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 N2 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 N2 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 N2 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, Karsten et al., 2008). These low oxygen conditions support denitrification and anammox that remove bioavailable nitrogen (N) from the water column (e.g. Codispoti et
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∗
ETNA) are usually above 40 µmol kg⁻¹. Oxygen concentrations within the oxygen minimum in the further increase this P deficit in the future (Duce et al., 2008). Atmospheric anthropogenic nitrogen into the open ocean could suggest a severe P limitation of primary producers (Ammerman et al., 2003; Mills et al., 2004). Additional input of at-
gestings 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 (Haus 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; Löschner 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 N₂-fixation (Deutsch et al., 2007). The conversion of readily available dissolved N₂ 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 N₂ fixation might be stimulated by an enhanced DOP supply under low N : P ratios (Franz et al., 2012).

Until recently, oceanic N₂ 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 N₂ 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 dia-
zotrophs are either outcompeted by fast growing phytoplank-
ton 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$_2$ fixa-
tion (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 (Amerman et al., 2003; Mills et al., 2004). Nevertheless, a diverse community of cyanobacte-
rial diazotrophs such as Trichodesmium (Capone et al., 1997;
Tyrrell et al., 2003), a variety of unicellular cyanobacterial di-
azotrophs (Groups A, B, C, diatom-symbionts; Falcon et al.,
2002; Langlois et al., 2005) as well as non-cyanobacterial di-
azotrophs such as different clades of proteobacteria are abun-
dant and widely distributed (e.g. Langlois et al., 2005, 2008).
Those diazotrophs have previously been demonstrated to ac-
tively 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 phos-
phate 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 un-
ravel 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 dia-
zotrophic community and their response to the chosen treat-
ment levels. The experimental design and response variables
were chosen in order to assess responses of the phytoplank-
ton community to possible changes in oceanic nutrient sto-
ichiometry as a consequence of ocean deoxygenation.

2 Methods

2.1 Experimental setup

In October 2012 we conducted two 8-day mesocosm ex-
periments 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 inter-
mediate bulk containers. Containers for water transport were
first rinsed with diluted HCl and several times with deionized
water. The experimental setup comprised 16 plastic meso-
cosm bags, which were distributed in four flow-through wa-
ter 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 bub-
bles. The volume inside each mesocosm was calculated af-
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$_2^-$), phos-
phate (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 con-
stant 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 cor-
nerpoints should have been repeated, but due to erroneous
nutrient levels in mesocosm 10 during varied N, this meso-
cosm also was adjusted to the center point conditions. Exper-
imental treatments were randomly distributed between the
four water baths. Initial sampling was carried out immedi-
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.

Figure 2. Temporal development of (a) NO$_3^-$ and NO$_2^-$, (b) PO$_4^{3-}$, (e) Chl a, (d) POC, (e) PON and (f) POP within all treatments of both experimental runs. Standard deviations are depicted as shaded error bands.

early 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$_3^-$ and PO$_4^{3-}$, 0.03 µmol L$^{-1}$ for NO$_2^-$ 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 precultured (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 $-20^\circ$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

\[
DOP = TDP - PO_4^{3-}
\]
Table 1. Primers and probes used in nifH TaqMan qPCR assays.

<table>
<thead>
<tr>
<th>Target group</th>
<th>Reverse primer (5′-3′)</th>
<th>Forward primer (5′-3′)</th>
<th>Probe (5′-3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filamentous (Fil)</td>
<td>GCAAATCCACCGCAAAACAC</td>
<td>TAGGCTGAGAAGAGAAAGTCTAGAAG</td>
<td>AAGGAGCTATTATACAGATCTA</td>
</tr>
<tr>
<td>UCYN-A</td>
<td>TCAAGGACACCGGGATCACAA</td>
<td>TAGGCTGAGAAGAGAAAGTCTAGAAG</td>
<td>TAATTCCTGCGTAAACAC</td>
</tr>
<tr>
<td>UCYN-B</td>
<td>TCAAGGACACCGGATGACTACCT</td>
<td>TGGCGGAAATGGTCTGCTGAGAA</td>
<td>CCAGAAGCTGATAGCTC</td>
</tr>
<tr>
<td>UCYN-C</td>
<td>GGATTCCTTCAGTTACTCTAGCT</td>
<td>TCTACCCGTTGAGCTCTACACTAA</td>
<td>AAATACCATCTCTACTAGCAG</td>
</tr>
<tr>
<td>GamAO</td>
<td>AACAGTCTTAGTTCTGAGCCCTTATTC</td>
<td>TTAAGTGTTCTACAGGTTGTG</td>
<td>TGGCAATGGCCTATTGGOG</td>
</tr>
<tr>
<td>Het I (Rich-Rizo)</td>
<td>AAATACACGCCCGCAACAAC</td>
<td>CGGTTCCCGTGTTGACGTT</td>
<td>TCCGGTGGTCGTCAGCTGCGT</td>
</tr>
<tr>
<td>Het II (Rich-Hemi)</td>
<td>AATGCCCGGACCGACACAC</td>
<td>TGTTACCGTGATGTAGCTTT</td>
<td>TCTGTTGGTCGTCAGCTGCGT</td>
</tr>
</tbody>
</table>

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 × 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 Oxisol (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 nifH 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 10^2 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 15N2 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 15N2 gas was (depending on the exact water volume in the Tedlar® bag) around 10 mL 15N2 per 1 L seawater. Dissolution of the 15N2 gas was achieved by “slapping” the bubble with a ruler. After complete dissolution of the added 15N2 gas (15N2-enriched seawater), an aliquot of the 15N2 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 15N2 and 13C-NaCO. Incubations were performed in 4.5 L polycarbonate bottles closed with Teflon®-coated butyl rubber septum caps. The 15N2 concentration in the prepared batches of enriched water was determined to be 250 µmol L−1, which translates in an 15N-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.
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 (∼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 ($r^2 = 0.31$, $p = 0.048$).
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 ($\pm 0.009$) $\mu$mol 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 $\mu$mol 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 ($\pm 0.038$) $\mu$mol 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 ($P_{\text{poolX}}$) at the end of the experiment ($P_{\text{poolX}}$ in varied $P$ $\sim 25\%$ of the initial P pool, $P_{\text{poolX}}$ in varied $N$ $\sim 14\%$). 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 N2 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 PO4-3 was depleted (Fig. 9). In mesocosm 12.00N/0.75P, PO4-3 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 PO4-3 depletion and highest POP accumulation exceeded values that could be explained by PO4-3 incorporation alone. Thus a potential impact of DOP on diazotrophy is hypothesized. In mesocosms without a significant increase in N2 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 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 NO3- 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 NO3-. N2 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 PO4-3 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 stoichiometric limitations...
Figure 7. Mean N\textsubscript{2} fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N\textsubscript{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.

4.2 The impact of bioavailable N on N\textsubscript{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\textsubscript{2} fixation in our experiments. Despite a wide spectrum of applied nitrate concentrations in varied N, no significant difference in N\textsubscript{2} fixation rates could be detected. Evidence from culture experiments also suggests that inorganic N compounds do not always repress N\textsubscript{2} fixation. While NO\textsubscript{3} addition in Trichodesmium spp. (Mulholland et al., 2001; Holl and Montoya, 2005) and NH\textsubscript{4} addition in Crocosphaera watsonii (Dekaezemacker and Bonnet, 2011) reduced N\textsubscript{2} fixation rates, NO\textsubscript{3} addition did not reduce N\textsubscript{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-
the size 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. However, it is not clear whether the ability to utilize DOP has been demonstrated for diazotrophs in the ETNA. This indicates that the ability to utilize DOP may give diazotrophs a competitive advantage when bioavailable forms of N are depleted and either PO$_4^{3-}$, apply 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 (Löschler 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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