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Changing nutrient stoichiometry affects phytoplankton production, DOP 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 N : P ratio could reach the euphotic layer, possibly influencing primary production in those waters. Previous mesocosm studies in the oligotrophic Atlantic Ocean identified N availability as controlling 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). Silicate was supplied at 15 µmol L^{-1} in all mesocosms. We monitored nutrient drawdown, bloom formation, biomass build up and diazotrophic feedback in response to variable nutrient stoichiometry. Our results confirmed N to be limiting to primary production. We found that excess P was channeled through particulate organic matter (POP) into the dissolved organic matter (DOP) pool. In mesocosms with low 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 was still available, indicating that bioavailable N does not necessarily has to have a negative impact on N_2 fixation. We observed a shift from a mixed cyanobacterial/proteobacterial dominated active diazotrophic community towards diazotrophic diatom 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.

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1 Introduction

Eastern boundary upwelling systems are characterized by cold, nutrient-rich water masses that are transported from intermediate water layers towards the surface. Resulting extensive primary production forms the basis for high biomass development and a productive food chain (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 phosphorus (P), silicate (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 phosphorus (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 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 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). Oxygen concentrations

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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 denitrification – with an accompanied decrease in N : P levels – 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 growth (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 stochiometry (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

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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 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.,

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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 N and 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.

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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. 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 silicate and measuring the resulting silicate concentration. The volume ranged from 105.5–145 liters. Experimental manipulation was achieved by adding different amounts of inorganic N and P. 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. In addition to this, "cornerpoints" were chosen, where both the N and P supply was changed. 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 LT 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 silicate. Table S1 gives the target nutrient concentrations and corresponding measured concentrations in the mesocosms.

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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 usually 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 feel justified in using the term mesocosm, as we conducted our experiment with a natural community consisting of at least 3 different trophic levels (bacteria, phytoplankton, microzooplankton).

2.2 Nutrients

Samples 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 according to Grasshoff et al. (1999).

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 (*Ana-*

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cystis 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 pre-combusted (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 analysed spectrophotometrically (Autoanalyzer QuAAtro Seal Analytic), following Bran and Luebbe AutoAnalyzer Method No. G-175-96 Rev. 13 (PO_4^{3-}).

DOP concentrations were calculated as:

DOP = total dissolved phosphorus (TDP) − dissolved inorganic phosphorus (DIP) (1)

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 mm × 8 mm × 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).

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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 2 and day 8 of both experiments as previously described by Mohr et al. (2010) and Großkopf et al. (2012). 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. 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).

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2.8 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 Supplement).

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

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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. NO_3^- and 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 (12.00N/0.25P in *varied P* and 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 (*varied P*: 6.35N/1.10P, 12.00N/1.25P, 12.00N/1.75P; *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.

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). 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 Supplement, Fig. S1).

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3.2 Particulate organic matter (POM) build-up 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 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 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 N supply (POC: $r^2 = 0.64$, $p = 0.006$; PON: $r^2 = 0.80$, $p < 0.0001$) while POP build-up showed a significantly positive regression coefficient to initial P 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 16.6 and 22.8, 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 15.9 and 31.9) 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} and increased in all mesocosms, except in the one with lowest initial P supply (12.00N/0.25P). Maximum 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

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concentrations increased progressively until the end of the experiment (Fig. 5). Maximum DOP build up (defined as described for maximum POM build up, Sect. 3.2) was significantly correlated to the initial P 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).

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*. 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.

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Changes in transcript abundance over time and Run ID (Fig. 8) showed mainly different response patterns between *nifH*_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 experiment, with a pronounced increase of *nifH*_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 (Figs. 7, 8, S3, and 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* (Figs. 8, S2).

In *varied P*, we observed an accumulation of DOP. In contrast, during *varied N, nifH* gene and transcript abundance of the *Richelia-Rhizosolenia* cluster was close to the detection limit and DOP build-up was rather negligible, thus a potential impact of DOP on diazotrophy was hypothesized. 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*, 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 P-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 build up exceeded values that could be explained by P incorporation alone. In mesocosms without a significant increase in N_2 fixation, POP and DOP con-

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centrations increased until the end of the experiment and no apparent uptake of DOP could be observed.

4 Discussion

4.1 Controls on primary production

In order to understand potential consequences of changes in nutrient regimes, it is necessary to determine the factors that control and limit primary production. In our experiments, amendments of N significantly increased chlorophyll concentrations and enhanced the buildup of POM, indicating the ability of the plankton community to rapidly and intensively react to N availability. These results indicate that the ultimate limiting nutrient for the phytoplankton community in our experiment was N. 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, N 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 N and P during nutrient addition bioassay experiments in the ETNA. In our experiment, however, only POP build up was positively affected by P supply. This argues against a secondary limitation by P, 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 producers 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 38.8 in *varied N* and 21.9 in *varied P* were measured. However, during growth phases in both experiments PON : POP ratios did never drop

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below 15.9. 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 WA 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 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). Reasons could be differences in the initial community composition that might lack organisms capable of assembling a P-rich growth machinery (Klausmeier et al., 2004; Arrigo, 2005) or other, yet unresolved factors. It has been reported that cellular N contents seems relatively inflexible in parts of the phytoplankton community, thus restricting the maintenance of metabolic processes at low dissolved inorganic nitrogen (DIN) concentrations (Moore et al., 2013). In contrast, 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 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 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

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spectrum of applied N concentrations in *varied N*, no significant change in N_2 fixation rates could be detected. Evidence from culture experiments also suggests that inorganic N compounds do not universally 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 predict high N_2 fixation rates in waters containing measurable amounts of fixed 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 P in mesocosm with N : P supply ratios below Redfield. Instead, excess P was absent and our data point towards a channeling of P through the particulate pool into DOP, as an increase in P supply significantly increased the concentration of DOP. Why phytoplankton synthesize and excrete higher levels of DOP under excess P conditions remains unclear, but enhanced P uptake (followed by DOP accumulation) is thought to hamper P limitation when sudden boosts in N are en-

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countered (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) showed the accumulation of DOP under excess 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 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 P* or DOP concentrations are sufficient.

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. Increasing diazotrophic transcript abundances of *Richelia intracellularis* in symbiosis with the diatom *Rhizosolenia* (Het I) were detected over the course of the same 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. 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 silicate 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 offshore transport.

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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 P 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 silicate and DOP, and not by the absence of fixed N compounds.

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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, 165–170, doi:10.1029/2003EO180001, 2003.

Arrigo, K. R.: Marine microorganisms and global nutrient cycles, Nature, 437, 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 mesocosms experiment (New Caledonia lagoon), Biogeosciences Discuss., 12, 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. Oceanogr., 48, 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. Cy., 23, 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, 1221–1229, doi:10.1126/science.276.5316.1221, 1997.

BGD
12, 9991–10029, 2015

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, 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, 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, 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. Oceanogr., 53, 1722–1733, doi:10.4319/lo.2008.53.5.1722, 2008.

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

Dekaezemacker, J. and Bonnet, S.: Sensitivity of N_2 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, 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. Cy., 15, 483–506, doi:10.1029/2000GB001291, 2012.

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, 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. Microb., 68, 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, 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 ma-

BGD
12, 9991–10029, 2015

rine surface waters are dominated by genes of non-cyanobacteria, edited by: Gilbert, J. A., PLoS ONE, 6, e19223, doi:10.1371/journal.pone.0019223, 2011.
Fernandez, C., Farías, L., and Ulloa, O.: Nitrogen fixation in denitrified marine waters, PLoS ONE, 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, 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, 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, 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, 1–17, 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, 146–157, doi:10.1093/plankt/fbs076, 2013.
Grasshoff, K., Kremling, K., and Ehrhardt, M.: Methods of Seawater Analysis, 3rd edn., edited by: Grasshoff, K., Kremling, K., and Ehrhardt, M., 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, 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, 361–364, doi:10.1038/nature11338, 2012.
Grover, J. P.: Resource Competition, Chapman & Hall, London, 1997.
Hansen, H. P. and Koroleff, F.: Determination of nutrients, in: Methods of Seawater Analysis, edited by: Grasshoff, K., Kremling, K., and Ehrhardt, M., Wiley-VCH Verlag GmbH, Weinheim, Germany, 159–228, 1999.

BGD
12, 9991–10029, 2015

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

Hecky, R. E., Campbell, P., and Hendzel, L. L.: The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans, Limnol. Oceanogr., 38, 709–724, 1993.

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

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

Ingall, E. and Jahnke, R.: Evidence for enhanced phosphorus regeneration from marine sediments overlain by oxygen depleted waters, Geochim. Cosmochim. Ac., 58, 2571–2575, doi:10.1016/0016-7037(94)90033-7, 1994.

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

Kalvelage, T., Jensen, M. M., Contreras, S., Revsbech, N. P., Lam, P., Günter, M., LaRoche, J., Lavik, G., and Kuypers, M. M. M.: Oxygen sensitivity of anammox and coupled n-cycle processes in oxygen minimum zones, PLoS ONE, 6, e29299, doi:10.1371/journal.pone.0029299.t003, 2011.

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

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

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

BGD
12, 9991–10029, 2015

Lagus, A.: Species-specific differences in phytoplankton responses to N and P enrichments and the N : P ratio in the Archipelago Sea, northern Baltic Sea, J. Plankton Res., 26, 779–798, doi:10.1093/plankt/fbh070, 2004.
Lam, P., Lavik, G., Jensen, M. M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez, D., Amann, R., Jetten, M. S. M., and Kuypers, M. M. M.: Revising the nitrogen cycle in the Peruvian oxygen minimum zone, P. Natl. Acad. Sci. USA, 106, 4752–4757, doi:10.1073/pnas.0812444106, 2009.
Langlois, R. J., LaRoche, J., and Raab, P. A.: Diazotrophic diversity and distribution in the tropical and subtropical Atlantic ocean, Appl. Environ. Microbiol., 71, 7910–7919, doi:10.1128/AEM.71.12.7910-7919.2005, 2005.
Langlois, R. J., Huemmer, D., and LaRoche, J.: Abundances and distributions of the dominant nifH phylotypes in the Northern Atlantic Ocean, Appl. Environ. Microbiol., 74, 1922–1931, doi:10.1128/AEM.01720-07, 2008.
Loescher, C. R., Großkopf, T., Desai, F. D., Gill, D., Schunck, H., Croot, P. L., Schlosser, C., Neulinger, S. C., Pinnow, N., Lavik, G., Kuypers, M. M. M., LaRoche, J., and Schmitz, R. A.: Facets of diazotrophy in the oxygen minimum zone waters off Peru, ISME J., 8, 2180–2192, doi:10.1038/ismej.2014.71, 2014.
Löscher, C. R., Kock, A., Könneke, M., LaRoche, J., Bange, H. W., and Schmitz, R. A.: Production of oceanic nitrous oxide by ammonia-oxidizing archaea, Biogeosciences, 9, 2419–2429, doi:10.5194/bg-9-2419-2012, 2012.
Mackey, K. R. M.: Phosphorus cycling in the red tide incubator region of Monterey Bay in response to upwelling, Front. Microbiol., 3, 1–14, doi:10.3389/fmicb.2012.00033, 2012.
Mahaffey, C., Reynolds, S., and Davis, C. E.: Alkaline phosphatase activity in the subtropical ocean: insights from nutrient, dust and trace metal addition experiments, Front. Mar. Sci., 1, 1–13, doi:10.3389/fmars.2014.00073, 2014.
Martin, P., Dyhrman, S. T., Lomas, M. W., Poulton, N. J., and Van Mooy, B. A. S.: Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus, P. Natl. Acad. Sci. USA, 111, 8089–8094, doi:10.1073/pnas.1321719111, 2014.
Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R.: Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic, Nature, 429, 292–232, doi:10.1038/nature02550, 2004.

BGD
12, 9991–10029, 2015

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

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

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

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

Mulholland, M. R. and Capone, D. G.: Stoichiometry of nitrogen and carbon utilization in cultured populations of Trichodesmium IMS101: implications for growth, Limnol. Oceanogr., 46, 436–443, doi:10.4319/lo.2001.46.2.0436, 2001.

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

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, 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, 291–294, doi:10.1038/nature01953, 2003.

Redfield, A. C.: The biological control of chemical factors in the environment, Am. Sci., 46, 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. Cy., 28, 805–824, doi:10.1002/2013GB004801, 2014.

BGD
12, 9991–10029, 2015

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., Publications Office of the European Union, Luxembourg, 95–112, 2010.

Ruttenberg, K. C.: Dissolved organic phosphorus production during simulated phytoplankton blooms in a coastal upwelling system, Front. Microbiol., 3, 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, 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, PLoS ONE, 10, 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, 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, J. Great Lakes Res., 16, 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. Oceanogr., 19, 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. vs. 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, 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.

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Subramaniam, A., Mahaffey, C., Johns, W., and Mahowald, N.: Equatorial upwelling enhances nitrogen fixation in the Atlantic Ocean, Geophys. Res. Lett., 40, 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. Oceanogr., 54, 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, 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, 405–416, doi:10.1093/plankt/25.4.405, 2003.
von Liebig, J.: Chemistry in its Application to Agriculture and Physiology, 3rd edn., edited by: Playfair, L., John Owen, Cambridge, 1842.
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. Oceanogr., 58, 2059–2075, doi:10.4319/lo.2013.58.6.2059, 2013.
Weber, T. and Deutsch, C.: Local vs. basin-scale limitation of marine nitrogen fixation, P. Natl. Acad. Sci. USA, 111, 8741–8746, doi:10.1073/pnas.1317193111, 2014.
Wood, S. N.: Stable and efficient multiple smoothing parameter estimation for generalized additive models, J. Am. Stat. Assoc., 99, 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. B Met., 73, 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, 3119–3124, doi:10.1128/AEM.66.7.3119-3124.2000, 2000.
Zehr, J. P. and Turner, P. J.: Nitrogen fixation: Nitrogenase genes and gene expression, Method. Microbiol., 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: Gail, M., Krickeberg, K., Samet, J. M., Tsiatis, A., and Wong, W., Springer, New York, 2009.

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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
GamA	AACAATGTAGATTTCCTGAGCCTTATTC	TTATGATGTTCTAGGTGATGTG	TTGCAATGCCTATTCG
Het I (Rich-Rizo)	AATACCACGACCCGCACAAC	CGGTTTCCGTGGTGTACGTT	TCCGGTGGTCCTGAGCCTGGTGT
Het II (Rich-Hemi)	AATGCCGCGACCAGCACAAC	TGGTTACCGTGATGTACGTT	TCTGGTGGTCCTGAGCCTGGTGT

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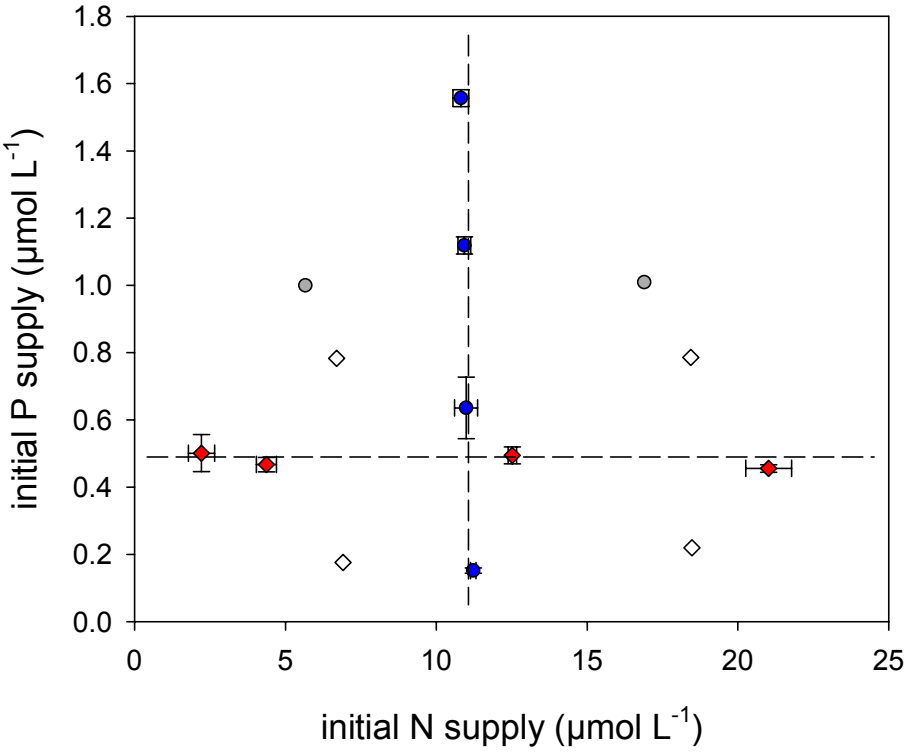

Figure 1. Experimental design and initial nutrient supply conditions during *varied P* (blue circles) and *varied N* (red diamonds). "Cornerpoints" during *varied P* and *varied N* are depicted as grey circles and white diamonds, respectively.

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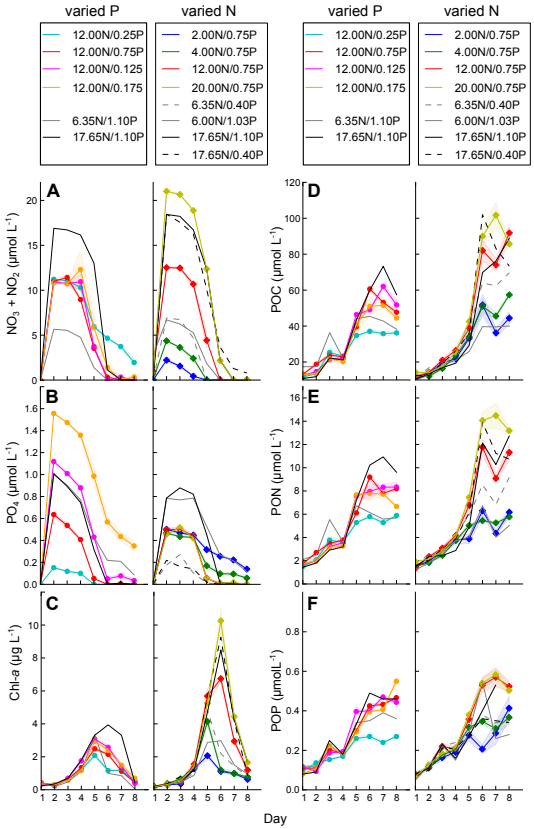

Figure 2. Temporal development of **(a)** NO_3^- and NO_2^-, **(b)** PO_4^{3-}, **(c)** Chl *a*, **(d)** POC, **(e)** PON and **(f)** POP within all treatments of both experimental runs. Standard errors are depicted as shaded error bands.

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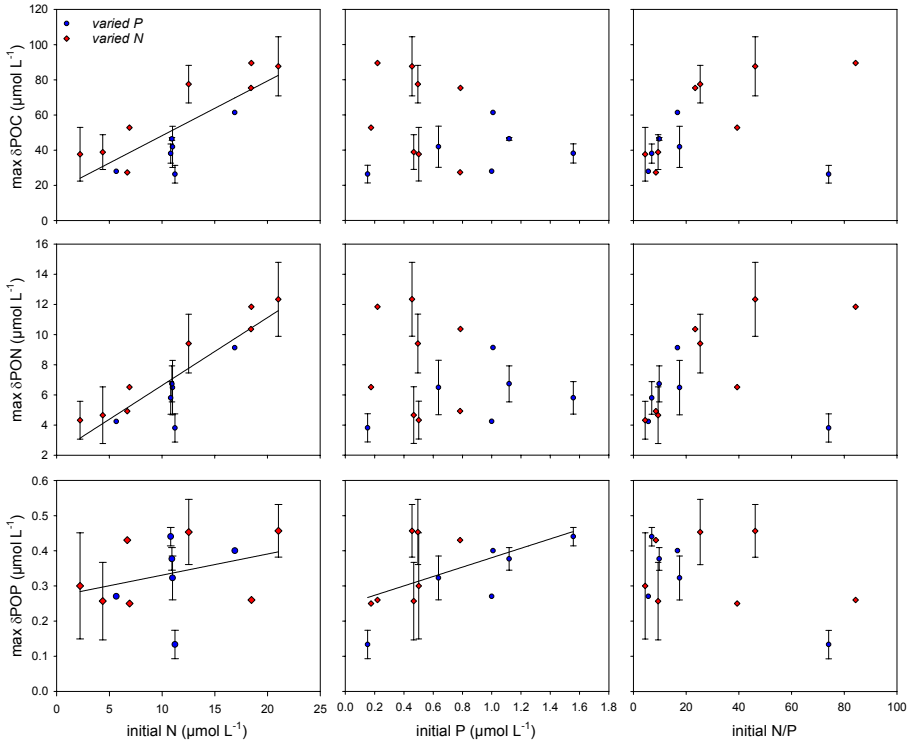

Figure 3. Maximum POC, PON and POP build-up as a function of the initial supply of N, P and N/P. Maximum δPOM is defined as peak POM concentration subtracted by the initial (day 1) POM concentration. Treatments in *varied P* are depicted as blue circles; treatments in *varied N* are depicted as red diamonds.

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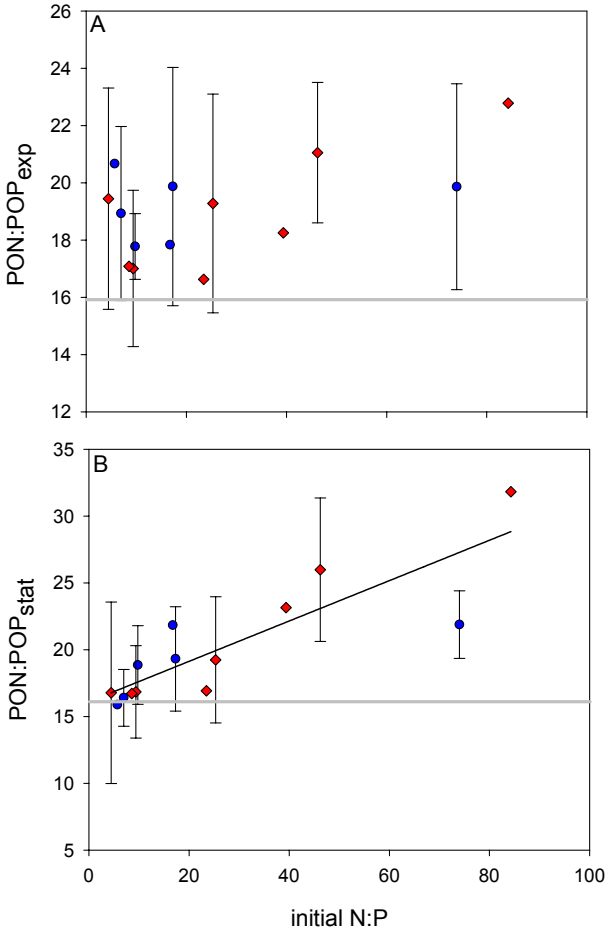

Figure 4. PON/POP stoichiometry during **(a)** the exponential growth phase and **(b)** the stationary growth phase of the experiment. The grey line visualizes the Redfield Ratio. The color code is the same as in Fig. 3.

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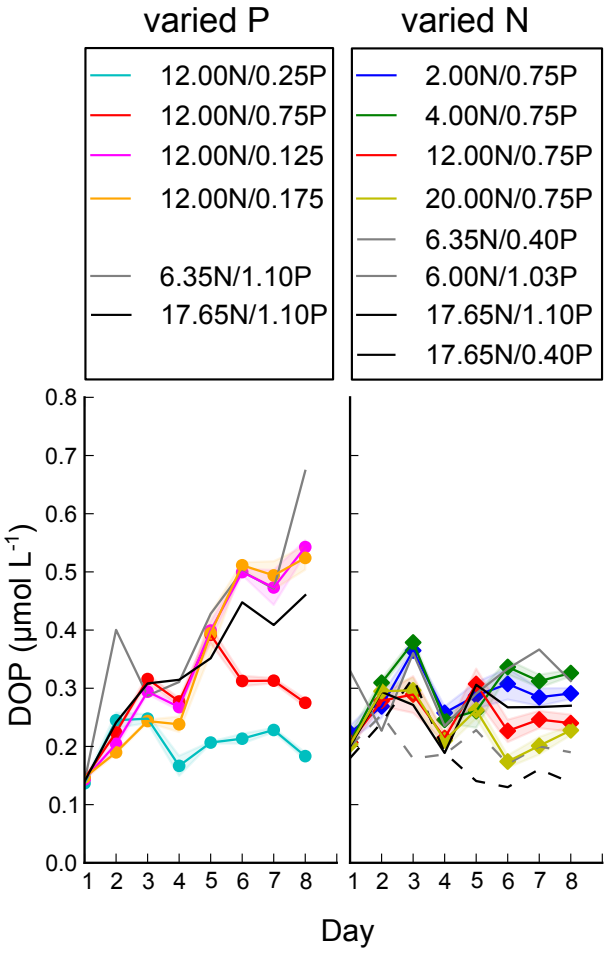

Figure 5. Temporal development of DOP with standard errors depicted as shaded error bands.

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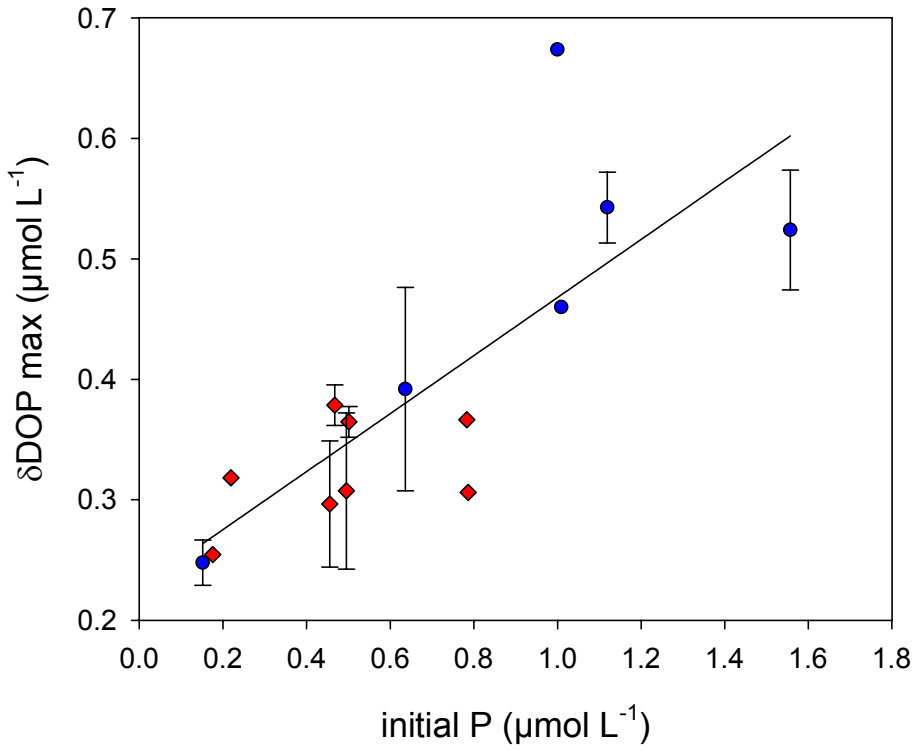

Figure 6. Maximum DOP build-up (defined as peak DOP concentration subtracted by the initial DOP concentration) as a function of initial P supply during *varied P* (blue circles) and *varied N* (red diamonds).

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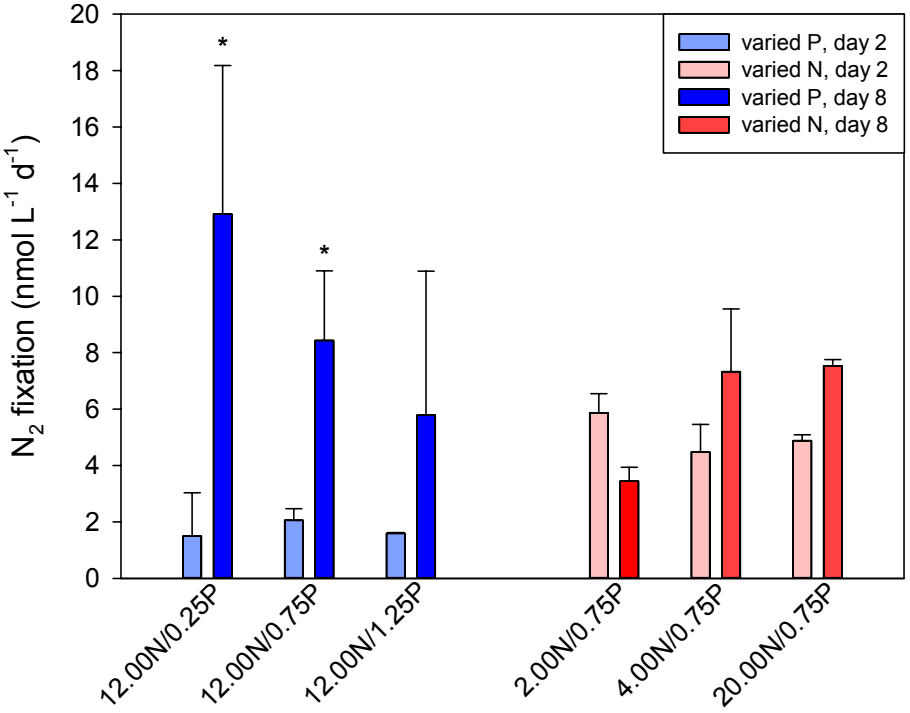

Figure 7. N_2 fixation rates measured on day 2 and day 8 of both experiments. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments. Asterisks indicate a significant difference between day 2 and day 8.

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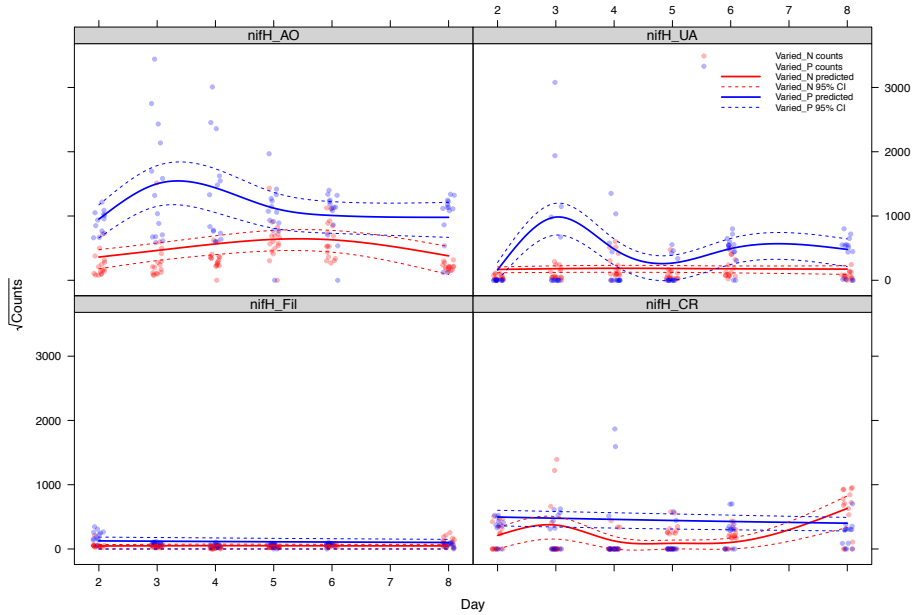

Figure 8. Selected gene count models over time. Predicted counts (solid lines) with 95 % confidence intervals (CI; dashed lines) are plotted along with measured count data. The predictive model is based on the original (untransformed) gene counts. For better visualization, values were square root-transformed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

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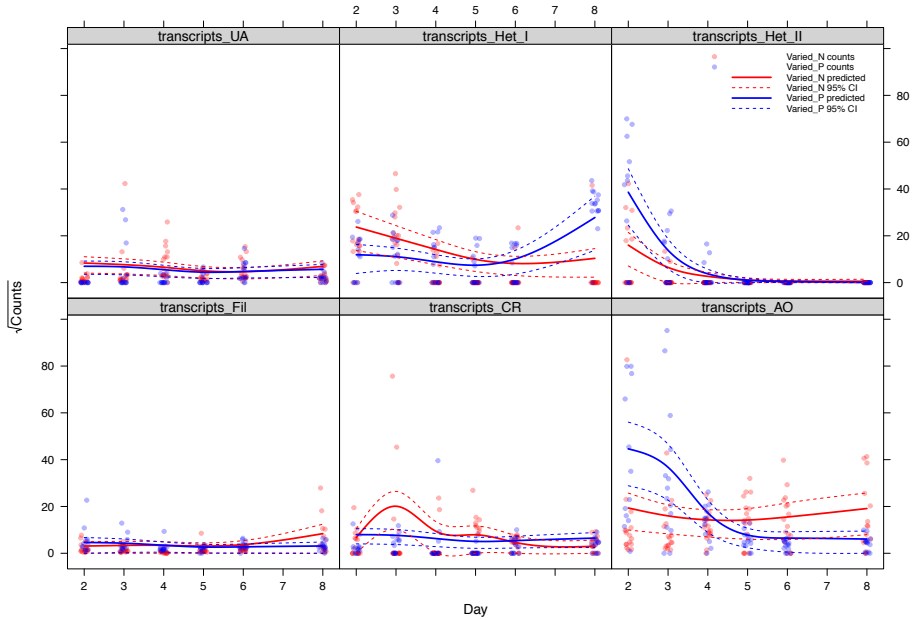

Figure 9. Selected transcript count models over time. Predicted counts (solid lines) with 95 % confidence intervals (CI; dashed lines) are plotted along with measured count data. The predictive model is based on the original (untransformed) transcript counts. For better visualization, values were square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

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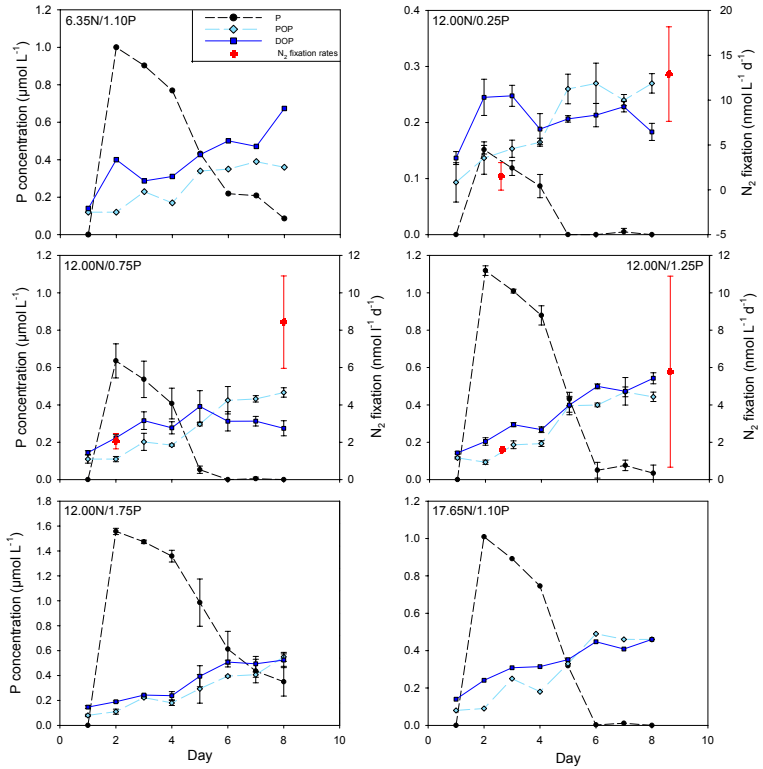

Figure 10. Dynamics of PO_4^{3-}, POP and DOP in all mesocosms. Because of the high variance between replicates we omitted N_2 fixation rates from un-replicated treatments.

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