We thank Dr Silvio Pantoja for his constructive criticism and valuable comments. In the following we address the points brought up, with editor comments in boldface and author responses in normal typeface.

1) Reviewer 1. "The authors report the N:P but not with P relative to one. I think this is confusing.. For example, page 10002, lines 14- 16. N:P 6.35/1.10, 12.00:1.25. It would be better if this was written as: 5.77 and 9.6 respectively and details of the concentration be inserted into a table, for example."

Response. "We prefer to report the N:P ratios as they are. That way, mesocosms with the same initial concentrations of N or P (center points) are easier to distinguish for the reader."

I agree with reviewer. We are not interested in a mesoscosm result by itself but inferences about ocean´s response, and for that it is easier for readers to have ratios relative to one since this article is based on deviations of canonical N:P. Please change accordingly.

We have changed N:P ratios in the text as suggested (e.g. lines 249-253). However, we cannot change the mesocosm treatment ID's as suggested. Due to our experimental design, there is more than one mesocosm in *varied N* and *varied P* with N:P ratios of 16:1 (see Table 1), which would be indistinguishable from each other if we only reported the N:P ratio relative to one.

2) "9. The authors switch between N and P and NO30 and PO43-. This needs to be fixed and made consistent throughout."

Response: We made this consistent throughout the manuscript."

I need to ask you to use proper nomenclature throughout the text. For general statements such as the ones in lines 47-52 of v2, it could be fine to use N or P, but regarding your results, you must refer to what you are experimenting with (nitrate or NO3-), but not N since nitrogen (N) involves a variety of chemical species you have not studied. One example is shown in conclusions in lines 328-329 of version 2 ("…ability of the plankton community to rapidly and intensively react to N availability. These results indicate that the ultimate limiting nutrient for phytoplankton production in our experiment was N.").

We have made the appropriate changes to the manuscript and figures.

3) Line 402 v2. Replace "measured" with "detected". It makes a more clear argument.

We changed this as suggested.

4) Reviewer 2. "Given the extensive measurements of the P pools (see e.g. Figure 10), it would have been useful to see an attempt at mass balance.

We addressed this issue by data presented in Fig 10. Mass balances were not subject of this manuscript but will be addressed in a follow up study. More extensive presentation of mass balances would be beyond the scope of this study."

Reviewer is referring to mass balance as part of the method. It cannot be "beyond the scope of this study" rather, how balance or lack of balance affects your interpretation.

We included a mass balance in version 2 of the submitted manuscript (see lines 287-291).

5) Why nitrate plus nitrite increase during the first day?

There is an increase because of our artificial nutrient addition between day 1 and 2.

6) Please write nitrate or NO3- (using subscripts and superscripts) throughout text and figures. Same format for nitrite, silicic acid.

We changed this accordingly.

We thank anonymous Referee #1 for his/her constructive criticism and valuable comments. In the following we address the points brought up, with referee comments in boldface and author responses in normal typeface.

1. The motivation for this study, ocean deoxygenation due to climate change, and thus reduction of the N:P ratio, is not the only process that will alter then N:P ratio in the ocean. Addition of anthropogenic nitrogen (e.g. see Kim et al 2014), as the potential to perturb the system by adding N in excess of P, thus intensifying or expanding phosphate limited regions and thus force the system the other way. They authors should really alter their motivation to cover both sides of the story here.
We agree that ocean deoxygenation is not the only process that will likely alter N:P ratios is the ocean. We clarified this in the introduction. However, in the eastern tropical North Atlantic (ETNA) N_2 fixation is supposed to be the dominant process for the input of new N over the next decades to centuries (Duce et al. 2008) compared to the North Pacific Ocean (Kim et al. 2014). Moreover, it is debatable how much of the atmospheric anthropogenic nitrogen input is affecting the open ocean of the ETNA. If the input is mostly restricted to the costal upwelling region, biological production would be fueled, export enhanced and degradation of organic matter at depth would increase. The oxygen inventory of the ETNA OMZ would shrink further, thereby enhancing N loss processes, leading to a decrease of N:P ratios in the water column. The fertilization with anthropogenic N would thus be compensated by a negative feedback cycle. In any case, the significance of atmospheric anthropogenic N inputs into the ETNA is unclear. The expansion of the oxygen minimum zone in the ETNA, on the other hand, has been ongoing for the past decades and is expected to continue in the future (Stramma et al. 2009). As the original goal and motivation for this experiment was to study changes in the N:P ratio due to ocean deoxygenation in the ETNA, we focused on the description of our experiment in this context.

2. The ability of nitrogen fixation to modify primary production is likely to be small. If you multiple nitrogen fixation rates by a C:N ratio of ~ 6, then compare the carbon fixed by diazotrophs to total carbon fixed, it is quite small. Instead, the switch to DDAs will impact carbon export, which is the potentially important here.
We agree and thank for this valuable input. The export of carbon might indeed be influenced by a shift of the diazotrophic community towards DDAs and included this point into the discussion. We interpret from our data that primary production might be affected by a shift within the diazotrophic community, especially in the oligotrophic open ocean of the ETNA, where diazotrophs are the dominant primary producers. We clarified this point in the manuscript.

3. The author needs to be clear when they refer to P limitation. It is likely phosphate limitation and not phosphorus limitation considering the ability of organisms to access DOP. Please be more explicit about this in the manuscript.
We agree with the referee and made the appropriate changes in the manuscript.

4. You argue that there is low O2 and N:P ratios (not levels as you have stated) below the MLD in mode water eddies. How typical do you think this is? Is this a wide spread occurrence and if so, how many mode water eddies exist in the Atlantic. It is not clear if this is just a local feature/unique phenomenon or widespread.
We changed “(…) with an accompanied decrease in N:P levels (...)“ to “(…) with an accompanied decrease in N:P ratios (...)“ (p. 9994, line 5-6).
Mode water eddies with very low oxygen concentrations were only recently discovered in the ETNA. These eddies can last several month, transporting shelf water signals offshore (Karstensen et al. 2015). In the ETNA, 5 such events were observed between 2007 and 2012 (Karstensen et al. 2015). We believe that the effect of mode water eddies is not basin-wide but rather of local importance. We clarified this in the manuscript.

5. What measures were taken to prevent contamination of the sample with either nutrients or trace metals? Was the nutrient or iron concentration at the oceanic sample collection site monitored between collection and use in the mesocoms?
Containers for water transport were first rinsed with diluted HCL and several times with deionized water. Nutrients in all mesocosms were measured before nutrient manipulation. NO_3^- and NO_2^-, PO_4^{3-} and $Si(OH)_4$ were all below the detection limit and far below the manipulation levels (see Fig. 2). We therefore conclude that no contamination with these nutrients occurred during water sampling, transport and mesocosm filling. This information was added to the manuscript.

6. Nutrients: considering you are reporting the change in nutrient concentrations over time and be- tween treatments, you should really report the precision of analysis as well as limits of detection in section 2.2. Also, the instrument and methods are described for DOP (section 2.4) but not nutrients. This needs to be fixed to be consistent. Again, what was the precision and limits of detection for DOP? This should be reported.
The precision of analysis and detection limits for nutrient and DOP analysis was added. The paragraph describing the nutrient measurements was changed as follows: "Samples (10 mL) for dissolved inorganic nutrients (NO−, NO−, PO3−, Si(OH)4) were taken daily from each mesocosm and measured directly using a QuAAtro Autoanalyzer according to Grasshoff et al. (1999). The detection limit of nutrient analyses were 0.01 µmol L^{-1} for NO_2^- and PO_4^{3-} and 0.03 µmol L^{-1} for NO_3^-."

7. In section 2.7, what was the atom percent enrichment of the 15N2 water added to the incubations and the final atom percent enrichment at the start of the incubations? This should be reported here.
The preparation of the $^{15}N_2$-enriched seawater was performed as described in Mohr et al. (2010). Degassed seawater was filled into evacuated gas-tight 3L Tedlar® bags without a headspace. Addition of $^{15}N_2$ gas was (depending on the exact water volume in the Tedlar bag) around 10 ml $^{15}N_2$ per 1 L seawater. Dissolution of the $^{15}N_2$ gas was achieved by 'slapping' the bubble with a ruler. After complete dissolution of the added $^{15}N_2$ gas ($^{15}N_2$-enriched seawater), an aliquot of the $^{15}N_2$ enriched water was collected for each preparation of enriched seawater and stored in an Exetainer, the isotopic composition was measured by membrane-inlet mass spectrometry. The $^{15}N_2$ concentration in the prepared batches of enriched water was determined to be 250 µmol L^{-1}, which translates in an ^{15}N-enrichment of about 2 % in the 4.5 L bottle incubations, when adding 100 mL enriched seawater (depending on temperature and salinity). These details were added to the methods section.

8. Section 2.8: Model selection. Why did you use a model here? What was the goal? This needs to be stated. Was it necessary? Also, the model description is quite confusing. What is gam and gamm? What is Akaike Information Criterion? This section needs to be edited to clarify the goal of the model and perhaps reduce the detail here and refer to other manuscripts where this sort of analysis has been done already. As a follow up on this, on getting to the end of this manuscript, I do not believe the modelling component adds any real value to this manuscript. The study is data rich and there are plenty of interesting and important points to make without including the model.
As both referees state that they don't see the added value of the model we introduce in the manuscript, we decided to remove it from our manuscript. Instead, we show the original transcript data obtained from the study (new Fig. 8) in order to avoid confusion and make the manuscript more intelligible to the reader (see also response to referee 2).

9. The authors switch between N and P and NO30 and PO43-. This needs to be fixed and made consistent throughout.
We made this consistent throughout the manuscript.

10. The authors report the N:P but not with P relative to one. I think this is confusing. For example, page 10002, lines 14- 16. N:P 6.35/1.10, 12.00:1.25. It would be better if this was written as: 5.77 and 9.6 respectively and details of the concentration be inserted into a table, for example.
We prefer to report the N:P ratios as they are. That way, mesocosms with the same initial concentrations of N or P (center points) are easier to distinguish for the reader.

11. I suggest changing the use of the word 'build up' to 'accumulation'. Also avoid using words like rise and drop, should be increase and decrease respectively.
We agree and made the appropriate changes in the manuscript.

12. Note that similar observations of the C:N:P ratio for POM were observed by Davis et al 2014 in GRL.
Thank you for this valuable input! We will add the suggested reference and the observations of this study to our discussion.

13. I suggest reducing the precision on ratios reported, e.g. change 38.8 to 39 and 21.9 to 22. The decimal places don't add value here.
We removed the decimal places as suggested.

We thank anonymous Referee #2 for his/her constructive criticism and valuable comments. In the following we address the points brought up, with referee comments in boldface and author responses in normal typeface.

In various places throughout the manuscript (e.g. Page 9993, line 26, Page 994, line 11 etc.) the authors refer to limitation when making inferences on the basis of ratios of available or supplied inorganic nutrients. Actually their own experiments suggest that this link is far from straight forward and I would encourage them to clarify where possible, maybe stating that the dissolved ratios indicate the 'potential for one nutrient to becoming limiting before the other' or sticking to the use of terms like 'deficiency', 'deficit', 'excess' etc. (see e.g. Page 9995, line 1).
We agree and revised the manuscript as recommended. For example, the sentence on Page 9993, line 18 was changed as follows: „Nevertheless, the nitracline tends to be deeper than the phosphocline in the ETNA (Hauss et al., 2013; Sandel et al., 2015), which also points towards a deficiency of N over P in the euphotic zone.

Experimental methods and statistical analysis need to be further described in places. In particular, although clear through consulting Table S1, the number of replicate mesocosms for individual treatments should be more clearly indicated to the reader, e.g. through stating in the text on Page 9997. Additionally, on Page 10001 the authors introduce a complex statistical model for the interpretation of the data without providing any justification for why this was required or chosen. Overall I was not sure why the statistical model was required as it appeared to largely be used just for the analysis of the nifH gene/transcript data and it wasn't clear that it added much to the interpretation of this data. Additionally it wasn't clear to me whether the analysis presented in Figure S1 was based on the GLM modelling performed or simple correlation analysis? Additionally, why is Figure S1 in supplementary rather than within main body of manuscript?
We added information about treatment replicates to the Material and Method section as follows: „In the first experiment, the P supply was changed at constant N supply (*varied P*) in thirteen of the sixteen units, while in the second experiment the N supply was changed at constant P supply (*varied N*) in twelve of the sixteen units. Each of these nutrient treatments was replicated 3 times. In addition to this, "cornerpoints" were chosen, where both the N and P supply was changed. The „cornerpoints" were not replicated."

Due to the fact that both referees don't see the added benefit in introducing a model to interpret our data, we decided to remove the model from our study and instead show the original transcript data (please also see the comment to referee 1).

Although an entirely feasible explanation, I think any potential causal link between the accumulation/availability of DOP and enhanced N2 fixation needs to be treated with caution on the basis of the data presented and experiment(s) performed. e.g. Page 10005, lines 11-20, an alternative interpretation might be that both the accumulation of DOP and the enhancement of N2 fixation are occurring within the 'varied P' experiments independently simply as a result of the addition of inorganic P. The authors may argue that the time series of DOP, P, POP, N2 fixation might argue against this (e.g. Figure 10), but given only 2 sampling time points for N2 fixation I would argue this remains equivocal. I would suggest the authors may simply wish to acknowledge this potential caveat.
We agree that the existence of only two sampling points for N_2 fixation has to be emphasized more when interpreting and discussing our data set. The text now reads:

"In our experiments a significant increase in N_2 fixation rates was only measured in *varied P*. In mesocosms with highest N_2 fixation rates, DIP was depleted after day 5 or 6 while POP increased until the end of the experiment. After DIP depletion, DOP concentrations declined, which indicates that DOP served as P source until the end of the

experiment. It has to be noted that N_2 fixation rates were only measured at the beginning and the end of our experiment and possible fluctuations over time cannot be accounted for. However, increasing diazotrophic transcript abundances of *Richelia intracellularis* in symbiosis with the diatom *Rhizosolenia* (Het I) were also detected over the course of the *variable P* experiment. While the diatom abundance was probably favored by replete amounts of silicate added at the beginning of the experiment, no increase in diatom-diazotroph associations (DDAs) was detected in the *varied N* experiment. Measured N_2 fixation rates and transcript abundances leads us to speculate that DDAs were favored in the *varied P* experiment, where diazotrophs in the mesocosms utilized DOP resources in order to supply P to themselves and/or their symbiont."

Concerning the alternative explanation suggested by the referee, we do not believe that N_2 fixation was solely enhanced by the addition of inorganic P in our experiment, since N_2 fixation was not measured in all treatments in *varied P*, but only in those treatments were inorganic P was depleted after a couple of days and DOP served as an alternative P source.

Given the extensive measurements of the P pools (see e.g. Figure 10), it would have been useful to see an attempt at mass balance.
We addressed this issue by data presented in Fig 10. Mass balances were not subject of this manuscript but will be addressed in a follow up study. More extensive presentation of mass balances would be beyond the scope of this study.

Page 9995, line 26: '. . .are regarded as key factors. . .'
This was changed.

Page 10004, line 11-15: This text does not appear to be fully consistent with the content of Figure 8? i.e. nifH Fil do not appear to be dominant for either experiment in this figure?
Due to the removal of the model from the manuscript (see comment above), Fig. 8 and 9 were dismissed. A new Fig. 8 was added to show the original transcript data. We ensured that the text describes the figure appropriately.

Page 10006, line 12 (and elsewhere): it is worth noting that the POC, PON, POP data reported will not just reflect that of 'primary producers' but actually will represent average values for the whole microbial community.
We agree and made the appropriate changes. For example, the sentences on Page 10006 now reads: "There is a large difference between the supply ratio of inorganic nutrients and the PON:POP ratio of the plankton community in our study."

Page 10008, line 24: The authors could be more specific here. They are specifically discussing excess inorganic P. Related, the authors should use the more specific term DIP to refer to dissolved inorganic P when appropriate throughout (compare Page 9999 line 13 with Page 10008, line 24).
As already stated in the response to referee 1, we made the use of DIP, PO_4^{3-} and P consistent throughout the manuscript in order to avoid confusion.

Page 10009, line 9: do the authors mean P* here? i.e. DIP – DIN/16 or some similar definition c.f. Deutsch et al. 2007? If so I don't think the term has been defined to this point in the manuscript.
Yes, we refer to P* as described by Deutsch et al, 2007. The term P* has been introduced in the Introduction, Page 9993, line 16.

Page 10009, line 29: '. . .locally prior to offshore transport.'
This has been corrected.

A number of the figure captions (and associated statistics) require work and/or better description and figures could be clarified in places:

Figure 1: please explain error bars (standard deviations? Standard errors?)
The figure caption now reads: “Experimental design and initial nutrient supply conditions during *varied P* (blue circles) and *varied N* (red diamonds). “Cornerpoints” during *varied P* and *varied N* are depicted as grey circles and white diamonds, respectively. Error bars denote the standard deviation of replicated (n=3) treatments.

Figure 2: shaded areas were a bit difficult to make out
We decreased the transparency of the shaded areas to make them better visible.

Figures 3, 4 & 6: error bars for data points need explanation, regression lines also need to be described in caption. Also were the fits model I or model II type regressions?
The figure captions now read:
“Figure 3: Maximum POC, PON and POP build-up as a function of the initial supply of N, P and N/P. Maximum δPOM is defined as peak POM concentration subtracted by the initial (day 1) POM concentration. Treatments in *varied P* are depicted as blue circles; treatments in *varied N* are depicted as red diamonds. Error bars denote the standard deviation of replicated (n=3) treatments. Regression lines (continuous lines) indicate linear correlations between the initial nutrient supply and POM accumulation.”

“Figure 4: PON/POP stoichiometry during (A) the exponential growth phase and (B) the stationary growth phase of the experiment. The grey line visualizes the Redfield Ratio. The color code, symbols and lines are the same as in Fig. 3.”

“Figure 6. Positive linear correlation between maximum DOP build-up (defined as peak DOP concentration subtracted by the initial DOP concentration) and initial P supply during *varied P* (blue circles) and *varied N* (red diamonds).”

The information about the type of regression was added to the Material and Methods section.

Figure 7: error bars again need description. Additionally what statistical test was being used here?
The figure caption now reads: “Figure 7: Mean N_2 fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Asterisks indicate a significant difference between day 2 and day 8 (paired t-test). Error bars denote the standard deviation.”

Figure 10: error bars.
The figure caption now reads: “Dynamics of PO_4^{3-}, POP and DOP in all mesocosms. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Error bars denote the standard deviation.”

Figure S1, caption and figure do not appear to match. Caption refers to ‘a’ and ‘b’ parts when there only appears to be one part in figure?
Please see comment above.

Changing nutrient stoichiometry affects phytoplankton production, DOP accumulation~~build up~~ and dinitrogen fixation – a mesocosm experiment in the eastern tropical North Atlantic

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Abstract:

Ocean deoxygenation due to climate change may alter redox-sensitive nutrient cycles in the marine environment. The productive eastern tropical North Atlantic (ETNA) upwelling region may be particularly affected when the relatively moderate oxygen minimum zone (OMZ) deoxygenates further and microbially-driven nitrogen (N) loss processes are promoted. Consequently, water masses with a low nitrogen to phosphorus (N:P)~~N:P~~ ratio could reach the euphotic layer, possibly influencing primary production in those waters. Previous mesocosm studies in the oligotrophic Atlantic Ocean identified nitrate~~N~~ availability as a control~~ling~~ of primary production, while a possible co-limitation of nitrate and phosphate ~~(P)~~ could not be ruled out. To better understand the impact of changing N:P ratios on primary production and~~ on~~ N_2 fixation in the ETNA surface ocean, we conducted land-based mesocosm experiments with natural plankton communities and applied a broad range of N:P ratios (2.67 – 48). Silicic acid~~ate~~ was supplied at 15 µmol L~~kg~~$^{-1}$ in all mesocosms. We monitored nutrient drawdown~~, bloom formation~~, biomass accumulation~~build up~~ and ~~diazotrophic feedback~~nitrogen fixation in response to variable nutrient stoichiometry. Our results confirmed nitrate~~N~~ to be ~~limiting to~~the key factor determining primary production. We found that excess phosphate~~P~~ was channeled through particulate organic matter (POP) into the dissolved organic matter (DOP) pool. In mesocosms with low inorganic phosphate~~P~~ availability, DOP was utilized while N_2 fixation increased, suggesting a link between those two processes. Interestingly this observation was most pronounced in mesocosms where ~~inorganic N~~nitrate was still available, indicating that bioavailable N does not necessarily ~~has to have a negative impact on~~suppress N_2 fixation. We observed a shift from a mixed cyanobacterial~~l~~/proteobacterial~~l~~ dominated active diazotrophic community towards a diatom-diazotrophic ~~diatom~~ association~~symbionts~~ of the *Richelia-Rhizosolenia* symbiosis. We hypothesize that a potential change in nutrient stoichiometry in the ETNA might lead to a general shift within the diazotrophic community, potentially ~~modifying primary productivity.~~ influencing primary productivity and carbon export.

1 Introduction

Eastern boundary upwelling systems are characterized by cold, nutrient-rich water masses that are transported from intermediate water layers towards the surface. The rResulting extensive primary production forms the basis for high biomass development and a productive food webchain (Pennington et al., 2006). At the same time, biological degradation at depth and weak interior ventilation cause permanently low oxygen concentrations in intermediate water masses (100 – 900 m, Karstensen et al., 2008). These low oxygen conditions support denitrification and anammox that remove bioavailable nitrogen (N) from the water column (e.g. Codispoti et al., 2001; Lam et al., 2009; Kalvelage et al., 2011). Oxygen minimum zones (OMZs) also influence the availability of inorganic phosphateorus (P), siliconate (Si) and trace elements such as iron (Fe), which are released at the sediment-water interface under oxygen deficient conditions (Ingall and Jahnke, 1994; Hensen et al., 2006). Subsequently, the elemental stoichiometry of inorganic nitrogen to phosphorusnutrients (N:P) in upwelled water masses is below the Redfield ratio of 16:1 (Redfield, 1958), which manifests itself as an excess of P (P*) relative to N ($P^* = PO_4^{3-} - NO_3^-/16$, after Deutsch et al. (2007).

In the Eastern Tropical North Atlantic (ETNA) nutrient concentrations and stoichiometry within the euphotic layer cover a wide range. Water masses in coastal regions feature low N:P ratios mainly as a result of benthic N-loss along with P leaching from the sediment (Trimmer and Nicholls, 2009; Jaeschke et al., 2010; Schafstall et al., 2010) suggesting an N limitation of primary production in OMZ-influenced surface waters (Deutsch et al. 2007). In the transition zone between coastal upwelling and open ocean, N:P ratios approach Redfield proportions (Moore et al., 2008). Nevertheless, the nitracline tends to be deeper than the phosphocline in the ETNA (Hauss et al., 2013; Sandel et al., 2015), which also points towards a deficiency of N over P in the euphotic zone N being the limiting nutrient for primary production in the euphotic zone. In the Central and West Atlantic, N:P ratios beyond 30:1 can be reached (Fanning, 1992; Moore et al., 2008), suggesting a severe P limitation of primary producers (Ammerman et al., 2003; Mills et al., 2004). Additional input of atmospheric anthropogenic nitrogen into the open ocean could further increase this P deficit in the future (Duce et al., 2008). Oxygen concentrations within the oxygen minimum in the ETNA are usually above 40 µmol kg^{-1} and thus considered too high to support N loss processes in the water column (Karstensen et al., 2008; Löscher et al., 2012; Ryabenko et al., 2012). However, recent observations of very low oxygen levels just below the mixed layer associated to anticyclonic modewater eddies suggest a potential for localized denitrification – with an accompanied decrease in N:P ratioslevels – in the open ocean of the ETNA (Karstensen et al.,2015).

Discrepancies from the canonical N:P ratio are known to influence productivity and composition of primary producers (Grover, 1997). Since the average elemental composition of C, N and P in seawater as well as in phytoplankton is 106:16:1, a deviation of dissolved inorganic nutrients from this ratio could indicate which nutrient is ultimately limiting for phytoplankton growthcan potentially become limiting before the other (Lagus, 2004; Moore et al., 2013). Transferring this concept to upwelling regions with inorganic N:P ratios below Redfield, one would expect that the limiting nutrient for phytoplankton growth in those areas is N. It has been shown, however, that certain functional ecotypes of phytoplankton differ in their required nutrient ratio, as specific cellular entities (e.g. chlorophyll, proteins or rRNA) of primary producers have a unique stoichiometric composition

deviating from the classical Redfield stoichiometry (Geider and La Roche, 2002; Quigg et al., 2003; Arrigo, 2005). Thus, surface waters adjacent to OMZs potentially provide a niche for certain types of primary producers, whose growth strategy and metabolic requirements are favored by low ratios of N:P. Arrigo (2005) refers to them as „bloomers“ and characterizes them as organisms adapted to exponential growth, which contain high amounts of ribosomes and P-rich rRNA. Those organisms build their biomass in non-Redfield-proportions and exhibit low cellular N:P ratios. The deficit in inorganic N of water masses adjacent to OMZs would thus be reduced by this non-Redfield production and N:P ratios further offshore would approach Redfield conditions.

Another concept of phytoplankton growth in N deficient waters is that inorganic nutrients are taken up in Redfield proportion by primary producers, which leaves the surface water masses enriched in P. Excess phosphate presence has been hypothesized to favor N_2-fixation (Deutsch et al., 2007). The conversion of readily available dissolved N_2 into bioavailable forms of fixed N by diazotrophs could replenish the N-deficit in surface waters adjacent to OMZs.

Previous bioassay studies that were conducted to identify controlling factors for primary production in the eastern Atlantic using inorganic N, P and dissolved Fe addition, determined N as the key limiting nutrient (e.g. (Graziano et al., 1996; Mills et al., 2004; Moore et al., 2008). These findings are in accordance with an on-board mesocosm study from the same area, where phytoplankton growth depended on the initial supply of N rather than on the N:P ratio and where a combined addition of N and P did not further increase biomass production compared to the addition of N sources alone (Franz et al., 2012). Additionally, the authors deduced that at low N:P ratios excess P was assimilated by non-diazotrophic phytoplankton and was channeled into dissolved organic phosphorus (DOP). As DOP might serve as an additional source of P for bacteria and phytoplankton (Mahaffey et al., 2014 and references therein) and is preferentially taken up by the filamentous diazotrophic cyanobacterium *Trichodesmium* (Dyhrman et al., 2006; Sohm and Capone, 2006), it has been proposed that N_2 fixation might be stimulated by an enhanced DOP supply under low N:P ratios (Franz et al., 2012).

Until recently, oceanic N_2 fixation was mainly attributed to phototrophic cyanobacteria, such as *Trichodesmium* or *Crocosphaera,* which are restricted to nutrient depleted surface to subsurface waters due to their light demand (Capone et al., 1997; Zehr and Turner, 2001). However, several groups of non-cyanobacterial diazotrophs and cyanobacterial symbionts have been detected in various oceanic regions, thus demonstrating the ubiquity and high diversity of diazotrophs (Foster et al., 2009; Farnelid et al., 2011; Loescher et al., 2014). Despite the growing awareness of diazotrophic diversity and distribution, the environmental conditions controlling diazotrophy are still not well understood. However temperature, Fe and P availability and dissolved oxygen concentrations are regarded as key factors for diazotrophic distribution and partly for active N_2 fixation (e.g. Sohm et al., 2011). The presence of high amounts of fixed N is thought to inhibit N_2 fixation (Weber and Deutsch, 2014), since diazotrophs are either outcompeted by fast growing phytoplankton species such as diatoms (Bonnet et al., 2009; Monteiro et al., 2011), or they themselves take up bioavailable forms of N rather than use the energy consuming process of N_2 fixation (Mulholland and Capone, 2001; Mulholland et al., 2001; Dekaezemacker and Bonnet, 2011).

In the ETNA, upwelling of N depleted waters along with high Fe input via Saharan dust deposition (Gao et al., 2001) sets a classical niche for N_2 fixation, while high N:P ratios beyond the upwelling

region of the ETNA point towards P limitation of diazotrophs (Ammerman et al., 2003; Mills et al., 2004). Nevertheless, a diverse community of cyanobacterial diazotrophs such as *Trichodesmium* (Capone et al., 1997; Tyrrell et al., 2003), a variety of unicellular cyanobacterial diazotrophs (Groups A, B, C, diatom-symbionts) (Falcon et al., 2002; Langlois et al., 2005) as well as non-cyanobacterial diazotrophs such as different clades of proteobacteria are abundant and widely distributed (e.g. (Langlois et al., 2005; 2008). Those diazotrophs have previously been demonstrated to actively fix N_2 in the ETNA (Langlois et al., 2005; 2008; Foster et al., 2009), showing highest rates in nutrient depleted surface to subsurface waters (Großkopf et al., 2012).

We investigated the effect of variable nitrate~~N~~ and phosphate~~P~~ supply on phytoplankton growth and addressed the diazotrophic response to changes in N:P stoichiometry over time in two consecutive mesocosm experiments. In order to extend the design of previous mesocosm experiments (Franz et al., 2012), N and P supply ratios were varied while keeping either nitrate or phosphate at constant concentrations. High N:P ratios were applied to investigate potential inhibition of ~~marine~~ N_2 fixation, while low N:P supply ratios were applied to unravel the role of excess P and consecutively formed DOP on primary production and diazotrophy. Direct N_2 fixation rate measurements as well as determination of *nifH* gene and transcript abundances were carried out to characterize the diazotrophic community and their response to the chosen treatment levels. The experimental design and response variables were chosen in order to assess responses of the phytoplankton community to possible changes in oceanic nutrient stoichiometry as a consequence of ocean deoxygenation.

2 Methods

2.1 Experimental Setup

In October 2012 we conducted two 8-day mesocosm experiments at the Instituto Nacional de Desenvolvimento das Pescas (INDP), Mindelo, Cape Verde. The night before the start of each experiment, surface water was collected with RV *Islândia* south of São Vicente (16°44.4'N, 25°09.4'W) and transported to shore using four 600 L food safe intermediate bulk containers. Containers for water transport were first rinsed with diluted HCl and several times with deionized water. The experimental setup comprised sixteen plastic mesocosm bags, which were distributed in four flow-through water baths. Blue, transparent lids were added to reduce the light intensity to approximately 20 % of surface irradiation. The collected water was evenly distributed among mesocosm bags by gravity, using a submerged hose to minimize bubbles. The volume inside each mesocosm was calculated after adding 1.5 mmol silicic acid~~ate~~ and measuring the resulting silicic acid~~ate~~ concentration. The volume ranged from 105.5–145 liters. Nutrients in all mesocosms were measured before nutrient manipulation. Nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and silicic acid ($Si(OH)_4$) were all below the detection limit and far below the manipulation levels (see Fig. 2). We therefore conclude that no contamination with these nutrients occurred during water sampling, transport and mesocosm filling. Experimental manipulation was achieved by adding different amounts of ~~inorganic N~~nitrate and phosphate~~P~~. In the first experiment, the phosphate~~P~~ supply was changed at constant nitrate~~N~~ supply (*varied P*) in thirteen of the sixteen units, while in the second experiment the nitrate~~N~~ supply was changed at constant phosphate~~P~~ supply (*varied N*) in twelve of the sixteen units. Each of these nutrient treatments was replicated 3 times. In addition to this, "cornerpoints" were chosen, where both the nitrate~~N~~ and phosphate~~P~~ supply was changed. The „cornerpoints" were not replicated. These treatments were repeated during both experiments (see Fig. 1 for experimental design). Four cornerpoints should have been repeated, but due to erroneous nutrient levels in mesocosm 10 during *varied N*, this mesocosm also was adjusted to the center point conditions. Experimental treatments were randomly distributed between the four water baths. Initial sampling was carried out immediately after filling of the mesocosms on day 1. After nutrient manipulation, sampling was conducted on a daily basis between 09:00 and 10:30 for days 2 to 8. Nutrient levels were set between 2 and 20 µmol L^{-1} for nitrate, 0.25 and 1.75 µmol L^{-1} for phosphate and 15 µmol L^{-1} for silicic acid~~ate~~. Table S1 gives the target nutrient concentrations and corresponding measured concentrations in the mesocosms.

It has to be noted, that no algal bloom developed in mesocosm 5 during *varied N* (target concentrations: 17.65 µmol L^{-1} NO_3^-, 0.40 µmol L^{-1} PO_4^{3+}). Thus, it was not included in the analysis and data are not presented.

Although we refer to our experimental approach as mesocosm experiment, this label might be disputable depending on the definition of the term mesocosm. Sometimes, experimental enclosures are only defined by size, where our approach would fall into the range of a microcosm experiment (<1 m^3; Riebesell et al., 2010). Independent of its size, a mesocosm can also be defined as a confined body of water, where environmental factors are manipulated at the community or ecosystem level (Stewart et al., 2013). In contrast, microcosm experiments are often used to manipulate factors at the population level and often lack the realism to extrapolate results to natural systems (Stewart et al., 2013). Although our experimental enclosures are limited in size, we consider it~~feel~~ justified ~~in~~

~~us~~using~~ing~~ the term mesocosm, as we conducted our experiments with ~~a~~ natural communities~~y~~ consisting of at least 3 ~~different~~ trophic levels (bacteria, phytoplankton, microzooplankton).

2.3 Nutrients

Samples (10 mL) for dissolved inorganic nutrients (NO_3^-, NO_2^-, PO_4^{3-}, $Si(OH)_4$) were taken daily from each mesocosm and measured directly using a QuAAtro Autoanalyzer (Seal Analytic) according to Grasshoff et al. (1999). The detection limits of nutrient analyses were 0.01 µmol L^{-1} for NO_2^- and PO_4^{3-}, 0.03 µmol L^{-1} for NO_3^- and 0.04 µmol L^{-1} for $Si(OH)_4$.

2.4 Chlorophyll *a*

For chlorophyll *a* (Chl *a*) analyses, water samples (0.5 – 1 L) were vacuum-filtered (200 mbar) onto Whatman GF/F filters (25 mm, 0.7 µm) before adding 1 ml of ultrapure water. Filters were immediately stored frozen for at least 24 hours. 9 ml acetone (100 %) was then added to each sample and the fluorescence was measured with a Turner Trilogy fluorometer, which was calibrated with a Chl *a* standard dilution series (*Anacystis nidulans*, Walter CMP, Kiel, Germany). Chl *a* concentrations were determined according to Parsons et al. (1984).

2.5 Dissolved organic phosphorus

Water samples for analyses were filtered through pre-combusted (450 °C, 5 hours) Whatman GF/F filters (25 mm, 0.7 µm). The filtrate was stored in acid-clean 60 ml HDPE bottles (5 % HCl~~L~~ for at least 12 hours) and frozen at -20 °C until further analysis.

Prior to analysis of total dissolved phosphorus (TDP) one metering spoon of the oxidizing reagent Oxisolv (Merck) was added to 40 ml of sample, which was hereupon autoclaved for 30 minutes. Samples were then analysed spectrophotometrically (Autoanalyzer QuAAtro Seal Analytic), following Bran and Luebbe AutoAnalyzer Method No. G-175-96 Rev. 13 (PO_4^{3-}). The detection limit was 0.2 µmol L^{-1} and analytical precision was ±8.3%.

DOP concentrations were calculated as:

DOP = total dissolved phosphorus (TDP) – dissolved inorganic ~~phosphorus~~ phosphate (~~DI~~PO_4^{3-}) (1)

2.6 Particulate organic matter

Particulate organic matter concentrations were determined by filtering 0.5 – 1 L seawater through pre-combusted (450 °C for 5 hours) Whatman GF/F filters (25 mm, 0.7 µm) under low pressure (200 mbar). Filters were immediately frozen and stored until analysis.

Prior to analysis, particulate organic carbon (POC) and nitrogen (PON) filters were fumed with HCl~~L~~ (37 %, for 24 hours) in order to remove inorganic carbon. After drying, filters were wrapped in tin cups (8 × 8 × 15 mm) and measured according to Sharp (1974) using an elemental analyzer (Euro EA, EuroVector, Milan, Italy).

For particulate organic phosphorus (POP) measurements, filters were autoclaved with the oxidation reagent Oxisolv (Merck) and 40 ml of ultrapure water for 30 min in a pressure cooker. Then,

orthophosphate was analyzed photometrically according to Hansen and Koroleff (1999).

Relationships of dissolved and particulate organic matter accumulation to the inorganic nutrient supply ratios were determined using Model I regression analyses (SigmaPlot, Systat).

2.7 Molecular methods

Samples for the extraction of DNA/RNA were taken by filtering a volume of 1–2 L (exact volumes and filtration times were determined and recorded continuously) of seawater through 0.2 µm polyethersulfon membrane filters (Millipore, Billerica, MA, USA). The filters were frozen and stored at -80 °C until analysis. Nucleic acid extraction was performed using the Qiagen DNA/RNA All prep Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted RNA was reverse transcribed to cDNA using the Superscript III First Strand synthesis Kit (Invitrogen) following the manufacturer's protocol with primers nifH2 and nifH3 (Langlois et al., 2005; Zani et al., 2000). *NifH* clusters were quantified from DNA and cDNA by quantitative Real Time PCRs as previously described by Church et al. (2005) and Langlois et al. (2008). TaqMan® qPCRs were set up in 12.5 µl reactions and were performed in technical duplicates in an ABI ViiA7 qPCR system (Life technologies, Carlsbad, CA, USA). For each primer and probe set, standard curves were obtained from dilution series ranging from 10^7 to 10 gene copies per reaction; standards were constructed using plasmids containing the target *nifH* gene. Sequences of primers and probes are given in Table 1. To confirm purity of RNA, non-template qPCRs were performed using the corresponding RNA.

2.8 $^{15}N_2$ seawater incubations

Seawater incubations were performed in triplicates from each mesocosm on day 1 and day 8 of both experiments as previously described by Mohr et al. (2010) and Großkopf et al. (2012). Degassed seawater was filled into evacuated gas-tight 3L Tedlar® bags without a headspace. Addition of $^{15}N_2$ gas was (depending on the exact water volume in the Tedlar® bag) around 10 ml $^{15}N_2$ per 1 L seawater. Dissolution of the $^{15}N_2$ gas was achieved by 'slapping' the bubble with a ruler. After complete dissolution of the added $^{15}N_2$ gas ($^{15}N_2$-enriched seawater), an aliquot of the $^{15}N_2$ enriched water was collected for each preparation of enriched seawater and stored in an Exetainer. Seawater samples were filled headspace-free; 100 ml of seawater were exchanged with previously degassed seawater containing a defined concentration $^{15}N_2$ and ^{13}C-NaCO. Incubations were performed in 4.5 L polycarbonate bottles closed with Teflon®-coated butyl rubber septum caps. The $^{15}N_2$ concentration in the prepared batches of enriched water was determined to be 250 μmol L^{-1}, which translates in an ^{15}N-enrichment of about 2 % in the 4.5 L bottle incubations, when adding 100 mL enriched seawater (depending on temperature and salinity). Water samples were incubated for 24 hours in the mesocosm water baths, thus at the same temperature and light regime, followed by a filtration on Whatman GF/F filters, which were analyzed using mass spectrometry as previously described in Loescher et al. (2014).

~~2.9 Statistical evaluation~~

~~Relationships of dissolved and particulate organic matter build up to the inorganic nutrient supply ratios were determined using regression analyses (SigmaPlot, Systat).~~

~~**Data selection.** Statistical modelling was performed with a subset of the dataset generated by removal of (i) variables with missing measures, (ii) incomplete observations, (iii) variables left with all-zero data. In order to minimize collinearity of explanatory variables in the statistical models, a set of environmental variables was chosen according to their variance inflation factor (VIF): Starting with a linear model that included all variables of interest, the variable with the highest VIF was iteratively determined and removed from the model until all remaining explanatory variables had a VIF <2.5. The set of explanatory variables with minimal collinearity was used in model selection (see supplemental material).~~

~~**Model selection.** Concentrations of genes and transcripts, respectively, as determined by qPCR, were fitted to the selected explanatory variables in generalized linear additive mixed models employing functions *gam* and *gamm* of the R package mgcv v1.8-4 (Wood, 2004; 2011). Multivariate analysis of counts was realized by a factor of gene/transcript names in the model. Temporal variation of gene/transcript counts was modeled by cubic spline smoothers. Each combination of gene/transcript and Run_ID (i.e. *varied N* or *varied P*) was given its own smoothing function. Other explanatory variables were standardized to zero mean and unit variance and added as covariates, or – if a non-linear response was to be expected – as cubic spline smoothers.~~

~~Candidate models were compared by means of the Akaike Information Criterion (AIC) and validated following the protocol of Zuur et al. (2009). Briefly, this comprised: (i) choice of an appropriate variance structure for the full model containing all selected explanatory variables and relevant interaction terms fitted with restricted maximum likelihood (REML); (ii) choice of the optimal fixed structure by subsequent removal of insignificant model terms fitted with maximum likelihood; (iii) refitting the model with REML and validation of model prerequisites. Regression models were visualized with the R packages lattice v0.20-30 and latticeExtra v0.6-26 (Sarkar, 2008).~~

3. Results

3.1 Bloom development and nutrient dynamics in the mesocosms

In both consecutive experiments (*varied P* and *N*) a bloom formation was observed following nutrient manipulation. Nitrate~~O_3^-~~ and phosphate~~PO_4^{3-}~~ were readily taken up by the plankton community and nutrient concentrations thus declined until the end of the experiment (Fig. 2). NO_3^- was fully depleted in all mesocosms at days 6–8 in both runs, except in the mesocosms with highest N:P ratios of 48:1 (treatment 12.00N/0.25P in *varied P*~~-~~) and 44:1 (treatment 17.65N/0.40P in *varied N*). Residual PO_4^{3-} was still detectable at the end of the experiments (day 8) in all mesocosms with initial N:P values <10 (treatments in *varied P*: 6.35N/1.10P, 12.00N/1.25P, 12.00N/1.75P; treatments in *varied N*: 2.00N/0.75P, 4.00N/0.75P, 6.00N/1.03P) indicating a limitation of primary productivity dependent on the N:P ~~stoichiometry~~ratio.

Although initial Chl *a* concentrations were slightly higher in *varied P* than in *varied N* (~0.38 µg L^{-1} and 0.2 µg L^{-1}, respectively), the increase in Chl *a* concentration was 5–10-fold until days 5/6 in *varied P* compared to 10–50-fold in *varied N*. After the bloom at days 5 and 6 Chl *a* declined again to 0.05–0.7 µg L^{-1} and 0.6–1.7 µg L^{-1} in *varied P* and *varied N*, respectively (Fig. 2). ~~In both runs, bloom formation~~

~~was initially independent from nutrient supply, however, the applied statistical model showed a slight positive correlation between Chl a and initial N:P (see supplemental material, figure S1).~~

3.2 Particulate organic matter (POM) ~~buil~~accumulation~~d-up~~ and stoichiometry

Temporal dynamics of POM were similar during both experiments. Initial concentrations of POC, PON and POP were 10–17 µmol L^{-1}, 1.5–2 µmol L^{-1} and 0.05–0.12 µmol L^{-1}, respectively (Fig. 2). In *varied P,* POC and PON reached a maximum on day 6, while POP increased until the end of the experiment. In *varied N* POM accumulation~~build up~~ also peaked on day 6 or 7 in most mesocosms, but differences between N:P treatments were more pronounced in *varied N* compared to *varied P*. Our results indicate that POM accumulation~~build up~~ was independent of the initial nutrient supply ratio in both experiments (Fig. 3). We observed a significantly positive regression coefficient between maximum POC and PON concentrations (defined as peak POC and PON concentration subtracted by the initial (day 1) POC and PON concentration) to the initial NO_3^- supply (POC: r^2 = 0.64, p = 0.0006; PON: r^2 = 0.80, p < 0.0001) while POP accumulation~~build up~~ showed a significantly positive regression coefficient to initial PO_4^{3-} supply (r^2 = 0.31, p = 0.048).

Mean PON:POP ratios during the exponential growth phase appeared to be independent of the initial N:P supply ratio in both experimental runs (Fig. 4). With ratios between 1~~76.6~~ and 2~~32.8~~, the PON:POP ratios were above, but close to Redfield proportion in all treatments during the first 5 days of the experiments, consistent with an observed initial uptake of N~~ and~~ P in Redfield proportions in all mesocosms. During the post bloom phase, mean PON:POP ratios were positively correlated with the initial nutrient supply ratio (r^2 = 0.73, p < 0.0001). Nevertheless, stoichiometry of POM (N:P between 1~~65.9~~ and 3~~21.9~~) ~~mostly~~ exceeded Redfield proportions, even in treatments with lowest N:P ratios.

3.3 Dissolved organic phosphorus dynamics

Initial DOP concentrations during *varied P* were 0.14 (± 0.009) µmol L^{-1}. In most mesocosms, except for the one with lowest initial PO_4^{3-} supply (12.00N/0.25P), DOP concentrations increased progressively until the end of the experiment (Fig. 5). ~~and increased in all mesocosms, except in the one with lowest initial P supply (12.00N/0.25P). Maximum~~Highest DOP concentrations of around 0.4 µmol L^{-1} were determined in mesocosm 12.00N/0.75P on day 5 and decreased again afterwards. ~~In all other mesocosms DOP concentrations increased progressively until the end of the experiment (Fig. 5).~~ Maximum DOP accumulation~~build up~~ (defined as described for maximum POM accumulation~~build up~~, section 3.2) was significantly correlated to the initial PO_4^{3-} supply (Fig. 5; r^2 = 0.63, p = 0.0007)~~., which was also in accordance with the applied statistical model (Fig. S1).~~

In *varied N* initial DOP concentrations in the mesocosms were 0.2 (± 0.038) µmol L^{-1} and increased slightly until day 3. Afterwards DOP concentrations remained rather constant, although with considerable variability in the data (Fig. 5).

A simple mass balance (Table S2) showed that part of the phosphorus pool, i.e. the sum of PO_4^{3-}, DOP and POP, remained unaccounted for ($P\ pool_X$) at the end of the experiment ($P\ pool_X$ in *varied P* ~25% of the initial P pool, $P\ pool_X$ in *varied N* ~14%). This undetermined P pool is most likely due to wall growth, which became visible towards the end of the experiment. However, only in two mesocosms the difference between P pools sizes on day 2 and day 8 was significant.

3.4 Importance of the *Richelia-Rhizosolenia* symbiosis for diazotrophy

Directly measured rates of N_2 fixation showed an increase with time in *varied P*, while no statistically significant increase could be observed in *varied N* (Fig. 6).

A molecular screening of the diazotrophic community in the initial water batch used for *varied P* using the *nifH* gene as functional marker gene showed a dominance of filamentous cyanobacterial diazotrophs related to *Trichodesmium* accounting for ~54% of the diazotrophic community (results from qPCR), followed by proteobacterial diazotrophs (~36%) in *varied P* (data not shown). The high abundance of filamentous cyanobacterial diazotrophs indicated the presence of a bloom in the initial water batch in *varied P*. In *varied N*, the initial community consisted mainly of proteobacterial diazotrophs (~88%), followed by UCYN-B (9%) and filamentous cyanobacteria (3%).

~~Predictability of gene abundances by time and Run ID (i.e. *varied N* or *varied P*) was assessed at the response scale by plotting the values fitted by the model with their 95% confidence bands (Fig. 7). Except for *nifH*_Fil, which displayed no temporal changes at all, gene count levels were generally higher in *varied P* compared to *varied N*. This was especially true for all time points in case of the gamma proteobacterial *nifH*_AO (significance bands overlapped by less than one half, Cumming et al., 2007). In unicellular cyanobacterial clusters *nifH*_UA (UCYN-A) and *nifH*_CR (UCYN-B), gene abundances differed only intermittently between the two experimental runs: While *nifH*_UA abundances were higher in *varied P* at days 3-4 and 6-8, *nifH*_CR abundances were higher in *varied P* at days 2 and 4-6.~~

Changes in transcript abundance over time ~~(Fig. 8) showed mainly different response patterns between~~were most intense for *Richelia-Rhizosolenia* (Het I) transcripts. At day 2, ~~*nifH*_~~Het I transcript abundances were higher in *varied N* conditions compared to *varied P*. This relation changed over the course of the experiments, with a pronounced increase of ~~*nifH*_~~Het I transcript abundances between day 6 and 8 in *varied P* (Fig. 8).

Thus, all classical *nifH* clusters (filamentous cyanobacteria, UCYN-A, -B, -C and proteobacteria diazotrophs) decreased in abundance of genes and gene transcripts down to the detection limit in both experiments ~~(Figs. 7, 8, S3, S4)~~, whereas diazotrophs of the *Richelia-Rhizosolenia* symbiosis ~~(Het I)~~ were the only diazotrophs that showed an increase in *nifH* transcripts over the course of the experiment, exclusively in *varied P* (Fig. 8~~, S2~~).

~~In *varied P*, we observed an accumulation of DOP. In contrast, d~~During *varied N, nifH* gene and transcript abundance of the *Richelia-Rhizosolenia* cluster was close to the detection limit and DOP accumulation~~build up~~ was rather negligible~~, thus a potential impact of DOP on diazotrophy was hypothesized~~. In contrast, we ~~In *varied P*, we~~ observed an accumulation of DOP~~:~~ ~~I In contrast,~~n *varied*

~~*To unravel a potential impact of DOP on N_2 fixation, we investigated temporal DOP patterns, which appeared strongly non-linear. At standard scores below -1 (~0.19 µM) DOP tended to contribute negatively to overall nifH transcript abundance levels, whereas at standard scores around -0.5 (~0.24 µM) the effect of DOP on nifH transcript abundance was positive. At higher concentrations, DOP tended again to a neutral or negative impact on transcript abundance indicating an optimum of DOP concentration on nifH transcript abundance.~~

In varied P. Here,, mesocosms with a significant increase in N_2 fixation (12.00N/0.25P and 12.00/0.75P) were also the ones where DOP was used as phosphorusP-source for biomass build up after PO_4^{3-} was depleted (Fig. 9). In mesocosm 12.00N/0.75P, PO_4^{3-} concentrations were below the detection limit after day 5. This coincided with a decrease of DOP after day 5, while POP concentrations increased until the end of the experiment. In mesocosm 12.00N/0.25P, POP also increased beyond the point of PO_4^{3-} depletion and highest POP accumulationbuild up exceeded values that could be explained by PO_4^{3-} incorporation alone. Thus a potential impact of DOP on diazotrophy is hypothesized. In mesocosms without a significant increase in N_2 fixation, POP and DOP concentrations increased until the end of the experiment and no apparent uptake of DOP could be observed.

4 Discussion

4.1 Controls on planktonPrimary pProduction

In order to understand potential consequences of changes in nutrient regimes, it is necessary to determine the factors that control and limit primary microbial production. In our experiments, amendments of NO_3^- significantly increased chlorophyll concentrations and enhanced the accumulationbuildup of POM, indicating the ability of the plankton community to rapidly and intensively react to nitrateN availability. These results indicate suggest that the ultimate limiting nutrient for the phytoplankton community production in our experiment was NO_3^-. N_2 fixation was measurable in all initial samples, which indicates the presence of a niche for diazotrophs in the Cape Verde region. For the upwelling region as well as for the oligotrophic open ocean of the ETNA, nitrateN limitation of the phytoplankton community has previously been reported (Davey et al., 2008; Moore et al., 2008; Franz et al., 2012). Additionally, Moore et al. (2008) observed a co-limitation of nitrateN and phosphateP during nutrient addition bioassay experiments in the ETNA. In our experiment, however, only POP accumulationbuild up was positively affected by PO_4^{3-} supply. This argues against a secondary limitation by phosphateP, but rather points towards a mechanism of accumulating and storing phosphate as polyphosphate within the cell (Schelske and Sicko-Goad, 1990; Geider and La Roche, 2002; Martin et al., 2014).

There is a large difference between the supply ratio of inorganic nutrients and the PON:POP ratio of primary producersthe plankton community in our study. Although initial N:P ratios in our mesocosms covered a wide range, PON:POP ratios reached maximum values of ~-21 in both experiments during the exponential growth phase. During stationary growth, maximum PON:POP values of 398.8 in *varied N* and 221.9 in *varied P* were measured. However, during growth phases in both experiments PON:POP ratios did never falldrop below 165.9. Very similar results were obtained by Franz et al (2012) off the Peruvian coast. However, two experiments conducted by Franz et al. (2012) in the ETNA and off West AfricaA showed a different response of the phytoplankton community. In these two cases, N:P supply ratio and PON:POP were highly correlated and PON:POP ratios as low as 6.0 (±+- 1.4) were observed in the stagnant phase. This shows that the stoichiometry of phytoplankton communities is flexible to a certain extent, but sometimes does not necessarily reach dimensions observed in laboratory experiments (Hecky et al., 1993) and implied by theoretical approaches (e.g. Geider and La Roche, 2002; Klausmeier et al., 2004). This may result from differences in the initial

community composition ~~tested that sometimes might~~if it lacks organisms able to assemble a P-rich growth machinery (Klausmeier et al., 2004; Arrigo, 2005) ~~or in other as yet unresolved factors~~. It has been reported that cellular N content~~s~~ seems relatively inflexible in ~~parts of the~~some phytoplankton ~~community~~groups, thus restricting the maintenance of metabolic processes at low dissolved inorganic nitrogen ~~(DIN)~~ concentrations (Moore et al., 2013). In contrast, phosphate~~P~~ requirements seem to be comparably flexible, as certain cellular components containing P (e.g. phospholipids) can be replaced by non-phosphorus containing compounds (Moore et al., 2013). This can also be deduced from our experiments, where higher N:P ratios lead to increasing PON:POP ratios, possibly due to the flexibility to substitute P compounds within the biomass. In contrast, lower N:P ratios lead to lower biomass accumulation, as the plasticity of PON:POP seems to be constrained by the availability of nitrate~~N~~ in our experiments.

4.2 The impact of bioavailable N on N_2 fixation

The ability of diazotrophs to grow independent of a fixed N source in principle gives them ~~in principle~~ an advantage to thrive under conditions where their competitors are limited by N availability. At the same time, diazotrophs are considered disadvantaged when competing with faster growing non-diazotrophs for nutrients under N replete conditions (Tyrrell, 1999; Ward et al., 2013). Contrary to this classical view, we could not detect a direct influence of reactive N compounds on N_2 fixation in our experiments. Despite a wide spectrum of applied nitrate~~N~~ concentrations in *varied N*, no significant ~~change~~ difference in N_2 fixation rates could be detected. Evidence from culture experiments also suggests that inorganic N compounds do not ~~universally~~ always repress N_2 fixation. While NO_3^- addition in *Trichodesmium* spp. (Mulholland et al., 2001; Holl and Montoya, 2005) and NH_4^+ addition in *Crocosphaera watsonii* (Dekaezemacker and Bonnet, 2011) reduced N_2 fixation rates, NO_3^- addition did not reduce N_2 fixation rates in *C. watsonii* and *Nodularia* spp. cultures (Sanz-Alférez and del Campo, 1994; Dekaezemacker and Bonnet, 2011). Moreover, recent field surveys demonstrated the occurrence of N_2 fixation in nutrient rich water masses of the eastern tropical South Pacific (ETSP) and equatorial Atlantic upwelling regions (Fernandez et al., 2011; Subramaniam et al., 2013; Loescher et al., 2014) and also modelling ~~efforts~~ studies predict high N_2 fixation rates in waters containing measurable amounts of ~~fixed~~ reactive N (Deutsch et al., 2012; Weber and Deutsch, 2014). Clearly, the degree of feedback concerning the inhibition of N_2 fixation by reactive N compounds is not universal and there is evidence that the absence of P and Fe in seawater is a stronger indicator for limitation of N_2 fixation than the presence of inorganic N compounds (Weber and Deutsch, 2014).

4.3 The role of excess P and DOP as controls on N_2 fixation

Deutsch et al. (2007) suggested that N_2 fixation is favored in upwelling regions, where N loss in adjacent OMZ waters and P leaching from the sediment lead to upwelling of waters enriched in P. This excess P is thought to be consumed by diazotrophs, thus replenishing the N-deficit in the vicinity of upwelling regions.

As nutrients were taken up in Redfield or above Redfield proportions in our experiments we would have expected excess phosphate~~P~~ in mesocosms with N:P supply ratios below Redfield. Instead, excess phosphate~~P~~ was absent and our data point towards a channeling of PO_4^{3-} through the

particulate pool into DOP, as an increase in PO_4^{3-} supply significantly increased the concentration of DOP. Why phytoplankton synthesize and excrete higher levels of DOP under excess phosphate~~P~~ conditions remains unclear, but enhanced PO_4^{3-}~~P~~ uptake (followed by DOP accumulation) is thought to hamper P limitation when sudden boosts in N are encountered (Mackey, 2012). In accordance with our study, mesocosm experiments from the ETNA and eastern tropical south Pacific (ETSP) open ocean (Franz et al., 2012) and measurements from shelf regions of the ETNA (Reynolds et al., 2014) and Celtic Sea (Davis et al., 2014) showed the accumulation of DOP under excess phosphate~~P~~ supply. Although the composition and bioavailability of the DOP pool needs to be further evaluated, DOP may act as a source of P for prokaryotic primary producers, either exclusively or in addition to PO_4^{3-}~~DIP~~ (Björkman and Karl, 2003; Dyhrman et al., 2006; Mahaffey et al., 2014; Reynolds et al., 2014). This indicates that the ability to utilize DOP may give diazotrophs a competitive advantage when bioavailable forms of N are depleted and either PO_4^{3-}~~*~~ or DOP concentrations are sufficient.

In our experiments a significant increase in N_2 fixation rates was only ~~measured~~ detected in *varied P*. In mesocosms with highest N_2 fixation rates, ~~DI~~PO_4^{3-} was depleted after day 5 or 6 while POP increased until the end of the experiment. After ~~DI~~PO_4^{3-} depletion, DOP concentrations declined, which indicates that DOP served as phosphorus~~P~~ source until the end of the experiment. It has to be noted that N_2 fixation rates were only measured at the beginning and the end of our experiment and possible fluctuations over time cannot be accounted for. However, ~~i~~increasing diazotrophic transcript abundances of *Richelia intracellularis* in symbiosis with the diatom *Rhizosolenia* (Het I) were also detected over the course of the ~~*same*~~ *variable P* experiment. While the diatom abundance was probably favored by replete amounts of silicic acid~~ate~~ added at the beginning of the experiment, no increase in diatom-diazotroph associations (DDAs) was detected in the *varied N* experiment. Measured N_2 fixation rates and transcript abundances lead~~This leads~~ us to speculate that DDAs were favored in the *varied P* experiment, where diazotrophs in the mesocosms utilized DOP resources in order to supply P to themselves and/or their symbiont. The ability to utilize DOP has previously been shown for *R. intracellularis* (Girault et al., 2013) and our observations suggest that they may not only provide their symbionts with N via N_2 fixation but also with P via DOP utilization.

DDAs in our experiment were favored by replete amounts of silicic acid~~ate~~ and DOP and were – in contrast to the classical view – not restrained by reactive N compounds. These findings suggest that DDAs have the potential to actively fix nitrogen in shelf waters of upwelling regions. Therefore, the N-deficit of upwelled water-masses could already be replenished locally prior to offshore transport.

A shift within the diazotrophic community towards DDAs could also exert controls on carbon export. Grazing, particle aggregation and export likely increase when filamentous and proteobacterial cyanobacteria are replaced by DDAs (e.g. Karl et al., 2008; 2012). The enhanced strength and efficiency of the biological pump would therefore increase the potential for carbon sequestration in the ETNA.

5 Conclusions and future implications for ETNA

Our findings add to the growing evidence that diminished N:P ratios in upwelling waters in the ETNA will either decrease the biomass of non-diazotrophic primary producers, specifically due to the decline of bioavailable N, or lead to a community shift towards primary producers that are able to adapt to

changing N:P conditions. As a considerable amount of DOP was produced under excess phosphateP conditions, changes in the N:P ratio of waters could exert profound control over DOP production rates in the ETNA. Our results indicate that enhanced DOP production in upwelling regions will likely fuel N_2 fixation, with an advantage for those diazotrophs capable of DOP utilization. We propose that N_2 fixation in the ETNA might not only be restricted to the oligotrophic open ocean but can occur in nutrient-rich upwelling regions as previously demonstrated for the tropical Pacific (Löscher et al., 2014) and the Atlantic equatorial upwelling (Subramanian et al., 2013), as N_2 fixation in DDAs seems to be favored by the presence of silicic acidate and DOP, and not by the absence of fixed N compounds.

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Authors' contribution

HH and RK designed the experiment with input from JM, CRL, AL, CB, UR, RAS; led the logistics and the study on site and provided nutrient and hydro-chemical datasets. JM, RK, AFR, AL, CB and HH conducted the sampling of particulate and dissolved matter. JM and AFR performed DOM and POM measurements, CRL performed N_2 fixation and molecular experiments and measurements. SCN performed the statistical modelling of the datasets. JM and CRL wrote the manuscript with input from all co-authors.

All data will be uploaded at www.pangaea.de upon publication.

References

Ammerman, J. W., Hood, R. R., Case, D. A. and Cotner, J. B.: Phosphorus deficiency in the Atlantic: An emerging paradigm in oceanography, Eos Trans. AGU, 84(18), 165–170, doi:10.1029/2003EO180001, 2003.

Arrigo, K. R.: Marine microorganisms and global nutrient cycles, Nature, 437(7057), 349–355, doi:10.1038/nature04158, 2005.

Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N., Charrière, B. and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled primary production and particulate export during the VAHINE mesocosm experiment (New Caledonia lagoon), Biogeosciences, 12(13), 4099–4112, doi:10.5194/bg-12-4099-2015, 2015. ~~Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N., Charrière, B. and Bonnet, S.: Dinitrogen fixation and dissolved organic nitrogen fueled primary production and particulate export during the VAHINE mesocosms experiment (New Caledonia lagoon), Biogeosciences Discuss., 12(5), 4273–4313, doi:10.5194/bgd-12-4273-2015, 2015.~~

Björkman, K. M. and Karl, D. M.: Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre, Limnol. Oceangr., 48(3), 1049–1057, doi:10.4319/lo.2003.48.3.1049, 2003.

Bonnet, S., Biegala, I. C., Dutrieux, P., Slemons, L. O. and Capone, D. G.: Nitrogen fixation in the western equatorial Pacific: Rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance, Global Biogeochem. Cycles, 23(3), GB3012, doi:10.1029/2008GB003439, 2009.

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B. and Carpenter, E. J.: Trichodesmium, a globally significant marine cyanobacterium, Science, 276(5316), 1221–1229, doi:10.1126/science.276.5316.1221, 1997.

Church, M. J., Jenkins, B. D., Karl, D. M. and Zehr, J. P.: Vertical distributions of nitrogen-fixing phylotypes at Stn Aloha in the oligotrophic North Pacific Ocean, Aquat. Microb. Ecol., 38(1), 3–14, doi:10.3354/ame038003, 2005.

Codispoti, L. A., Brandes, J. A., Christensen, J. P., Devol, A. H., Naqvi, S., Paerl, H. W. and Yoshinari, T.: The oceanic fixed nitrogen and nitrous oxide budgets: Moving targets as we enter the anthropocene? Sci. Mar., 65(S2), 85–105, doi:10.3989/scimar.2001.65s285, 2001.

~~Cumming, G., Fidler, F. and Vaux, D. L.: Error bars in experimental biology, J. Cell Biol., 177(1), 7–11, doi:10.1083/jcb.200611141, 2007.~~

Davey, M., Tarran, G. A., Mills, M. M., Ridame, C., Geider, R. J. and LaRoche, J.: Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic, Limnol. Oceangr., 53(5), 1722–1733, doi:10.4319/lo.2008.53.5.1722, 2008.

Davis, C. E., Mahaffey, C., Wolff, G. A. and Sharples, J.: A storm in a shelf sea: Variation in phosphorus distribution and organic matter stoichiometry, Geophys. Res. Lett., 41(23), 8452–8459, doi:10.1002/2014GL061949, 2014.

De Baar, H.: von Liebig's law of the minimum and plankton ecology (1899–1991), Progr. Oceanog., 33(4), 347–386, doi:10.1016/0079-6611(94)90022-1, 1994.

Dekaezemacker, J. and Bonnet, S.: Sensitivity of N2 fixation to combined nitrogen forms (NO_3^- and NH_4^+) in two strains of the marine diazotroph *Crocosphaera watsonii* (Cyanobacteria), Mar. Ecol. Prog. Ser., 438, 33–46, doi:10.3354/meps09297, 2011.

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(7124), 163–167, doi:10.1038/nature05392, 2007.

Deutsch, C., Gruber, N., Key, R. M., Sarmiento, J. L. and Ganachaud, A.: Denitrification and N_2

fixation in the Pacific Ocean, Global Biogeochem. Cycles, 15(2), 483–506, doi:10.1029/2000GB001291, 2012.

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(7124), 163–167, doi:10.1038/nature05392, 2007.

Duce, R. A., LaRoche, J., Altieri, K., Arrigo, K. R., Baker, A. R., Capone, D. G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R. S., Geider, R. J., Jickells, T., Kuypers, M. M., Langlois, R., Liss, P. S., Liu, S. M., Middelburg, J. J., Moore, C. M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L. L., Uematsu, M., Ulloa, O., Voss, M., Ward, B. and Zamora, L.: Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean, Science, 320(5878), 893–897, doi:10.1126/science.1150369, 2008.

Dyhrman, S. T., Chappell, P. D., Haley, S. T., Moffett, J. W., Orchard, E. D., Waterbury, J. B. and Webb, E. A.: Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*, Nature, 439(7072), 68–71, doi:10.1038/nature04203, 2006.

Falcon, L. I., Cipriano, F., Chistoserdov, A. Y. and Carpenter, E. J.: Diversity of diazotrophic unicellular cyanobacteria in the tropical North Atlantic Ocean, Appl. Environ. Microbiol., 68(11), 5760–5764, doi:10.1128/AEM.68.11.5760-5764.2002, 2002.

Fanning, K. A.: Nutrient Provinces in the Sea - Concentration Ratios, Reaction-Rate Ratios, and Ideal Covariation, J. Geophys. Res., 97(C4), 5693–5712, doi:10.1029/92JC00007, 1992.

Farnelid, H., Andersson, A. F., Bertilsson, S., Abu Al-Soud, W., Hansen, L. H., Sorensen, S., Steward, G. F., Hagstrom, A. and Riemann, L.: Nitrogenase Gene Amplicons from Global Marine Surface Waters Are Dominated by Genes of Non-Cyanobacteria, edited by J. A. Gilbert, PLoS ONE, 6(4), doi:10.1371/journal.pone.0019223, 2011.

Fernandez, C., Farías, L. and Ulloa, O.: Nitrogen Fixation in Denitrified Marine Waters, PLoS ONE, 6(6), e20539, doi:10.1371/journal.pone.0020539.s007, 2011.

Foster, R. A., Subramaniam, A. and Zehr, J. P.: Distribution and activity of diazotrophs in the Eastern Equatorial Atlantic, Environ. Microbiol., 11(4), 741–750, doi:10.1111/j.1462-2920.2008.01796.x, 2009.

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(11), 4629–4643, doi:10.5194/bg-9-4629-2012, 2012.

Gao, Y., Kaufman, Y. J., Tanre, D., Kolber, D. and Falkowski, P. G.: Seasonal distributions of aeolian iron fluxes to the global ocean, Geophys. Res. Lett., 28(1), 29–32, doi:10.1029/2000GL011926, 2001.

Geider, R. and La Roche, J.: Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis, Eur. J. Phycol., 37(1), 1–17, doi:10.1017/S0967026201003456, 2002.

Girault, M., Arakawa, H. and Hashihama, F.: Phosphorus stress of microphytoplankton community in the western subtropical North Pacific, J. Plankton Res., 35(1), 146–157, doi:10.1093/plankt/fbs076, 2013.

Grasshoff, K., Kremling, K. and Ehrhardt, M.: Methods of Seawater Analysis, 3rd ed., edited by K. Grasshoff, K. Kremling, and M. Ehrhardt, Wiley ‐ VCH Verlag GmbH, Weinheim, Germany. 1999.

Graziano, L. M., Geider, R. J., Li, W. and Olaizola, M.: Nitrogen limitation of North Atlantic phytoplankton: Analysis of physiological condition in nutrient enrichment experiments, Aquat. Microb. Ecol., 11(1), 53–64, doi:10.3354/ame011053, 1996.

Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., Lavik, G., Schmitz, R. A., Wallace, D. W. R. and LaRoche, J.: Doubling of marine dinitrogen-fixation rates based on direct measurements, Nature, 488(7411), 361–364, doi:10.1038/nature11338, 2012.

615 Grover, J. P.: Resource competition. Chapman & Hall, London, 1997.

616 Hansen, H. P. and Koroleff, F.: Determination of nutrients, in Methods of Seawater Analysis, edited by
617 K. Grasshoff, K. Kremling, and M. Ehrhardt, pp. 159–228, Wiley‐VCH Verlag GmbH, Weinheim,
618 Germany, 1999.

619 Hauss, H., Franz, J.M.S., Hansen, T., Struck, U. and Sommer U.: Relative inputs of upwelled and
620 atmospheric nitrogen to the eastern tropical North Atlantic food web: Spatial distribution of δ^{15}N in
621 mesozooplankton and relation to dissolved nutrient dynamics. Deep-Sea Res. I, 75, 135–145,
622 doi:10.1016/j.dsr.2013.01.010, 2013.

623 Hecky, R. E., Campbell, P. and Hendzel, L. L.: The Stoichiometry of Carbon, Nitrogen, and
624 Phosphorus in Particulate Matter of Lakes and Oceans, Limnol. Oceangr., 38(4), 709–724, 1993.

625 Hensen, C., Zabel, M. and Schulz, H. N.: Early Diagenesis at the Benthic Boundary Layer: Oxygen,
626 Nitrogen, and Phosphorus in Marine Sediments, in Marine Geochemistry, edited by H. D. Schulz and
627 M. Zabel, pp. 207–240, Springer. 2006.

628 Holl, C. M. and Montoya, J. P.: Interactions between nitrate uptake and nitrogen fixation in continuous
629 cultures of the marine diazotroph *Trichodesmium* (Cyanobacteria), J. Phycol., 41(6), 1178–1183,
630 doi:10.1111/j.1529-8817.2005.00146.x, 2005.

631 Ingall, E. and Jahnke, R.: Evidence for Enhanced Phosphorus Regeneration From Marine Sediments
632 Overlain by Oxygen Depleted Waters, Geochim. Cosmochim. Acta, 58(11), 2571–2575,
633 doi:10.1016/0016-7037(94)90033-7, 1994.

634 Jaeschke, A., Abbas, B., Zabel, M., Hopmans, E. C., Schouten, S. and Damste, J. S. S.: Molecular
635 evidence for anaerobic ammonium-oxidizing (anammox) bacteria in continental shelf and slope
636 sediments off northwest Africa, Limnol. Oceangr., 55(1), 365–376, doi:10.4319/lo.2010.55.1.0365,
637 2010.

638 Kalvelage, T., Jensen, M. M., Contreras, S., Revsbech, N. P., Lam, P., Günter, M., LaRoche, J., Lavik,
639 G. and Kuypers, M. M. M.: Oxygen Sensitivity of Anammox and Coupled N-Cycle Processes in
640 Oxygen Minimum Zones, PLoS ONE, 6(12), e29299, doi:10.1371/journal.pone.0029299.t003, 2011.

641 Karl, D. M. and Letelier, R. M.: Nitrogen fixation-enhanced carbon sequestration in low nitrate, low
642 chlorophyll seascapes, Mar. Ecol. Prog. Ser., 364, 257–268, doi:10.3354/meps07547, 2008.

643 Karl, D. M., Church, M. J., Dore, J. E., Letelier, R. M. and Mahaffey, C.: Predictable and efficient
644 carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation, PNAS,
645 109(6), 1842–1849, doi:10.1073/pnas.1120312109, 2012.

646 Karstensen, J., Stramma, L. and Visbeck, M.: Oxygen minimum zones in the eastern tropical Atlantic
647 and Pacific oceans, Progr. Oceanog., 77(4), 331–350, doi:10.1016/j.pocean.2007.05.009, 2008.

648 Karstensen, J., Fiedler, B., Schütte, F., Brandt, P., Körtzinger, A., Fischer, G., Zantopp, R., Hahn, J.,
649 Visbeck, M., and Wallace, D.: Open ocean dead zones in the tropical North Atlantic Ocean,
650 Biogeosciences, 12(8), 2597-2605, doi:10.5194/bg-12-2597-2015, 2015.

651 Klausmeier, C. A., Litchman, E., Daufresne, T. and Levin, S. A.: Optimal nitrogen-to-phosphorus
652 stoichiometry of phytoplankton, Nature, 429(6988), 171–174, doi:10.1038/nature02508, 2004.

653 Lagus, A.: Species-specific differences in phytoplankton responses to N and P enrichments and the
654 N:P ratio in the Archipelago Sea, northern Baltic Sea, J. Plankton Res., 26(7), 779–798,
655 doi:10.1093/plankt/fbh070, 2004.

656 Lam, P., Lavik, G., Jensen, M. M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez, D.,
657 Amann, R., Jetten, M. S. M. and Kuypers, M. M. M.: Revising the nitrogen cycle in the Peruvian
658 oxygen minimum zone, PNAS, 106(12), 4752–4757, doi:10.1073/pnas.0812444106, 2009.

659 Langlois, R. J., Huemmer, D. and LaRoche, J.: Abundances and distributions of the dominant nifH

660 phylotypes in the Northern Atlantic Ocean, Appl. Environ. Microbiol., 74(6), 1922–1931,
661 doi:10.1128/AEM.01720-07, 2008.

662 Langlois, R. J., LaRoche, J. and Raab, P. A.: Diazotrophic diversity and distribution in the tropical and
663 subtropical Atlantic ocean, Appl. Environ. Microbiol., 71(12), 7910–7919,
664 doi:10.1128/AEM.71.12.7910-7919.2005, 2005.

665 Liebig, von, J.: Chemistry in its application to agriculture and physiology, 3rd ed., edited by L. Playfair,
666 John Owen, Cambridge. 1842.

667 Loescher, C. R., Großkopf, T., Desai, F. D., Gill, D., Schunck, H., Croot, P. L., Schlosser, C.,
668 Neulinger, S. C., Pinnow, N., Lavik, G., Kuypers, M. M. M., LaRoche, J. and Schmitz, R. A.: Facets of
669 diazotrophy in the oxygen minimum zone waters off Peru, ISME J, 8(11), 2180–2192,
670 doi:10.1038/ismej.2014.71, 2014.

671 Löscher, C. R., Kock, A., Könneke, M., LaRoche, J., Bange, H. W. and Schmitz, R. A.: Production of
672 oceanic nitrous oxide by ammonia-oxidizing archaea, Biogeosciences, 9(7), 2419–2429,
673 doi:10.5194/bg-9-2419-2012, 2012.

674 Mackey, K. R. M.: Phosphorus cycling in the red tide incubator region of Monterey Bay in response to
675 upwelling, Front. Microbiol., 3(33), 1–14, doi: 10.3389/fmicb.2012.00033, 2012.

676 Mahaffey, C., Reynolds, S. and Davis, C. E.: Alkaline phosphatase activity in the subtropical ocean:
677 insights from nutrient, dust and trace metal addition experiments, Front. Mar. Sci., 1(73), 1–13,
678 doi:10.3389/fmars.2014.00073, 2014.

679 Martin, P., Dyhrman, S. T., Lomas, M. W., Poulton, N. J. and Van Mooy, B. A. S.: Accumulation and
680 enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus, PNAS,
681 111(22), 8089–8094, doi:10.1073/pnas.1321719111, 2014.

682 Mills, M. M., Ridame, C., Davey, M., La Roche, J. and Geider, R.: Iron and phosphorus co-limit
683 nitrogen fixation in the eastern tropical North Atlantic, Nature, 429(6989), 292–232, doi:
684 10.1038/nature02550, 2004.

685 Mohr, W., Großkopf, T., Wallace, D. W. R. and LaRoche, J.: Methodological Underestimation of
686 Oceanic Nitrogen Fixation Rates, edited by Z. Finkel, PLoS ONE, 5(9), e12583,
687 doi:10.1371/journal.pone.0012583, 2010.

688 Monteiro, F. M., Dutkiewicz, S. and Follows, M. J.: Biogeographical controls on the marine nitrogen
689 fixers, Global Biogeochem. Cycles, 25(2), GB2003, doi:10.1029/2010GB003902, 2011.

690 Moore, M. C., Mills, M. M., Langlois, R., Milne, A., Achterberg, E. P., La Roche, J. and Geider, R.:
691 Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and
692 productivity in the oligotrophic sub-tropical North Atlantic Ocean, Limnol. Oceangr., 53(1), 291–305,
693 doi: 10.4319/lo.2008.53.1.0291, 2008.

694 Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P. W., Galbraith, E. D.,
695 Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., La Roche, J., Lenton, T. M., Mahowald, N. M.,
696 Maranon, E., Marinov, I., Moore, J. K., Nakatsuka, T., Oschlies, A., Saito, M. A., Thingstad, T. F.,
697 Tsuda, A. and Ulloa, O.: Processes and patterns of oceanic nutrient limitation, Nat. Geosci., 6(9), 701–
698 710, doi:10.1038/ngeo1765, 2013.

699 Mulholland, M. R. and Capone, D. G.: Stoichiometry of nitrogen and carbon utilization in cultured
700 populations of Trichodesmium IMS101: Implications for growth, Limnol. Oceangr., 46(2), 436–443,
701 doi:10.4319/lo.2001.46.2.0436, 2001.

702 Mulholland, M. R., Ohki, K. and Capone, D. G.: Nutrient controls on nitrogen uptake and metabolism
703 by natural populations and cultures of *Trichodesmium* (Cyanobacteria), J. Phycol., 37(6), 1001–1009,
704 doi:10.1046/j.1529-8817.2001.00080.x, 2001.

705 Parsons, T. R., Maita, Y. and Lalli, C. M.: A manual of chemical and biological methods for seawater

analysis, 1984, Pergamon, Oxford. 1984.

Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Calienes, R. and Chavez, F. P.: Primary production in the eastern tropical Pacific: A review, Progr. Oceanog., 69(2-4), 285–317, doi:10.1016/j.pocean.2006.03.012, 2006.

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., Schofield, O., Morel, F. M. and Falkowski, P. G.: The evolutionary inheritance of elemental stoichiometry in marine phytoplankton, Nature, 425(6955), 291–294, doi: 10.1038/nature01953, 2003.

Redfield, A. C.: The Biological Control of Chemical Factors in the Environment, American Scientist, 46(3), 205–221, 1958.

Reynolds, S., Mahaffey, C., Roussenov, V. and Williams, R. G.: Evidence for production and lateral transport of dissolved organic phosphorus in the eastern subtropical North Atlantic, Global Biogeochem. Cycles, 28(8), 805–824, doi:10.1002/2013GB004801, 2014.

Riebesell, U., Lee, K. and Nejstgaard, J. C.: Pelagic mesocosms, in: Guide to best practices for ocean acidification research and data reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L. and Gattuso, J.-P., Luxembourg: Publications Office of the European Union, 95–112, 2010.

Ruttenberg, K. C.: Dissolved organic phosphorus production during simulated phytoplankton blooms in a coastal upwelling system, Front. Microbiol.,3(274), 1–12, doi: 10.3389/fmicb.2012.00274 , 2012.

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(1), 203–215, doi:10.5194/bg-9-203-2012, 2012.

Sandel, V., Kiko, R., Brandt, P., Dengler, M., Stemmann, L., Vandromme, P., Sommer, U. and Hauss, H.: Nitrogen Fuelling of the Pelagic Food Web of the Tropical Atlantic, edited by A. C. Anil, PLoS ONE, 10(6), e0131258, doi:10.1371/journal.pone.0131258, 2015.

Sanz-Alférez, S. and del Campo, F. F.: Relationship between nitrogen fixation and nitrate metabolism in the *Nodularia* strains M1 and M2, Planta, 194(3), 339–345, 1994.

~~Sarkar, D.: Lattice, Springer New York, New York, NY. 2008.~~

Schafstall, J., Dengler, M., Brandt, P. and Bange, H.: Tidal-induced mixing and diapycnal nutrient fluxes in the Mauritanian upwelling region, J. Geophys. Res., 115, C10014, doi:10.1029/2009JC005940, 2010.

Schelske, C. L. and Sicko-Goad, L.: Effect of Chelated Trace Metals on Phosphorus Uptake and Storage in Natural Assemblages of Lake Michigan Phytoplankton, Journal of Great Lakes Research, 16(1), 82–89, doi:10.1016/S0380-1330(90)71400-1, 1990.

Sharp, J. H.: Improved analysis for “particulate” organic carbon and nitrogen from seawater, Limnol. Oceangr., 19(6), 984–989, doi:10.4319/lo.1974.19.6.0984, 1974.

Sohm, J. A. and Capone, D. G.: Phosphorus dynamics of the tropical and subtropical north Atlantic: Trichodesmium spp. versus bulk plankton, Mar. Ecol. Prog. Ser., 317, 21–28, doi:10.3354/meps317021, 2006.

Sohm, J. A., Webb, E. A. and Capone, D. G.: Emerging patterns of marine nitrogen fixation, Nat. Rev. Microbiol., 9(7), 499–508, doi:10.1038/nrmicro2594, 2011.

Stewart, R. I. A., Dossena, M., Bohan, D. A., Jeppesen, E., Kordas, R. L., Ledger, M. E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J. B., Suttle, B., Thompson, R., Trimmer, M. and Woodward, G.: Mesocosm Experiments as a Tool for Ecological Climate-Change Research, Adv. Ecol. Res, 48, 71–181, 2013.

Subramaniam, A., Mahaffey, C., Johns, W. and Mahowald, N.: Equatorial upwelling enhances nitrogen

fixation in the Atlantic Ocean, Geophys. Res. Lett., 40(9), 1766–1771, doi:10.1002/grl.50250, 2013.

Trimmer, M. and Nicholls, J. C.: Production of nitrogen gas via anammox and denitrification in intact sediment cores along a continental shelf to slope transect in the North Atlantic, Limnol. Oceangr., 54(2), 577–589, doi:10.4319/lo.2009.54.2.0577, 2009.

Tyrrell, T.: The relative influences of nitrogen and phosphorus on oceanic primary production, Nature, 400(6744), 525–531, doi:10.1038/22941, 1999.

Tyrrell, T., Maranon, E., Poulton, A. J., Bowie, A. R., Harbour, D. S. and Woodward, E.: Large-scale latitudinal distribution of *Trichodesmium spp.* in the Atlantic Ocean, J. Plankton Res., 25(4), 405–416, doi:10.1093/plankt/25.4.405, 2003.

Ward, B. A., Dutkiewicz, S., Moore, C. M. and Follows, M. J.: Iron, phosphorus, and nitrogen supply ratios define the biogeography of nitrogen fixation, Limnol. Oceangr., 58(6), 2059–2075, doi:10.4319/lo.2013.58.6.2059, 2013.

Weber, T. and Deutsch, C.: Local versus basin-scale limitation of marine nitrogen fixation, PNAS, 111(24), 8741–8746, doi:10.1073/pnas.1317193111, 2014.

~~Wood, S. N.: Stable and efficient multiple smoothing parameter estimation for generalized additive models, J. Am. Statist. Assoc., 99(467), 673–686, doi:10.1198/016214504000000980, 2004.~~

~~Wood, S. N.: Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models, J. R. Stat. Soc. Ser. B Stat. Methodol., 73(1), 3–36, doi:10.1111/j.1467-9868.2010.00749.x, 2011.~~

Zani, S., Mellon, M. T., Collier, J. L. and Zehr, J. P.: Expression of nifH genes in natural microbial assemblages in Lake George, New York, detected by reverse transcriptase PCR, Appl. Environ. Microbiol., 66(7), 3119–3124, 10.1128/AEM.66.7.3119-3124.2000, 2000.

Zehr, J. P. and Turner, P. J.: Nitrogen fixation: Nitrogenase genes and gene expression, Methods in Microbiology, 30, 271–286, doi:10.1016/S0580-9517(01)30049-1, 2001.

~~Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M.: Mixed Effects Models and Extensions in Ecology with R, edited by M. Gail, K. Krickeberg, J. M. Samet, A. Tsiatis, and W. Wong, Springer, New York. 2009.~~

Tables

Table 1: Primers and Probes used in *nifH* TaqMan qPCR assays.

Target Group	Reverse Primer (5'-3')	Forward Primer (5'-3')	Probe (5'-3')
Filamentous (Fil)	GCAAATCCACCGCAAACAAC	TGGCCGTGGTATTATTACTGCTATC	AAGGAGCTTATACAGATCA
UCYN-A	TCAGGACCACCGGACTCAAC	TAGCTGCAGAAAGAGGAACTGTAGAAG	TAATTCCTGGCTATAACA
UCYN-B	TCAGGACCACCAGATTCTACACACT	TGCTGAAATGGGTTCTGTTGAA	CGAAGACGTAATGCTC
UCYN-C	GGTATCCTTCAAGTAGTACTTCGTCTAGCT	TCTACCCGTTTGATGCTACACACTAA	AAACTACCATTCTTCACTGCAG
GamAO	AACAATGTAGATTTCCTGAGCCTTATTC	TTATGATGTTCTAGGTGATGTG	TTGCAATGCCTATTCG
Het I (Rich-Rizo)	AATACCACGACCCGCACAAC	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCGTGT
Het II (Rich-Hemi)	AATGCCGCGACCAGCACAAC	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCGTGT

Figure captions

Figure 1: Experimental design and initial nutrient supply conditions during *varied P* (blue circles) and *varied N* (red diamonds). "Cornerpoints" during *varied P* and *varied N* are depicted as grey circles and white diamonds, respectively. Error bars denote the standard deviation of replicated (n=3) treatments.

Figure 2: Temporal development of (A) NO_3^- and NO_2^-, (B) PO_4^{3-}, (C) Chl a, (D) POC, (E) PON and (F) POP within all treatments of both experimental runs. Standard ~~errors~~ deviations are depicted as shaded error bands.

Figure 3: Maximum POC, PON and POP accumulation~~build-up~~ as a function of the initial supply of NO_3^-, PO_4^{3-} and N/P. Maximum δ POM is defined as peak POM concentration subtracted by the initial (day 1) POM concentration. Treatments in *varied P* are depicted as blue circles; treatments in *varied N* are depicted as red diamonds. Error bars denote the standard deviation of replicated (n=3) treatments. Regression lines (continuous lines) indicate significant linear correlations between the initial nutrient supply and POM accumulation.

Figure 4: PON/POP stoichiometry during (A) the exponential growth phase and (B) the stationary growth phase of the experiment. The grey line visualizes the Redfield Ratio. The color code, symbols and lines are the same as in Fig. 3~~The color code is the same as in Fig. 3~~.

Figure 5: Temporal development of DOP with standard ~~errors~~ deviations depicted as shaded error bands.

Figure 6: Positive linear correlation between maximum DOP accumulation (defined as peak DOP concentration subtracted by the initial DOP concentration) and initial PO_4^{3-} supply during *varied P* (blue circles) and *varied N* (red diamonds).~~Maximum DOP build-up (defined as peak DOP concentration subtracted by the initial DOP concentration) as a function of initial P supply during *varied P* (blue circles) and *varied N* (red diamonds).~~

Figure 7: Mean N_2 fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Asterisks indicate a significant difference between day 2 and day 8 (t-test). Error bars indicate the standard deviation.

Figure 8: Temporal development of transcript abundances for (A) *Richelia-Rhizosolenia* (Het I) and filamentous cyanobacteria related to *Trichodesmium* (Fil). Standard deviations are depicted as shaded error bands.

~~Figure 8: Selected gene count models over time. Predicted counts (solid lines) with 95% confidence intervals (CI; dashed lines) are plotted along with measured count data. The predictive model is based on the original (untransformed) gene counts. For better visualization, values were square root-transformed prior to plotting.~~

~~Figure 9: Selected transcript count models over time. Predicted counts (solid lines) with 95% confidence intervals (CI; dashed lines) are plotted along with measured count data. The predictive model is based on the original (untransformed) transcript counts. For better visualization, values were square root-transformed prior to plotting.~~

Figure 9~~10~~: Dynamics of PO_4^{3-}, POP and DOP and N_2 fixation rates in ~~all~~ mesocosms during *varied P*. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments.