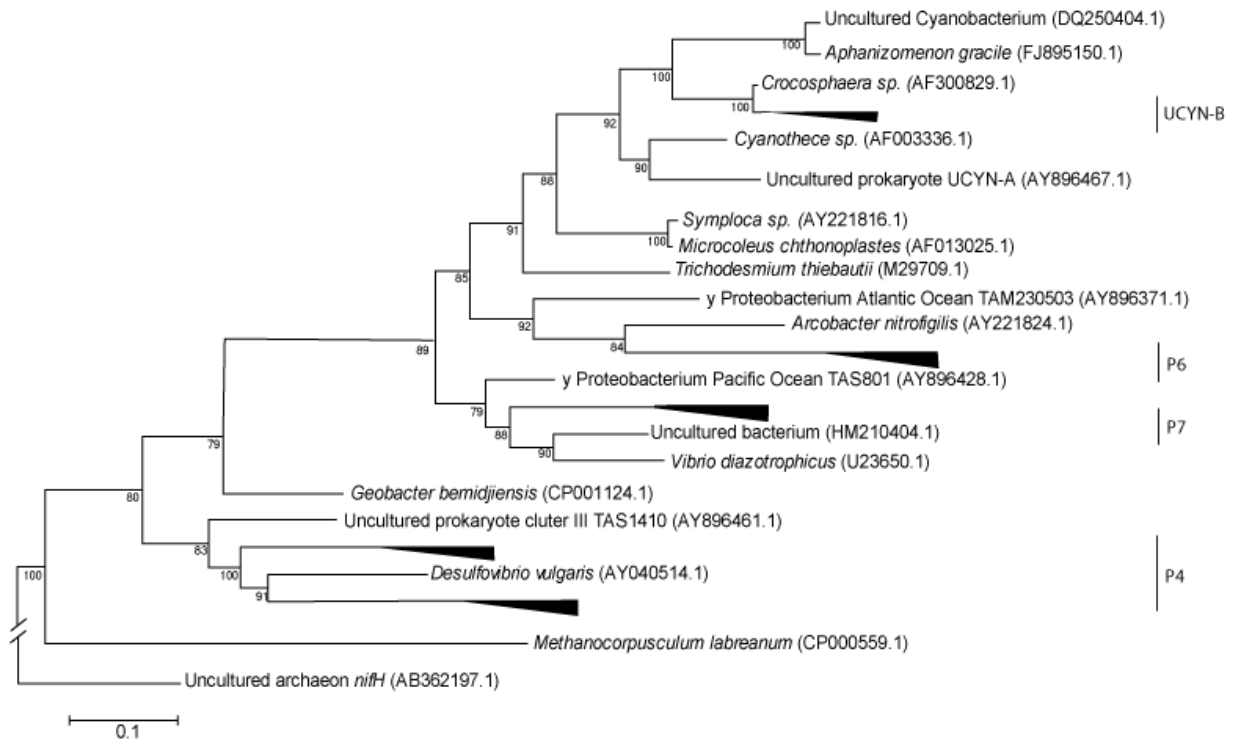


Figure 4: Phylogenetic diversity in *nifH* cDNA libraries, black triangles denote detected clusters present in samples from M90 and M91. The tree was constructed from a ClustalW alignment as a neighbor joining tree, bootstrap values are given (% of 1000 bootstraps) below branches, P4, P6 and P7 are clusters previously identified in that region (Löscher et al., 2014).

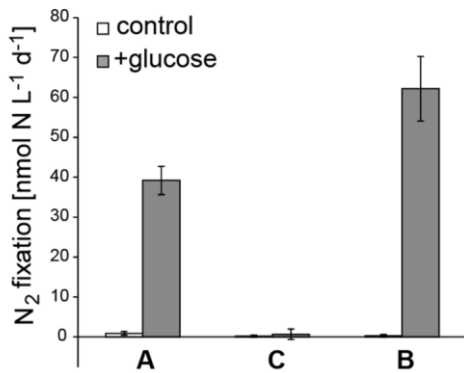


Figure 5: N<sub>2</sub> fixation in response to glucose fertilization experiment performed in eddy A1 (100 m), B (125 m) and C (100 m); samples were derived from the eddy center stations water depth.

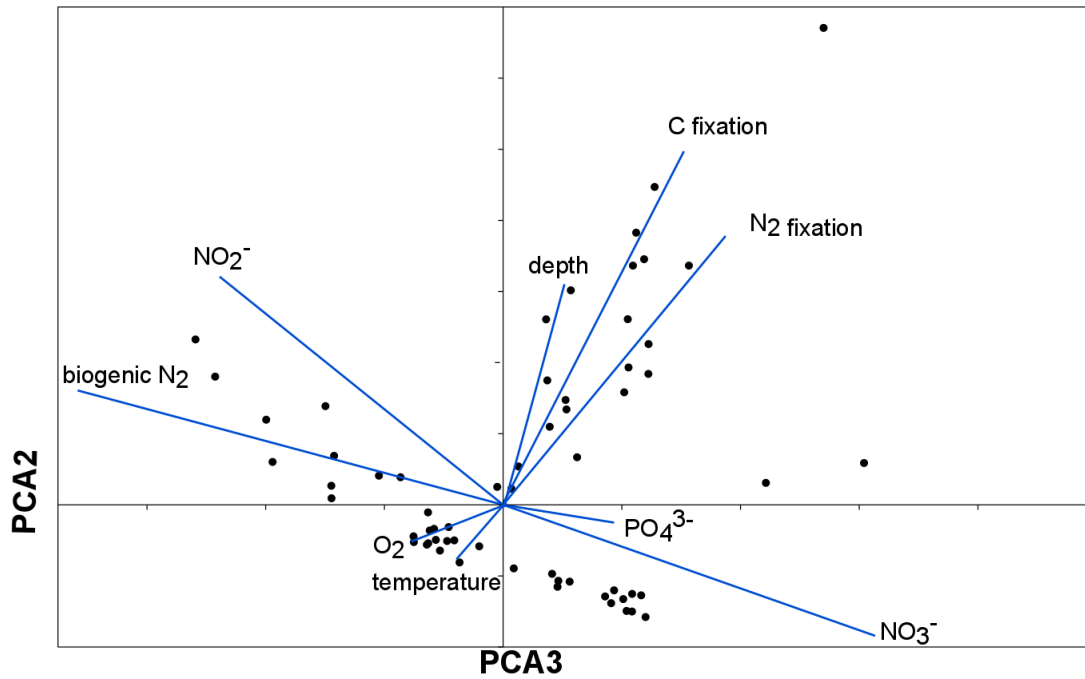


Figure 6: Principal component analysis correlation biplot shows relations between N<sub>2</sub> fixation and environmental variables. Strongest negative correlations are present between N<sub>2</sub> fixation and O<sub>2</sub> and N<sub>2</sub> fixation and temperature. N<sub>2</sub> fixation is positively correlated with C fixation as indicated by the direction of vectors. Black dots denote single samples (n=58).