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Changing nutrient stoichiometry affects phytoplankton production, DOP accumulation 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) ratio could reach the euphotic layer, possibly influencing primary production in those waters. Previous mesocosm studies in the oligotrophic Atlantic Ocean identified nitrate availability as a control of primary production, while a possible co-limitation of nitrate and phosphate could not be ruled out. To better understand the impact of changing N : P ratios on primary production and 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 was supplied at 15 µmol L^{-1} in all mesocosms. We monitored nutrient drawdown, biomass accumulation and nitrogen fixation in response to variable nutrient stoichiometry. Our results confirmed nitrate to be the key factor determining primary production. We found that excess phosphate was channeled through particulate organic matter (POP) into the dissolved organic matter (DOP) pool. In mesocosms with low inorganic phosphate 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 nitrate was still available, indicating that bioavailable N does not necessarily suppress N_2 fixation. We observed a shift from a mixed cyanobacteria–proteobacteria dominated active diazotrophic community towards a diatom-diazotrophic association 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 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 resulting extensive primary production forms the basis for high biomass development and a productive food web (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 phosphate (P), silicon (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 nutrients (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. 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 ratios – 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 N and P in seawater as well as in phytoplankton is 16 : 1, a deviation of dissolved inorganic nutrients from this ratio could indicate which nutrient can 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 and phosphate 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 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, Cabo Verde. The night before the start of each experiment, surface water was collected with RV *Islandia* 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 16 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 and measuring the resulting silicic acid concentration. The volume ranged from 105.5–145 L. 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 nitrate and phosphate. In the first experiment, the phosphate supply was changed at constant nitrate supply (*varied P*) in 13 of the 16 units, while in the second experiment the nitrate supply was changed at constant phosphate supply (*varied N*) in 12 of the 16 units. Each of these nutrient treatments was replicated 3 times. In addition, "cornerpoints" were chosen, where both the nitrate and phosphate 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 immedi-

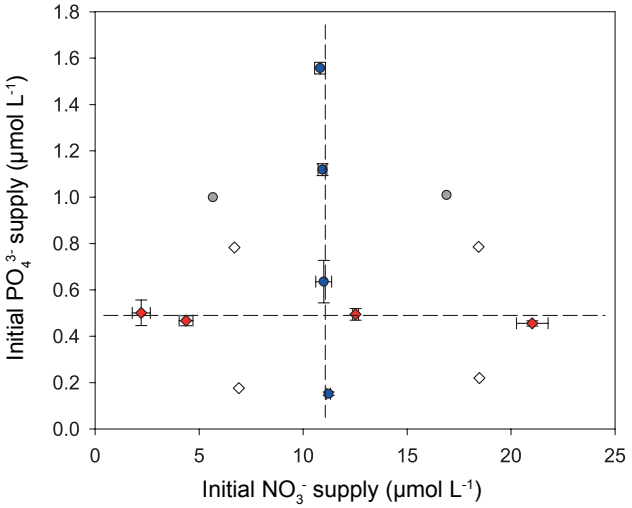

Figure 1. Experimental design and initial nutrient supply conditions during *varied P* (blue circles) and *varied N* (red diamonds). "Cornerpoints" during *varied P* and *varied N* are depicted as gray circles and white diamonds, respectively. Error bars denote the standard deviation of replicated ($n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

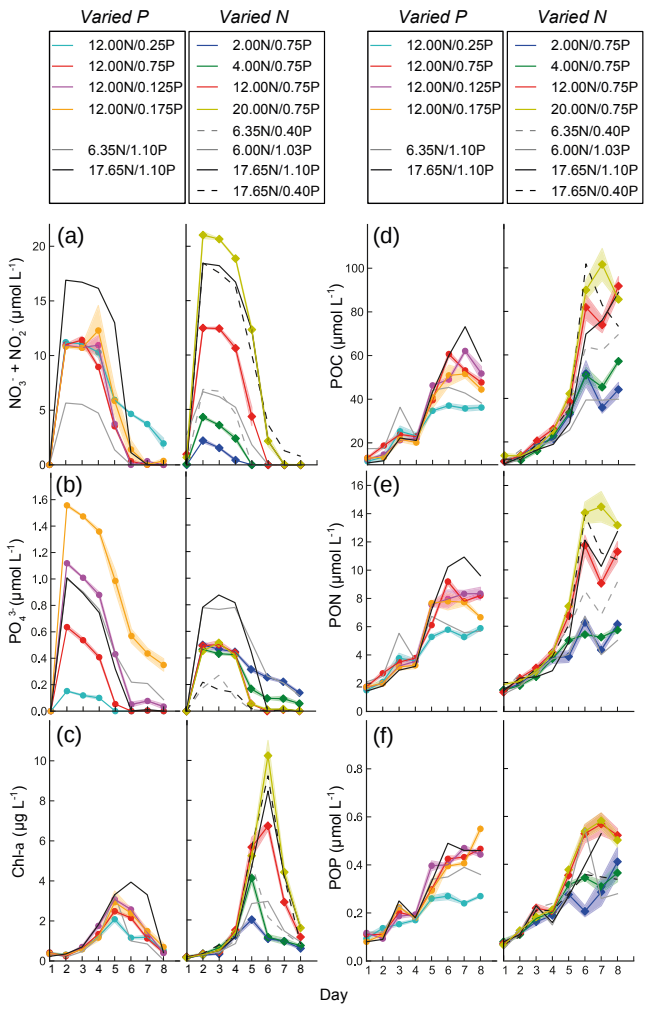

Figure 2. Temporal development of **(a)** NO_3^- and NO_2^-, **(b)** PO_4^{3-}, **(c)** Chl *a*, **(d)** POC, **(e)** PON and **(f)** POP within all treatments of both experimental runs. Standard deviations are depicted as shaded error bands.

ately after filling of the mesocosms on day 1. After nutrient manipulation, sampling was conducted on a daily basis between 09:00 and 10:30 Cape Verde Time (CVT) 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. Table S1 in the Supplement 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 justified using the term mesocosm, as we conducted our experiments with natural communities consisting of at least three trophic levels (bacteria, phytoplankton, microzooplankton).

2.2 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 $Si(OH)_4$.

2.3 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 h. 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).

2.4 Dissolved organic phosphorus

Water samples for analyses were filtered through precombusted (450 °C, 5 h) Whatman GF/F filters (25 mm, 0.7 µm). The filtrate was stored in acid-clean 60 mL HDPE bottles (5 % HCl for at least 12 h) 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 min. Samples were then analyzed 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

$$DOP = TDP - PO_4^{3-} \quad (1)$$

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	AAGGAGCTTATACAGATCTA
UCYN-A	TCAGGACCACCGGACTCAAC	TAGCTGCAGAAAGAGGAACTGTAGAAG	TAATTCCTGGCTATAACAAC
UCYN-B	TCAGGACCACCAGATTCTACACACT	TGCTGAAATGGGTTCTGTTGAA	CGAAGACGTAATGCTC
UCYN-C	GGTATCCTTCAAGTAGTACTTCGTCTAGCT	TCTACCCGTTTGATGCTACACACTAA	AAACTACCATTCTTCACTTAGCAG
GamAO	AACAATGTAGATTTCCTGAGCCTTATTC	TTATGATGTTCTAGGTGATGTG	TTGCAATGCCTATTCG
Het I (Rich-Rizo)	AATACCACGACCCGCACAAC	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT
Het II (Rich-Hemi)	AATGCCGCGACCAGCACAAC	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGTGT

2.5 Particulate organic matter

Particulate organic matter concentrations were determined by filtering 0.5–1 L seawater through pre-combusted (450 °C for 5 h) 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 (37 %, for 24,h) in order to remove inorganic carbon. After drying, filters were wrapped in tin cups ($8 \times 8 \times 15$ 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).

2.6 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.

2.7 $^{15}N_2$ 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 3 L 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 was 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 h 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).

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 and phosphate were readily taken up by the plankton community and nutrient concentrations thus declined

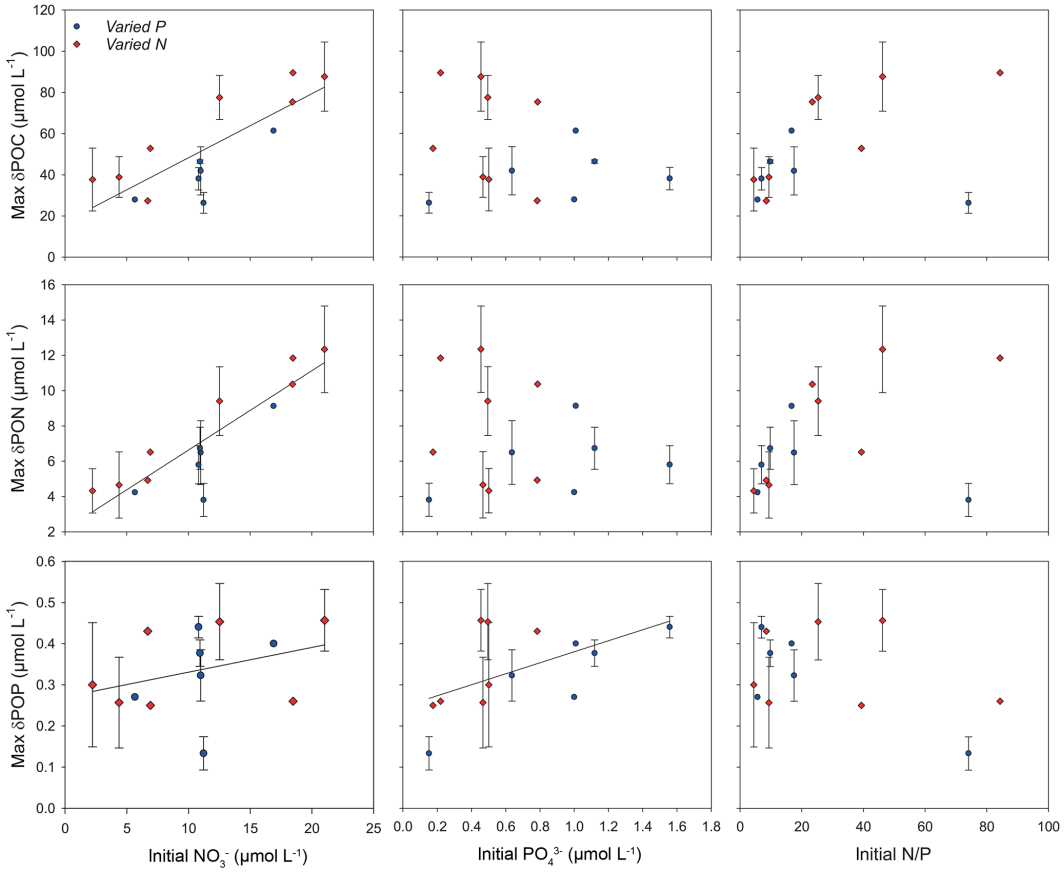

Figure 3. Maximum POC, PON and POP accumulation as a function of the initial supply of NO_3^-, PO_4^{3-} and N/P. Maximum δPOM is defined as peak POM concentration subtracted by the initial (day 1) POM concentration. Treatments in *varied P* are depicted as blue circles; treatments in *varied N* are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

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 ratio.

Although initial Chl *a* concentrations were slightly higher in *varied P* than in *varied N* ($\sim$ 0.38 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 and 0.6–1.7 µg L^{-1} in *varied P* and *varied N*, respectively (Fig. 2).

3.2 Particulate organic matter (POM) accumulation and stoichiometry

Temporal dynamics of POM were similar during both experiments. Initial concentrations of POC, PON and POP were 10–17, 1.5–2 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 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 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 showed a significantly positive regression coefficient to initial PO_4^{3-} supply ($r^2 = 0.31$, $p = 0.048$).

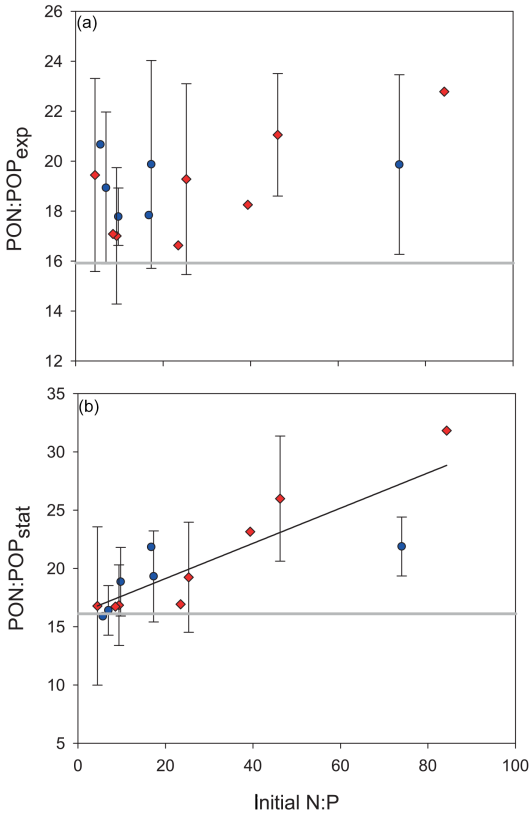

Figure 4. PON/POP stoichiometry during **(a)** the exponential growth phase and **(b)** the stationary growth phase of the experiment. The gray line visualizes the Redfield Ratio. The color code, symbols and lines are the same as in Fig. 3.

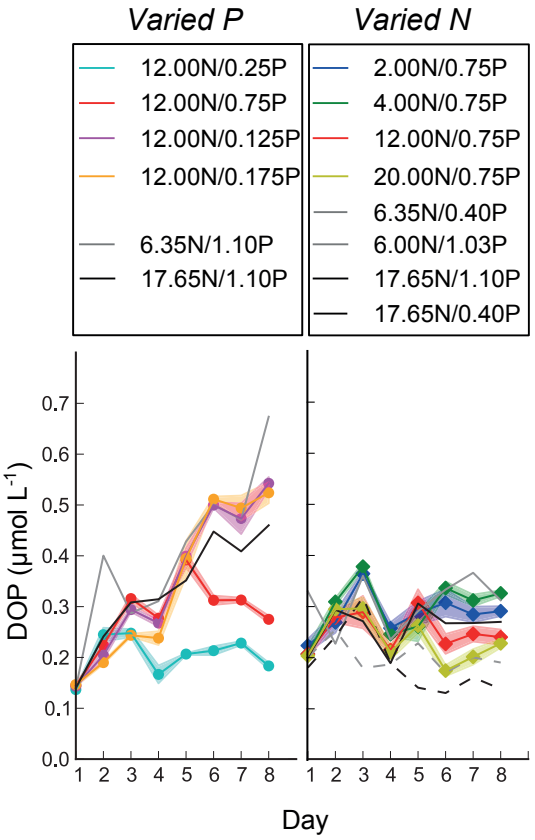

Figure 5. Temporal development of DOP with standard deviations depicted as shaded error bands.

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 17 and 23, 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 : 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 16 and 32) 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). 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. Maximum DOP accumulation (defined as described for maximum POM accumulation, Sect. 3.2) was significantly correlated to the initial PO_4^{3-} supply (Fig. 6; $r^2 = 0.63$, $p = 0.0007$).

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. 7).

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 %).

Changes in transcript abundance over time were most intense for *Richelia-Rhizosolenia* (Het I) transcripts (Fig. 8). At day 2, 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 Het I transcript abundances between day 6 and 8 in *varied P*.

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, whereas diazotrophs of the *Richelia-Rhizosolenia* symbiosis were the only diazotrophs that showed an increase in *nifH* transcripts over the course of the experiment, exclusively in *varied P* (Fig. 8). During *varied N*, *nifH* gene and transcript abundance of the *Richelia-Rhizosolenia* cluster was close to the detection limit and DOP accumulation was rather negligible. In contrast, we observed an accumulation of DOP 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 phosphorus-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 accumulation 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.

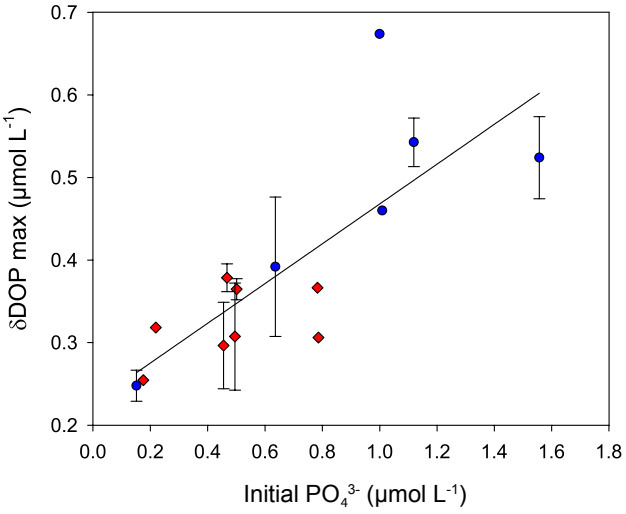

Figure 6. Positive linear correlation between maximum DOP accumulation (defined as peak DOP concentration subtracted by the initial DOP concentration) and initial PO_4^{3-} supply during *varied P* (blue circles) and *varied N* (red diamonds).

4 Discussion

4.1 Controls on plankton production

In order to understand potential consequences of changes in nutrient regimes, it is necessary to determine the factors that control and limit microbial production. In our experiments, amendments of NO_3^- significantly increased chlorophyll concentrations and enhanced the accumulation of POM, indicating the ability of the plankton community to rapidly and intensively react to nitrate availability. These results suggest that the ultimate limiting nutrient for phytoplankton 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 Cabo Verde region. For the upwelling region as well as for the oligotrophic open ocean of the ETNA, nitrate 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 nitrate and phosphate during nutrient addition bioassay experiments in the ETNA. In our experiment, however, only POP accumulation was positively affected by PO_4^{3-} supply. This argues against a secondary limitation by phosphate, 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 the 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 39 in *varied N* and 22 in *varied P* were measured. However, during growth phases in both experiments PON : POP ratios never fell below 16. 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 Africa 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 stoichiom-

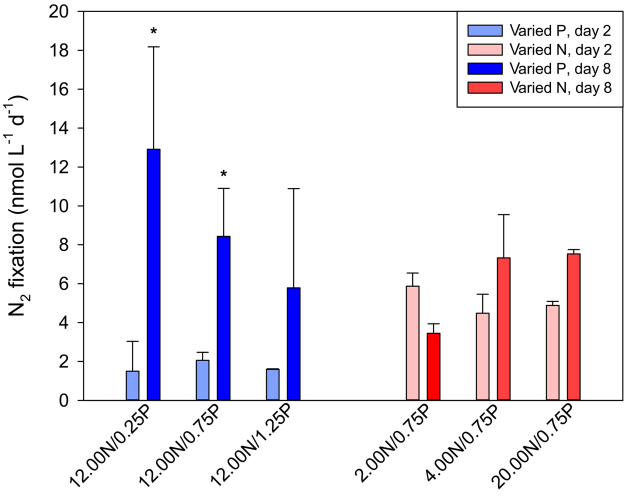

Figure 7. Mean N_2 fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Asterisks indicate a significant difference between day 2 and day 8 (*t* test). Error bars indicate the standard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

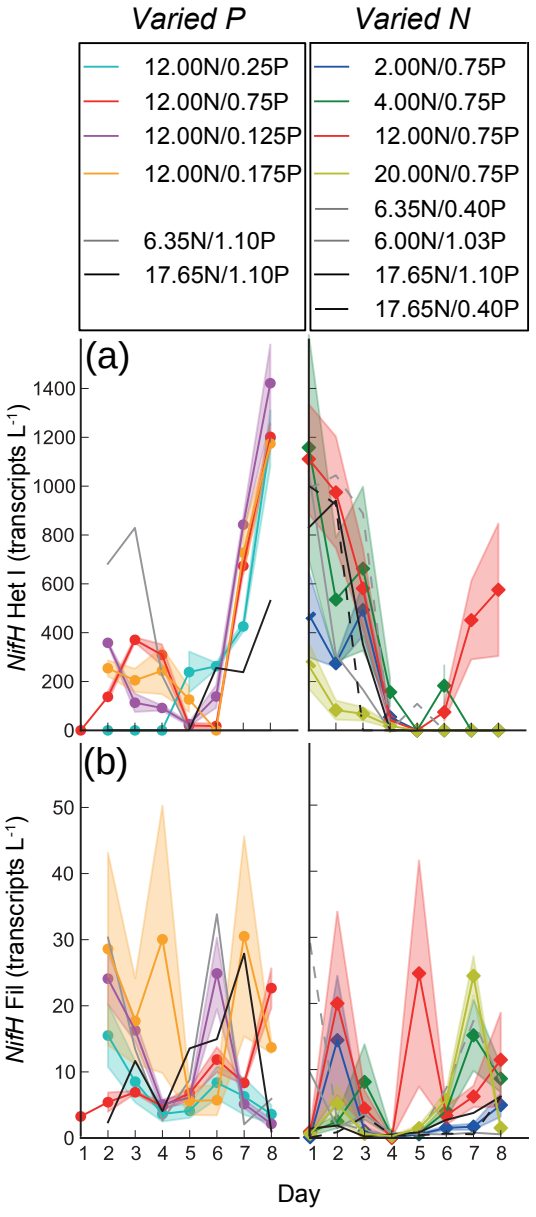

Figure 8. Temporal development of transcript abundances for **(a)** *Richelia-Rhizosolenia* (Het I) and filamentous cyanobacteria related to *Trichodesmium* (Fil). Standard deviations are depicted as shaded error bands.

etry of phytoplankton communities is flexible to a certain extent, but 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 if it lacks organisms able to assemble a P-rich growth machinery (Klausmeier et al., 2004; Arrigo, 2005). It has been reported that cellular N content seems relatively inflexible in some phytoplankton groups, thus restricting the maintenance of metabolic processes at low dissolved inorganic nitrogen concentrations (Moore et al., 2013). In contrast, phosphate 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 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 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 concentrations in *varied N*, no significant difference in N_2 fixation rates could be detected. Evidence from culture experiments also suggests that inorganic N compounds do not 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

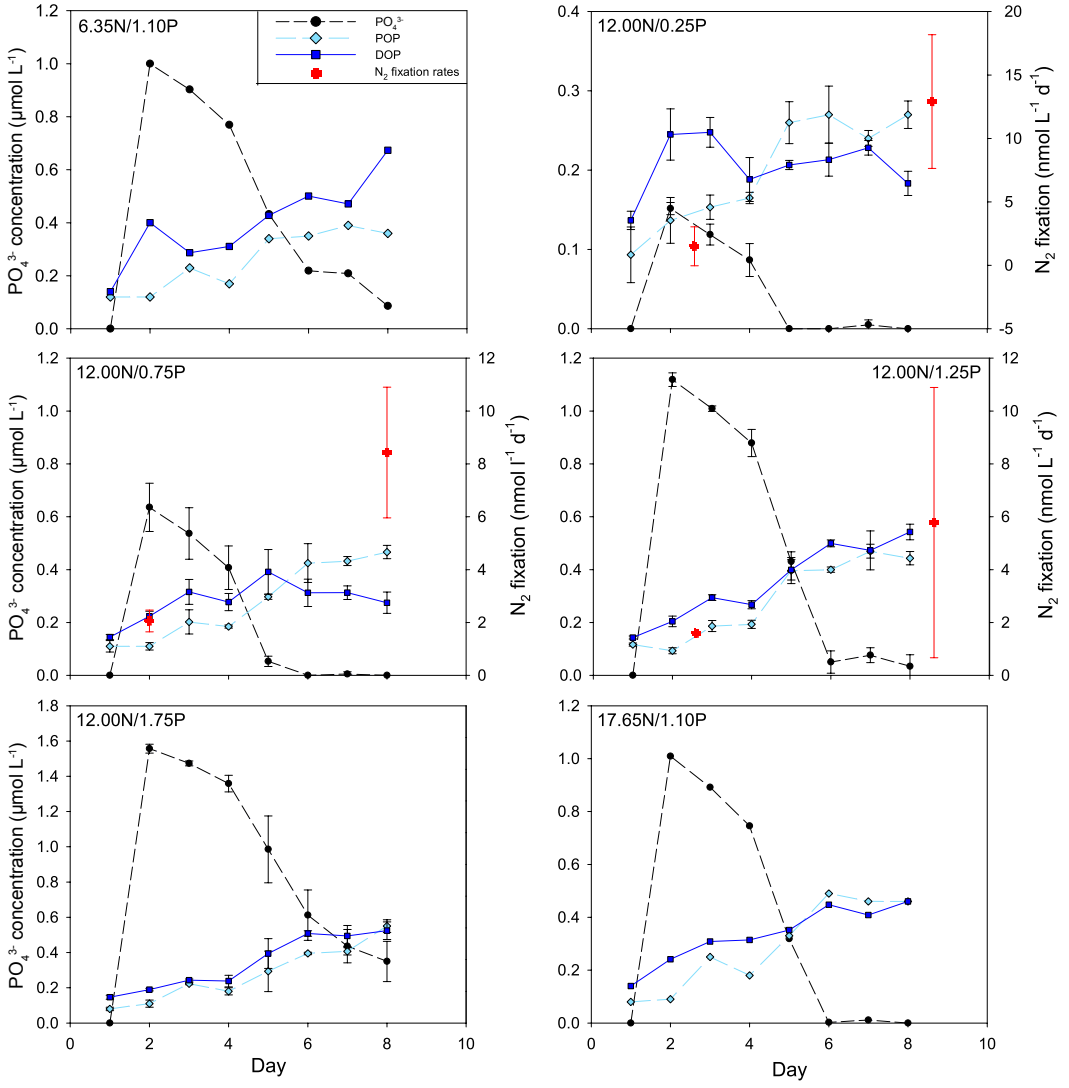

Figure 9. Dynamics of PO_4^{3-}, POP and DOP and N_2 fixation rates in mesocosms during *varied P*. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatment.

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 modeling studies predict high N_2 fixation rates in waters containing measurable amounts of 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 in mesocosms with N : P supply ratios below Redfield. Instead, excess phosphate 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 syn-

thesize and excrete higher levels of DOP under excess phosphate conditions remains unclear, but enhanced PO_4^{3-} 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 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-} (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 detected in *varied P*. In mesocosms with highest N_2 fixation rates, PO_4^{3-} was depleted after day 5 or 6 while POP increased until the end of the experiment. After PO_4^{3-} depletion, DOP concentrations declined, which indicates that DOP served as phosphorus 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 *varied P* experiment. While the diatom abundance was probably favored by replete amounts of silicic acid 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 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 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. Berthelot et al., 2015; Karl and Letelier, 2008; Karl et al., 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 implication for the 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 phosphate 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 acid and DOP, and not by the absence of fixed N compounds.

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Author contributions. H. Hauss and R. Kiko designed the experiment with input from J. Meyer, C. R. Löscher, A. Loginova, C. Borchard, U. Riebesell, R. A. Schmitz; led the logistics and the study on site and provided nutrient and hydro-chemical data sets. J. Meyer, R. Kiko, A. F. Reichel, A. Loginova, C. Borchard and H. Hauss conducted the sampling of particulate and dissolved matter. J. Meyer and A. F. Reichel performed DOM and POM measurements, C. R. Löscher performed N_2 fixation and molecular experiments and measurements. J. Meyer and C. R. Löscher wrote the manuscript with input from all co-authors. All data will be uploaded at www.pangaea.de upon publication.

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