# Ocean acidification and warming in the Baltic Sea: Effects on diazotrophy and pelagic biogeochemistry

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*To Isobel and Blair*

# Summary

Nitrogen (N) is an essential element for cellular functioning in all living organisms. However, the most abundant form of nitrogen ($N_2$ gas) in not bioavailable, or fixed, and therefore N availability restricts primary production in large parts of the ocean. Dinitrogen ($N_2$)-fixing, or diazotrophic, organisms possess a nitrogenase enzyme which converts $N_2$ into bioavailable forms. Aquatic $N_2$-fixation is a source of new nitrogen, hence where fixed N availability conditions, $N_2$-fixation can relieve the N-supply bottleneck in the non-diazotrophic organisms and support increased production - provided there is enough phosphate, light, and warm temperatures to sustain the energetically demanding $N_2$-fixation.

The Baltic Sea is a semi-enclosed water body under considerable anthropogenic pressure due to the highly populated drainage basin and limited water exchange. The spring-bloom draws down inorganic nutrients leading to seasonal N-limitation and provision of a diazotrophic niche with excess phosphate, increasingly stratified water column and warming sea surface temperatures. This is a seasonal niche occupied by filamentous diazotrophic cyanobacteria such as *Nodularia spumigena* and *Aphanizomenon flos-aquae*, which commonly form extensive surface blooms during the summer period. $N_2$-fixation is particularly important process in the region as it balances N loss processes and supports an estimated 20 - 45% of primary productivity during the summer season.

Increasing atmospheric $CO_2$ concentrations due to anthropogenic activity leads not only to warming of the atmosphere and oceans, but also to measurable shifts in seawater carbonate chemistry, termed ocean acidification. Single-strain culture studies have shown that $N_2$-fixation and diazotroph growth is sensitive to changes in the seawater temperature and $CO_2$ concentrations. Until now only a few short-term experiments have been completed to probe changes in fitness of diazotrophic species in situ. In addition, comparatively little is known about the response of low nutrient plankton communities to ocean acidification as more commonly nutrient induced blooms have been studied.

This doctoral dissertation presents the results from two independent mesocosm studies on naturally present summer plankton communities in the Baltic Sea. The aim was to investigate the impact of ocean acidification (increased $CO_2$ concentration and decreased pH) as well as the combination of ocean acidification and ocean warming (increased seawater temperature) on the abundance and activity of diazotrophic organisms and on N-limited plankton communities.

In the first study, pelagic mesocosms were deployed off the south-western tip of Finland in

the Archipelago Sea. To observe differences in organic matter pools and fluxes under realistic ocean acidification scenarios, $CO_2$ concentrations were adjusted to give a range between 365 and 1231 µatm (average during study period) and the plankton community and biogeochemical elemental pools were sampled over the 47-day long study. Approximately three weeks after the initial $CO_2$-manipulation, $CO_2$-related differences in pelagic particulate and dissolved matter pools became clear. These differences were sustained for a further three weeks until the end of the experiment. Higher particulate matter and dissolved organic carbon and chlorophyll *a* concentrations, and lower dissolved inorganic phosphate ($PO_4^{3-}$) concentrations under increased $CO_2$ concentrations were driven by the positive response of picophytoplankton (<2 µm). These $CO_2$-related differences in the water column could not be traced into the sinking particle flux within the study period. There were no significant differences in *A. flos-aquae* abundances, the dominant filamentous diazotrophic cyanobacterium present, or in diazotroph activity. Hence the positive response of plankton community biomass, could not be attributed to changes in fixed N supply.

In the second study using the indoor mesocosm facility in Kiel, the interactive effects of elevated $CO_2$ and temperature on new N inputs through diazotrophy were studied in four week long experiment. Here the dominant diazotrophic filamentous cyanobacteria was *N. spumigena*. There was a strong negative effect of $pCO_2$ on *N. spumigena* abundances which was exacerbated in the higher temperature treatment. This supports results from culture experiments with *N. spumigena* and shows that the negative response to increased $CO_2$ concentrations may not be overridden by biotic interactions such as grazing pressure and resource competition within the plankton community.

In both studies, abundances of filamentous diazotrophic cyanobacteria were too low to distinguish any potential influence on biogeochemical element pools. However, there were noticeable effects of temperature and $CO_2$ on one of the two common filamentous diazotrophic cyanobacteria species. Growth of *N. spumigena* may become restricted at the summer bloom peak in future, even though the period where blooms occur during summer may expand. How this interacts with shifts in $pCO_2$ and spring bloom dynamics remains unclear. The more coastal-dwelling species, *A. flos-aquae*, seemed better adapted to variable $CO_2$ concentrations, indicating that future $CO_2$-related changes in abundance in this species are not expected. Previous culture studies have also shown a diverse response of diazotrophic taxa.

The results included in this dissertation indicate that picoplankton may be able to sustain higher biomass under ocean acidification despite very low N availability. Hence, there is potential that this sustained response in picoplankton may shift food web structure with consequences for long-term changes in organic matter fluxes.

# Zusammenfassung

Stickstoff (N) ist ein essenzielles Element für alle Zellfunktionen in Organismen. Die häufigste Form von Stickstoff, das molekulare Gas $N_2$, ist biologisch nicht verfügbar, wodurch die Primärproduktion in großen Gebieten der Ozeane limitiert ist. Stickstofffixierende, sogenannte diazotrophe, Organismen können jedoch mittels des Enzyms Nitrogenase das reichlich vorhandene $N_2$in biologisch verfügbaren Stickstoff umwandeln. Dieser wiederum kann auch von nicht-diazotrophen Organismen aufgenommen werden. Stickstofffixierung ist eine neue Quelle von Stickstoff und ermöglicht demzufolge eine Minderung der Stickstofflimitierung einer ganzen Planktongemeinschaft und eine Steigerung der Primärproduktion - vorausgesetzt genügend Phosphat, Licht und entsprechend hohe Temperaturen sind gegeben, um den hohen energetischen Ansprüchen der Stickstofffixierer gerecht zu werden.

Die Ostsee ist durch ihr stark besiedeltes Wassereinzugsgebiet und den limitierten Wasseraustausch erheblich menschlichen Einflüssen ausgesetzt. Während der Frühjahrsblüte des Planktons werden die gelösten anorganischen Nährstoffe in der Wassersäule aufgezehrt, wodurch eine saisonale Stickstofflimitierung auftritt. Die entstandene Stickstofflimitierung, zusammen mit einem Phosphatüberschuss, einer zunehmend stratifizierten Wassersäule und warmen Wassertemperaturen an der Oberfläche, bilden eine saisonale Nische für Diazotrophe, die in der Ostsee von fadenförmigen Cyanobakterien, wie zum Beispiel Nodularia spumigena und *Aphanizomenon flos-aquae* besetzt wird. Diese Cyanobakterien bilden im Sommer oft ausgedehnte Blüten an der Meeresoberfläche. Stickstofffixierung durch Cyanobakterien ist ein besonders wichtiger Prozess in der Ostsee, da sie den Stickstoffverlust in den tieferen, sauerstoffarmen Schichten ausgleicht und 20-45% der Primärproduktion im Sommer unterstützt.

Die ansteigenden anthropogenen $CO_2$-Emissionen führen nicht nur zu einer Erwärmung der Atmosphäre und der Ozeane, sondern zusätzlich zu messbaren Änderungen in der Karbonatchemie des Meerwassers, der sogenannten Ozeanversauerung. Studien mit Reinkulturen von diazotrophen Organismen haben gezeigt, dass Stickstofffixierung und das Wachstum dieser Organismengruppe empfindlich auf Veränderungen der $CO_2$-Konzentration ($pCO_2$) und Temperatur reagieren. Die Fitness der Diazotrophen in-situ wurde bisher jedoch in nur wenigen Kurzzeitexperimenten erforscht. Zudem ist wenig darüber bekannt, wie Nährstoff-limitierte Planktongemeinschaften auf Ozeanversauerung reagieren, da in der Vergangenheit hauptsächlich Nährstoffinduzierte Blüten untersucht wurden.

In dieser Dissertation wurde sowohl der Einfluss von Ozeanversauerung (ansteigender $pCO_2$ und sinkender pH-Wert im Seewasser) als auch die Kombination von Ozeanversauerung mit

Ozeanerwärmung auf zwei Planktongemeinschaften der Ostsee im Sommer untersucht.

In zwei unabhängigen Mesokosmen-Studien wurden natürlich vorkommende Planktongemeinschaften der Ostsee während der Sommermonate untersucht. Ziel der beiden Studien war es, den Einfluss von $CO_2$-Konzentration auf die Stickstoff-limitierte Planktongemeinschaft und die diazotrophe Organismen zu untersuchen.

In der ersten Studie wurden pelagische Mesokosmen im Archipel vor der Südwestspitze Finnlands ausgesetzt. Um Unterschiede in der Menge und dem Umsatz des organischen Materials unter realistischen Szenarien der Ozeanversauerung zu untersuchen, wurden die Mesokosmen auf verschiedene $CO_2$-Konzentrationen (von durchschnittlich 365 bis 1231 µatm) eingestellt und die Planktongemeinschaft 47 Tage lang beprobt. Nach mehr als drei Wochen zeigten sich deutliche $CO_2$-bedingte Unterschiede im partikulären und gelösten organischen Material. Höhere $CO_2$-Konzentrationen führten zu höheren Konzentrationen im organischen Material und im Chlorophyll a sowie zu niedrigeren Phosphatkonzentrationen in der Wassersäule. Verantwortlich für diese Unterschiede war das Picophytoplankton (< 2 µm), welches unter erhöhten $CO_2$-Konzentrationen schon früh im Experiment erhöhte Abundanzen erreichte. Die $CO_2$-bedingten Unterschiede in der Wassersäule konnten jedoch nicht im absinkenden Partikelfluss beobachtet werden. Abundanz und Aktivität der diazotrophen Cyanobakterien, überwiegend *A. flos-aquae*, zeigten ebenfalls keine signifikanten Unterschiede. Daher kann die erhöhte Biomasseproduktion der Planktongemeinschaft nicht auf eine erhöhte Stickstoffverfügbarkeit durch Diazotrophe zurückgeführt werden.

In einer zweiten Studie wurde über vier Wochen der kombinierte Effekt von erhöhten $CO_2$-Konzentrationen und Temperatur auf die Bereitstellung fixierten Stickstoffs durch Diazotrophe in einer natürlichen Planktongemeinschaft untersucht. Die dominante stickstofffixierende Art in der Planktongemeinschaft war das fädige Cyanobakterium *N. spumigena*. Höhere $CO_2$-Konzentrationen und erhöhte Temperatur führten zu geringeren *N. spumigena* Abundanzen. Dies unterstützt Ergebnisse aus Experimenten mit *N. spumigena*-Reinkulturen und zeigt, dass die negative Reaktion auf erhöhte $CO_2$-Konzentrationen und Temperatur trotz Fraßdruck und Nährstoffkonkurrenz innerhalb der Planktongemeinschaft sichtbar ist.

In beiden Studien war der Biomasseanteil der Cyanobakterien zu niedrig, um die Stoffkreisläufe signifikant zu beeinflussen. Allerdings zeigten sich deutliche Temperatur- und $CO_2$-Effekte auf eine der beiden stickstofffixierenden Arten. Das Wachstum von *N. spumigena*, dem dominanten Stickstofffixierer der offenen Ostsee, könnte in Zukunft deutlich eingeschränkt sein, auch wenn sich die möglichen Blütezeiten im Sommer durch die globale Erwärmung tendenziell ausdehnen werden. Die überwiegend in Küstengewässern zu findende Art *A. flos-aquae* scheint an erhöhte $CO_2$-Konzentrationen angepasst zu sein, sodass nach jetzigem Kenntnisstand keine zukünftigen $CO_2$-bedingten Änderungen zu erwarten sind. Auch bisherige Studien mit Reinkulturen zeigen, dass verschiedene Cyanobakterien-Arten unterschiedlich auf $CO_2$-Änderungen reagieren.

Die Ergebnisse dieser Arbeit deuten auch darauf hin, dass Picoplankton trotz Ozeanversauerung und Stickstofflimitierung in der Lage ist, eine hohe Biomasse zu erhalten. Diese nachhaltige Reaktion des Picoplanktons könnte die Struktur des marinen Nahrungsnetzes und die marinen Stoffkreisläufe in Zukunft dauerhaft beeinflussen.

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# 1 | Introduction

## 1.1 Phytoplankton and biogeochemical cycling in the aquatic realm

### 1.1.1 Phytoplankton and organic matter production

As primary producers of the ocean, phytoplankton harness light energy to fix carbon dioxide ($CO_2$) into organic carbon through photosynthesis, the first step in the energy cascade of the pelagic food web. These microscopic organisms are a crucial part of the food web in marine and freshwater ecosystems accounting for around 50% of global primary production (Field et al., 1998). This energy, converted into organic matter, can then be transferred through secondary production and the trophic cascade to higher organisms such as fish, as well as support microbial community turnover (Azam et al., 1983).

Aquatic photosynthesis is not only an important process in terms of energy transfer in the food web but also has a defining influence on the cycling of key elements such as carbon (C), nitrogen (N), phosphorus (P) as well as trace metals such as iron (Fe). The fixed organic matter can sink out of the euphotic zone where the majority is remineralised back to dissolved inorganic species in the deep ocean layers. Only a small fraction reaches the sea bed and remains effectively stored in the deep ocean on geological time-scales. The exact fraction is regionally dependent but is generally within the range of 0.1 to 10% (Sarmiento and Gruber, 2006). This fixation and sinking of carbon from the surface to the deep ocean layers is called the biological carbon pump (Volk and Hoffert, 1985). Thus carbon fixation by phytoplankton is effectively mediating surface ocean and lower atmospheric $CO_2$ concentrations. N and P are vital elements in the biosphere as they are key elemental components governing reactivity and biological function in essential biomolecules and cellular building blocks such as nucleic acids, structural proteins and phospholipids (Geider and La Roche, 2002). The stoichiometric relationship in organic matter composition in the ocean in relation to inorganic nutrients generally follows the characteristic 'Redfield ratio' (Redfield, 1958) of 106C:16N:1P as illustrated by Eqn. 1.1, which shows this stoichiometry as a chemical equation for photosynthetic production (forward reaction) and respiration of organic matter (reverse reaction):

$$106\,CO_2 + 16\,HNO_3 + H_3PO_4 + 122\,H_2O \overset{light}{\rightleftharpoons} (CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 138\,O_2 \quad (1.1)$$

This stoichiometry of organic matter composition implies a strong link between the availability of the macronutrients nitrate ($NO_3^-$) and phosphate ($PO_4^{3-}$) and pelagic productivity and carbon cycling in the aquatic environment. Deviations in C:N:P from the Redfield ratio however do occur on a regional or species level due to differences in environmental conditions, physiological requirements between dominant plankton species and ambient inorganic N:P availability (Geider and La Roche, 2002; Klausmeier et al., 2004). In turn, nutrient availability affects the species and average chemical composition of the plankton assemblage (Hunt and Matveev, 2005; Van den Brink et al., 1994; Gervais and Riebesell, 2001; Franz et al., 2012) with consequences for energy transfer to higher trophic levels within the marine foodweb (Malzahn et al., 2007).

**The concept of resource limitation**

As implied by the stoichiometry in Eqn. 1.1, the supply of either P or N can also limit net organic matter production in phytoplankton. Liebig's Law of the minimum (von Liebig, 1855) suggests that organic matter yield is controlled by the availability of the scarcest resource i.e. the limiting resource for growth. This principle is commonly inferred to describe macronutrient limitation in the aquatic environment between $NO_3^-$ and $PO_4^{3-}$ based on the Redfield ratio, where N:P >16 is considered P-limited and N:P < 16 N-limited, but can also be used to describe micronutrient or trace metal limitation, or in the case of diatoms, dissolved silicate limitation.

Resource limitation can also restrict rates of biological processes, in particular those driven by enzymatic reactions. This kinetically based idea was proposed by Blackman (1905). A clear example of this is light as a limiting resource for photosynthesis. Increasing light availability increases the photosynthetic rate and hence phytoplankton growth until growth plateaus, then declines once light levels are too high, thereby forming the characteristic photosynthesis-irradiance (P vs. I) curves. This concept is in line with the Monod model (Monod, 1950) and Michaelis-Menten kinetics, a simple approach frequently used to empirically describe microbial growth or enzyme activity as a function of substrate availability e.g. rates of carboxylation and oxygenation in RuBISCO as a function of $CO_2$ and $O_2$ availability in photosynthetic organisms (Farquhar et al., 1980).

### 1.1.2 Overview of key pelagic nitrogen pools in the aquatic environment

On a global scale, N is regarded as the limiting macronutrient for primary production in the surface ocean (Falkowski, 1997; Tyrrell, 1999), particularly on short time-scales. This is because most elemental N is not present in a bioavailable or 'fixed' state, and is thus inaccessible to most organisms. Numerous different N species and oxidation states exist ranging from ammonium (-III) to gaseous $N_2$ (0) to inorganic species (nitrate, V) as well as organic compounds such as amino acids and urea, of which all but $N_2$ are regarded as fixed N (Table 1.1). Transformations between these various chemical species and redox states underlies microbial N cycling during metabolism and growth. This includes the production of key cellular components such as amino and nucleic acids, energy transfer molecules (ATP: adenosine triphosphate) and light harvesting pigments (e.g. Chl *a*). Both $NO_3^-$ and the reduced N form of ammonium ($NH_4^+$) are of particular importance for phytoplankton because they can be directly assimilated into biomass.

In 1967, Dugdale and Goering distinguished between new and regenerative production based on the N supply mechanisms. Both new and regenerative production can support carbon fixation. However, only new production through N supply by upwelled $NO_3^-$ or $N_2$-fixation from atmospheric $N_2$ can relieve N-limitation or influence organic matter export (Karl et al., 2002), whereas regenerative production is supported by turnover of $NH_4^+$ through organic N remineralisation within the euphotic zone (Dugdale and Goering, 1967). Generally productivity in plankton communities is dominated by $NO_3^-$ supply (i.e. new production) during the winter and early spring bloom before inorganic N pools are exhausted. Consequently, organic matter remineralisation, mediated by either zooplankton or bacteria, supplies $NH_4^+$ to primary producers during summer and autumn (Quéguiner et al., 1986).

**Table 1.1:** Summary of selected key N species found in the marine environment and their oxidation states and classification as fixed or non-fixed.

| Oxidation state | Chemical species | Species name | Fixed N |
|---|---|---|---|
| -III | $NH_4^+$, $R{-}NH_3$ | ammonia, organic N | fixed |
| -I | $NH_2OH$ | hydroxylamine | fixed |
| 0 | $N_2$ | dinitrogen gas | not fixed |
| I | $N_2O$ | nitrous oxide | fixed |
| II | NO | nitric oxide | fixed |
| III | $NO_2^-$ | nitrite | fixed |
| V | $NO_3^-$ | nitrate | fixed |

### 1.1.3 Nitrogen fixation and the diazotrophic niche

N is highly abundant in elemental form as gaseous $N_2$ constituting up to 78% in our atmosphere and >99% of total N atoms in the ocean ($1 \times 10^7$ Tg N as $N_2$ vs. $6.6 \times 10^5$ Tg N as fixed N in ocean, Gruber 2008). However, the strength of the N≡N bond and instability of the chemical intermediates confers a high energy requirement to overcome this energetic barrier as implied by the strong forcing conditions required in the commercial Haber-Bosch process ($500\,^{\circ}\mathrm{C}$ and up to 1000 atm pressure with presence of catalyst, Manahan 2006). Biological fixation of $N_2$ into bioavailable N forms occurs both on land and in the ocean, but only by specialised bacteria and archaea possessing the nitrogenase enzyme complex which reduces $N_2$ to $NH_3$. Organisms with this capability are generally called diazotrophs. This is an energetically demanding process, even for these specialised organisms, with high adenosine triphosphate (16 ATP, Eqn. 1.2) and iron (Fe) requirements for the multiple enzyme redox centres (Howard and Rees, 1996).

$$\mathrm{N_2 + 8\,e^- + 8\,H^+ + 16\,ATP + 6\,H_2O \longrightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i} \tag{1.2}$$

Consequently, warm water temperatures (usually >20°C, (Breitbarth et al., 2007; Luo et al., 2014)), high ambient inorganic phosphate and Fe availability (Berman-Frank et al., 2007; Monteiro et al., 2011) and high light environments (Kononen et al., 1996; Luo et al., 2014) are required to satisfy the specific nutritional and high energetic demands for $N_2$-fixation. Diazotrophs can grow and reproduce successfully under these conditions, which are commonly described as the ecological niche for $N_2$-fixing organisms. While there is some evidence that $N_2$-fixing organisms can also utilise inorganic N sources ($NO_3^-$ and $NH_4^+$) and persist under high inorganic N concentrations (Fernandez et al., 2011), their relatively slow growth rates compared to other competing phytoplankton and high energetic investment in $N_2$-fixation means they no longer have their competitive advantage in the plankton community. Hence, diazotrophy is only an advantageous characteristic when N availability limits growth and primary production of the non-diazotrophic phytoplankton in the community (Tyrrell, 1999).

The idea of a diazotrophic niche is generally supported by current knowledge on the distribution of autotrophic diazotrophic organisms in the global ocean. Fixed N availability is low in the tropics and sub-tropics and thus $N_2$-fixation is particularly influential in harnessing the ubiquitous atmospheric N source (Saito et al., 2011). Prominent communities of diazotrophic cyanobacteria are primarily found in surface waters of tropical regions, where there is high light, warm water temperatures (>16°C), and in regions of high dust (i.e. Fe) deposition (Sohm et al. (2011) and references therein). Evidence is, however, stronger for the role of irradiance andtemperature (i.e. stratification) on $N_2$-fixation than for other environmental controls (Luo et al., 2014).

## 1.2 Study area of the thesis: the Baltic Sea

### 1.2.1 Hydrological and environmental characteristics of the Baltic Sea

The Baltic Sea is situated in northern Europe, spanning latitudes from 53°N to 66°N. It is a semi-enclosed marginal sea with the only connection to the North Sea through a few narrow, shallow channels in the Danish Straits. In general the water body is very shallow with just 12% of the total area under 100 m deep, with Landsort Deep the deepest part of the Baltic Sea at 459 m deep (Leppäranta and Myrberg 2009). This shallow nature confers many important characteristics to water mass exchange and oxygenation of deep basins in the Baltic Sea.

At only 18 m deep, the shallow and narrow Darss Sill in the Danish Straits presents the greatest barrier to saline water inflow from the North Sea via the Kattegat to the adjacent basins in the southern end of the Baltic Sea. This dense seawater of salinity 15 - 25 collects in the deep basins (Voipio, 1981). In combination with freshwater input from rivers in northern and coastal areas which remains in the surface layer, this leads to steep salinity gradients from north to south as well as maintaining the strong, permanent halocline (Leppäranta and Myrberg, 2009) present at around 60 m deep (Schiewer, 2008). The Baltic Sea is classified as a brackish water body with a surface water salinity of between 6.5 - 8, much lower than the open ocean (East Gotland Basin, Leppäranta and Myrberg, 2009). Although the permanent halocline restricts physical water mass exchange, sinking organic matter can sink below the halocline. As it is remineralised through aerobic bacterial processes, this consumes oxygen, thereby depleting the deeper waters in oxygen. Periods of persistent westerly winds can lead to sporadic, short and intensive pulses of saline oxygenated water over the Darss Sill and into the Baltic Sea (Leppäranta and Myrberg, 2009). While there is always a small degree of subsurface inflow and exchange, this is the only process which substantially replenishes oxygen below the halocline.

#### Anthropogenic pressures in the Baltic Sea ecosystem

Around 85 million people in 14 countries live in the drainage basin which is almost four times larger than the sea itself (Hannerz and Destouni, 2006) meaning that anthropogenic activity from agriculture, urban centres, recreational activities, fishing activities and ship traffic have a large influence on the water quality in the Baltic Sea. The anthropogenic influence on the Baltic Sea has intensified over the past two centuries (Elmgren, 2001; HELCOM, 2013; Viitasalo et al., 2015). Substantial nutrient run-off and ensuing changes in phytoplankton productivity have

been of particular concern in the region due to the importance of the biological diversity, stable ecological state, and recreational area in this primarily coastal ecosystem (HELCOM, 2009).

### 1.2.2 N supply and seasonal plankton succession

The Baltic Sea is a region where fixed N concentrations are low in summer and limit net autotrophic production in the surface layers (Granéli et al., 1990), similar to the open ocean (Section 1.1.2). Nutrient supply in the Baltic Sea is not strictly in Redfield proportion as hypoxia in the bottom water drives preferential phosphate release under hypoxia from the sediments and N loss processes of annamox and denitification below the halocline and at the oxic/anoxic interface (Risgaard-Petersen et al., 2005; Lam and Kuypers, 2011). Hence mixing events, such as coastal upwelling (Kahru et al., 1995), bring up P-rich and N-deplete deep water. Hence the generally diatom-dominated spring bloom (Wasmund et al., 1998) draws down inorganic N leaving an excess of inorganic P (Granéli et al. 1990, Fig. 1.1).

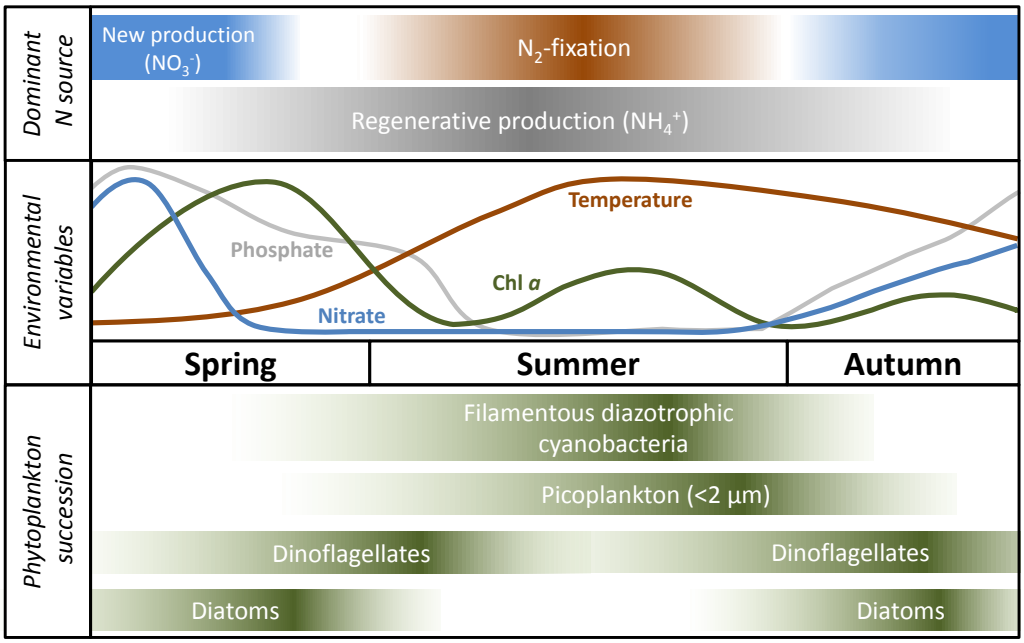


**Figure 1.1:** Schematic summarising common variations in environmental variables, dominant N source and succession of select phytoplankton groups in different regions in the Baltic Sea between spring and autumn. Based on data obtained from Andersson et al. (1996); Wasmund and Siegel (2008). In some regions, the order of succession of diatoms and dinoflagellates may be reversed e.g. Lignell et al. (1993).

The residual phosphate, increasingly stratified water column with warm surface waters and high light availability during the summer, leads to a seasonal diazotrophic niche (Section 1.1.3), which supports the development of annual blooms of diazotrophic filamentous cyanobacteria. Due to their buoyancy regulation, these organisms tend to form large aggregates, which accumulate in the surface. These scums occur regularly over large areas of the Baltic Sea and are highly visible as indicated in this satellite photo as the light green swirls against the dark water background (Fig. 1.2).

These common annual blooms are generally dominated by filamentous diazotrophic cyanobacteria with two main genera: *Nodularia spumigena* in the open Baltic Sea and *Aphanizomenon flos-aquae* in more coastal areas (Fig. 1.3, Olli et al., 2015). This geographical distinction between these two genera may be attributed to dissolved phosphate (Degerholm et al., 2006; Olli et al., 2015) or salinity and solar irradiance (Lehtimaki et al., 1997). Both of these genera have heterocysts, specialised compartments to fix N. This spatially separates the nitrogenase enzyme from carbon fixation and associated $O_2$ production from C-fixation in carboxysomes in the neighbouring vegetative cells as the nitrogenase enzyme is irreversibly inhibited by $O_2$ (Postgate, 1998). In contrast, other autotrophic diazotrophs use temporal rather than physical separation of C-fixation and the $O_2$-sensitive $N_2$-fixation (Berman-Frank et al., 2003). *N. spumigena* is a toxic species, known outside of scientific circles due to the hepatotoxins it produces which can lead to beach closures during major blooms.


**Figure 1.2:** Satellite image of a surface bloom of filamentous cyanobacteria in the southern Baltic Sea taken on 27/7/2012 by the MODIS satellite. Source: M. Kahru.

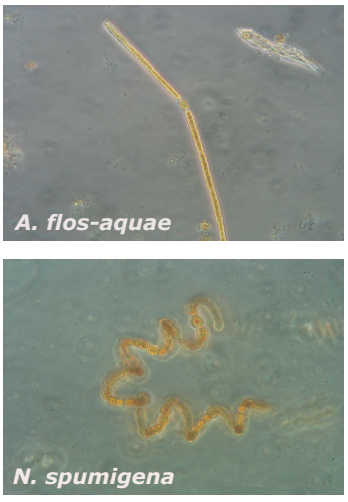


**Figure 1.3:** Microscopy photographs of *Aphanizomenon flos-aquae* and *Nodularia spumigena*, two common, bloom-forming filamentous $N_2$-fixing cyanobacteria in the Baltic Sea. Source: A. Stuhr.

Estimations vary, however, new N input through $N_2$-fixation in the Baltic Sea is reportedly on the same order of magnitude as riverine inputs and atmospheric N deposition (Fig. 1.4, Voss et al. 2011) indicating the importance of diazotrophic organisms in supporting N turnover in the region. In addition, $N_2$-fixation counteracts the nitrogen lost via anammox and denitrification in the anoxic layers below the halocline and in the sediments (Fig. 1.4). This may also act as a natural feedback system promoting organic matter production, oxygen consumption, phosphate release from the sediments under anoxia which in turn increases the niche for $N_2$-fixing filamentous cyanobacteria (Vahtera et al., 2007).

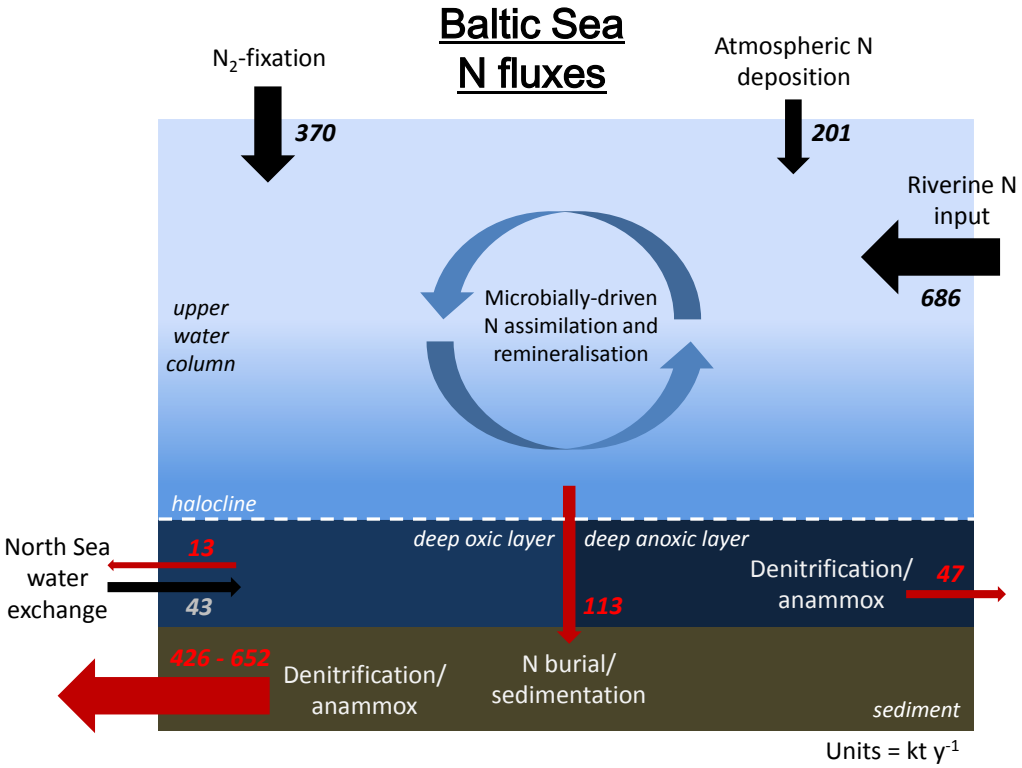


**Figure 1.4:** Key N fluxes in Baltic Sea as summarised by Voss et al. (2011). N sources to the Baltic Sea are indicated as black and N loss processes in red text and arrows. The budget is not balanced, possibly due to underestimated N loss processes.

## 1.3 Ocean acidification and ocean warming

### 1.3.1 Historical basis and basic underlying principles

Since the beginning of the Industrial Revolution in the 18th century, considerable amounts of fossil fuel carbon has been burned resulting in increased atmospheric $CO_2$ concentrations from around 280 ppm reaching over 400 ppm in 2014 (Tans and Keeling, 2015). Increased atmospheric $CO_2$ concentration works in a similar way to a greenhouse and traps heat energy in the earth system, observed as an increase in air temperature (i.e. global warming). Due to the high thermal capacity of water and large volume, a major proportion of the heat energy is transferred to the oceans resulting in increased water temperature, termed 'ocean warming'. In addition to the warming effect, around 30% of anthropogenic $CO_2$ emitted to the atmosphere ends up in the oceans (Sabine et al., 2004), leading to measurable changes in seawater chemistry. $CO_2$ dissolves in seawater to form a weak acid which dissociates (Eqn. 1.3), releasing $H^+$ and causing a shift in the carbonate system in seawater.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2\,H^+ + CO_3^{2-} \tag{1.3}$$

This rapid influx of $CO_2$ has been clearly observed in major oceans from the Pacific to the eastern and western Atlantic Oceans (Rhein et al., 2013). This change in seawater chemistry is detectable above seasonal variation over a number of decades (Tans and Keeling, 2015), and is at a rate of increase not observed over the geological history (Hönisch et al., 2012). Continued emission of $CO_2$ is expected to increase atmospheric $CO_2$ concentrations to over 1000 µatm with mean projected increases in surface air temperature of 4°C and a decrease of around 0.3 in seawater pH by the year 2100 (Collins et al., 2013; Ciais et al., 2013).

### 1.3.2 Ocean acidification and warming in the Baltic Sea

In addition to the anthropogenic pressures mentioned in Section 1.2.1. Additionally, the Baltic Sea is recognised as a hotspot for both ocean warming and acidification which may change the baseline ecosystem ecology (Elmgren, 2001). Model-based projections suggest that summer surface seawater temperature will likely further increase by between 2 and 4°C by the end of this century (HELCOM, 2013) and average pH decrease of around 0.3 – 0.4 (Fig. 1.5, Omstedt et al. 2012) under 'Business as usual' (scenario BAU-A2).

The respective decrease in surface water pH from ocean acidification is more difficult to accurately predict than for the open ocean because may be partially negated through changes in riverine alkalinity inputs (Schneider et al., 2015) or extent of anoxia in the deeper basins (Havenhand, 2012). Indeed, change in pH will not be uniformly distributed as regional differences in alkalinity modulate $CO_2$ uptake and the pH decrease (HELCOM, 2013). Nevertheless, it appears as though the rate of change in the Baltic Sea proper, where the terrestrial influence is less than coastal regions, pH is decreasing at a rate faster than in the open ocean (Fig. 1.5). Baltic Sea surface water temperature has increased in all regions since 1990 on the order of 1°C per decade (Lehmann et al., 2011), much higher than projections (see above).

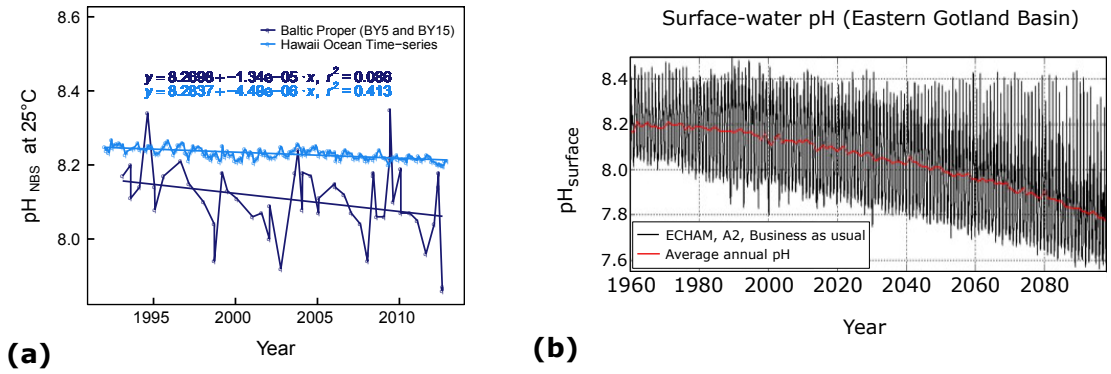


**Figure 1.5:** **(a)** Measured pH in Baltic Sea and in tropical North Pacific Ocean (HOT times series). Data adapted from Dore et al. (2009) and The International Council for Exploration of the Sea (2014). **(b)** Modelled pH data indicating projected future pH decrease, figure adapted from Omstedt et al. (2012).

### 1.3.3 Biotic response to ocean acidification and warming

These physical and chemical changes in seawater from ocean acidification and warming affect the metabolism and pH-sensitive processes and structures in aquatic organisms. Planktonic organisms are too small for internal temperature regulation thus their metabolism is affected directly by the ambient environmental temperature. A concerted research effort has been made over the past two decades which have shown physiological processes in phytoplankton such as C-fixation, biomineralisation, and enzyme-mediated organic matter degradation are temperature, pH and/or $CO_2$-sensitive as comprehensively summarised in two recent publications (Pörtner et al., 2014; Riebesell and Tortell, 2011). RuBISCO is a key enzyme in the fixation of $CO_2$ which is capable of both carboxylation (C-fixation) and photorespiration (remineralisation) activity, however diffusion of $CO_2$ into the cell alone may not provide sufficient carbon for efficient C-fixation (Raven, 1993). Therefore, many phytoplankton invest in carbon concentrating mechanisms to increase intracellular $CO_2$ and saturate the RuBISCO enzyme. This increase in $CO_2$ at carboxylating site saturates the enzyme RuBISCO and leads to reduced photorespiration at high $CO_2$ (Raven and Beardall, 2003). Ocean acidification will increase the concentrations of dissolved $CO_2$ and hence diffusion into the cell, leading to potential fertilisation of C-fixation and reducing the need for CCM activity. Some organisms may profit more than others due to different affinities of RuBISCO and the energetic relief that increased $CO_2$ availability reducing the need for CCM, indicating likely changes in competitiveness between phytoplankton species (Dutkiewicz et al., 2015; Reinfelder, 2011). Nonetheless any stimulating effect of increased $CO_2$ availability will be mediated concurrently by the effect of pH on cellular pH homeostasis (Taylor et al., 2011; Bach et al., 2011).

For diazotrophic organisms studies have shown that $H_2$ production as a by-product of nitrogenase activity in the photosynthetic bacterium, *Rhodobacter sulfidophilus*, is pH dependent (Peng et al., 1987), with peak enzyme activity for $N_2$-fixation between pH 7 and 8.2 (Pham and Burgess, 1993). Furthermore, in hetercystous diazotrophic cyanobacteria, such as *N. spumigena*, $N_2$-fixation rates may be negatively affected by pH due to intercellular transfer of carbon

and nitrogen between the heterocysts and vegetative cells (Czerny et al., 2009). C-fixation also provides energy for $N_2$-fixation. $N_2$-fixation is also expected to be affected by increased $CO_2$ hence may provide a natural new N source to relieve N limitation and stimulate primary production. Table 1.2 is a current overview of published studies showing how the observed response of $N_2$-fixation activity both between and within diverse diazotrophic genera to increased $CO_2$ has been far from consistent. How diazotrophic organisms respond to ocean acidification may therefore be dependent on differences in $O_2$ exclusion strategies and cell morphology, or due to adaptation of CCMs to the carbonate chemistry of their respective ecological niches as suggested by Eichner et al. (2014).

**Table 1.2:** Summary of reported responses of biomass normalised $N_2$-fixation rates to increased $CO_2$/decreased pH (OA = ocean acidification) in single-strain isolates of a variety of diazotrophic organisms. HL indicates high light and LL indicates low light conditions and the direction of the arrows correspond to the observed direction of the response.

| Genus | Isolated from | Cell morphology | OA response | OW response | Reference |
|---|---|---|---|---|---|
| *Trichodesmium* | marine, tropical | filamentous, non-heterocystous | | ⇔ | Mulholland and Bernhardt (2005) |
| | | | ⇑ | | Barcelos e Ramos et al. (2007) |
| | | | ⇑ | ⇔ | Hutchins et al. (2007) |
| | | | ⇑ | | Levitan et al. (2007) |
| | | | ⇑ | | Kranz et al. (2010) |
| | | | ⇑ | | Garcia et al. (2011) |
| | | | +Fe ⇔<br>- Fe ⇓ | | Shi et al. (2012) |
| | | | ⇑ | | Hutchins et al. (2013) |
| | | | | ⇑ | Fu et al. (2014) |
| | | | ⇑ | | Hutchins et al. (2015) |
| *Crocosphaera* | marine, tropical | unicellular | ⇑ | | Fu et al. (2008) |
| | | | ⇑ | | Hutchins et al. (2013) |
| | | | HL ⇑<br>LL ⇔ | | Garcia et al. (2013b) |
| | | | | | Garcia et al. (2013a) |
| | | | | ⇑ | Fu et al. (2014) |
| *Cyanothece* | marine | unicellular | | ⇑ | Brauer et al. (2013) |
| | | | ⇔ | | Eichner et al. (2014) |
| *Calothrix* | marine | symbiotic | ⇑ | | Eichner et al. (2014) |
| *Nodularia* | brackish | filamentous, heterocystous | ⇓ | | Czerny et al. (2009) |
| | (Baltic Sea) | | ⇑ | | Wannicke et al. (2012) |
| | | | ⇔ | | Karlberg and Wulff (2013) |
| | | | ⇓ | | Eichner et al. (2014) |
| *Aphanizomenon* | freshwater | filamentous, heterocystous | ⇓ | | Yamamoto and Nakahara (2005) |

Additionally, temperature strongly modulates $N_2$-fixation and diazotroph abundances, as previously described in Section 1.1.3. However as optimum temperature for $N_2$-fixation was commonly below 30°C, interpretation of these studies in terms of ocean warming is complex therefore results in Table 1.2 should be interpreted with caution. For example, organisms can also increase the geographical distribution as populations may shift polewards to escape physiologically intolerable warm temperatures due to shifting isotherms as suggested for *Trichodesmium* by Breitbarth et al. (2007) and indicated in model simulations (Dutkiewicz et al., 2015). Even less is known about the interactive effects of $CO_2$ and temperature. It has been shown that the physiological and growth response of phytoplankton to $CO_2$ can be modulated by changing temperature in single-strain cultures (Sett et al., 2014; Fu et al., 2007). There are comparatively few studies compared to those investigating the individual effects of each driver. Nutrient concentration and other resource availability such as changing light fields through increased stratification may also interplay and complicate projections of the biological response of plankton to ocean acidification and warming (Gunderson et al., 2016).

## 1.4 Thesis Outline

### 1.4.1 Overview

Diazotrophic N is a source of new N, giving rise to the description of diazotrophs as 'gatekeepers of marine productivity' by Berman-Frank et al. (2003). Thus, increases in new N inputs such as from increased $N_2$-fixation under ocean acidification and warming may stimulate primary production in the surface ocean through relief of N-limitation (Eppley and Peterson, 1979; Karl et al., 2002). In terms of global change, the combination and interaction of individual species responses to ocean acidification and ocean warming on all trophic levels will determine the sustained ecosystem scale response.

Until now, the majority of published studies of the impacts of ocean acidification on plankton communities have occured on short time-scales ranging from a few days until four weeks and have utilised nutrient replete conditions (see Appendix, Table 5.1 for a current overview of published studies). Only two short-term studies have included diazotrophic organisms. Hence, knowledge of the response of diazotrophic organisms in nutrient limited plankton communities to ocean acidification and warming over a growing season is currently lacking in our understanding of the impacts of global change in the marine environment. Changes in nutrient availability may influence the relative importance of autotrophic (biomass production by primary producers) vs. heterotrophic (remineralisation by consumers) activity as well as shape the plankton community as some species have better strategies for nutrient assimilation.

In this doctoral dissertion, an experimental approach of between 4 to 8 weeks using large-scale mesocosms (volume > 1000 L) was selected to investigate the impact of ocean acidification (increased $CO_2$/decreased pH) on two different summer plankton communities in the Baltic Sea with different dominant filamentous diazotrophic cyanobacteria (*A. flos-aquae* and *N. spumigena*). Both studies took place in seasons where fixed N availability was low and environmental conditions were favourable for development of filamentous diazotrophic cyanobacteria. The aim was to determine whether inputs of new diazotrophic N may be affected by ocean acidification, as

observed in single-strain culture studies, as this may affect new production in the wider plankton community under the prevailing N-limited conditions.

Chapter 2 presents an overview of organic matter pools in the in situ $CO_2$ manipulation mesocosm experiment using the natural plankton community present in the Archipelago Sea, on the south-western tip of Finland. Treatment $fCO_2$ ranged from ambient (365 µatm) up to 1231 µatm. Bioavailable nitrogen concentrations were very low and capped phytoplankton biomass. Particulate and dissolved matter pools as well as phytoplankton pigment concentrations were sampled over the 47 day study period to investigate the influence of ocean acidification on biogeochemical elemental cycling and organic matter partitioning.

In Chapter 3, $N_2$-fixation rates and the abundance of the dominant filamentous diazotrophic cyanobacteria *Aphanizomenon flos-aquae* were followed to assess any influence of $CO_2$ on growth and activity of diazotrophic organisms in the same mesocosm study as Chapter 2. This chapter aimed to determine the magnitude of diazotrophic N inputs to indicate if this was a relevant process for the N inventory during the study period.

Chapter 4 reports on the contribution of diazotrophy to the N cycle during an indoor mesocosm study in the Kiel Fjord in the south-western Baltic Sea, where both $CO_2$ and temperature were manipulated to investigate the response of the filamentous diazotrophic cyanobacterum, *Nodularia spumigena*, to ocean acidifiation and warming. Filamentous diazotrophic cyanobacteria were followed over the 28 days after initial $CO_2$-manipulation to probe the single and interactive effects of increased $CO_2$ and temperature on diazotrophic N inputs in a contrasting region with a different dominant cyanobacterium to Chapter 3.

### 1.4.2 List of first-author papers

This doctoral thesis is based on the following three manuscripts which are each considered one chapter of the thesis:

I **Paul, A. J.**, Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M. and Riebesell, U.: Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community, *Biogeosciences*, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015

II **Paul, A. J.**, Achterberg, E. P., Bach, L. T., Boxhammer, T., Czerny, J., Haunost, M., Schulz, K.-G., Stuhr, A. and Riebesell, U.: No measureable effect of ocean acidification on nitrogen biogeochemistry in a Baltic Sea plankton community, *Biogeosciences Discussions*, 12, 17507-17541, doi:10.5194/bgd-12-17507-2015, 2015

III **Paul, A. J.**, Sommer, U., Paul, C., and Riebesell, U.: Growth of key diazotrophic species negatively affected by ocean acidification and warming.
*To be submitted*

### 1.4.3 Declaration of contribution

**Manuscript I:**

Idea: Allanah Paul, Lennart Bach, Ulf Riebesell

Data acquisition: Allanah Paul, Eric Achterberg, Lennart Bach, Tim Boxhammer, Jan Czerny, Dana Hellemann, Silke Lischka, Michael Meyerhöfer, Monika Nausch, Kai Schulz, Michael Sswat, Yves Trense, Ulf Riebesell

Data interpretation and manuscript preparation: Allanah Paul with comments from all co-authors

**Manuscript II:**

Idea: Allanah Paul, Ulf Riebesell

Data acquisition: Allanah Paul, Eric Achterberg, Lennart Bach, Tim Boxhammer, Mathias Haunost, Kai Schulz, Annegret Stuhr

Data interpretation and manuscript preparation: Allanah Paul with comments from all co-authors

**Manuscript III:**

Idea: Allanah Paul, Ulf Riebesell

Data acquisition: Allanah Paul, Carolin Paul, Ulrich Sommer

Data interpretation and manuscript preparation: Allanah Paul with comments from all co-authors

# References


Andersson, A., Hajdu, S., Haecky, P., Kuparinen, J., and Wikner, J.: Succession and growth limitation of phytoplankton in the Gulf of Bothnia (Baltic Sea), Marine Biology, 126, 791–801, doi:10.1007/BF00351346, 1996.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, T. F.: The ecological role of water-column microbes in the sea, Marine Ecology Progress Series, 10, 257 – 263, 1983.

Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnology and Oceanography, 56, 2040–2050, doi:10.4319/lo.2011.56.6.2040, 2011.

Barcelos e Ramos, J., Biswas, H., Schulz, K., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochemical Cycles, 21, GB2028, doi:10.1029/2006GB002898, 2007.

Berman-Frank, I., Lundgren, P., and Falkowski, P.: Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria, Research in Microbiology, 154, 157–164, doi:10.1016/S0923-2508(03)00029-9, 2003.

Berman-Frank, I., Quigg, A., Finkel, Z. V., Irwin, A. J., and Haramaty, L.: Nitrogen-fixation strategies and Fe requirements in cyanobacteria, Limnology and Oceanography, 52, 2260–2269, doi:10.4319/lo.2007.52.5.2260, 2007.

Brauer, V. S., Stomp, M., Rosso, C., van Beusekom, S. A., Emmerich, B., Stal, L. J., and Huisman, J.: Low temperature delays timing and enhances the cost of nitrogen fixation in the unicellular cyanobacterium *Cyanothece*, The ISME Journal, 7, 2105–2115, doi:10.1038/ismej.2013.103, 2013.

Breitbarth, E., Oschlies, A., and LaRoche, J.: Physiological constraints on the global distribution of *Trichodesmium* - Effect of temperature on diazotrophy, Biogeosciences, 4, 53–61, doi:10.5194/bg-4-53-2007, 2007.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., LeQuee C., Myneni, R., Piao, S., and Thornton, P.: Carbon and Other Biogeochemical Cycles, p. 465–570, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, doi:10.1017/CBO9781107415324.015, 2013.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A., and Wehner, M.: Long-term Climate Change: Projections, Commitments and Irreversibility, p. 1029–1136, Cambridge University Press,

Cambridge, United Kingdom, Cambridge, United Kingdom and New York, NY, USA, doi:10.1017/CBO9781107415324.024, 2013.

Czerny, J., Ramos, J., and Riebesell, U.: Influence of elevated $CO_2$ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, Biogeosciences, 6, 1865–1875, doi:10.5194/bg-6-1865-2009, 2009.

Degerholm, J., Gundersen, K., Bergman, B., and Soderback, E.: Phosphorus-limited growth dynamics in two Baltic Sea cyanobacteria, *Nodularia* sp and *Aphanizomenon* sp., Fems Microbiology Ecology, 58, 323–332, doi:10.1111/j.1574-6941.2006.00180.x, 2006.

Dore, J. E., Lukas, R., Sadler, D. W., Church, M. J., and Karl, D. M.: Physical and biogeochemical modulation of ocean acidification in the central North Pacific, Proceedings of the National Academy of Sciences, 106, 12 235–12 240, doi:10.1073/pnas.0906044106, 2009.

Dugdale, R. and Goering, J.: Uptake of new and regenerated forms of nitrogen in primary productivity, Limnology and Oceanography, 12, 196–206, doi:10.4319/lo.1967.12.2.0196, 1967.

Dutkiewicz, S., Morris, J. J., Follows, M. J., Scott, J., Levitan, O., Dyhrman, S. T., and Berman-Frank, I.: Impact of ocean acidification on the structure of future phytoplankton communities, Nature Climate Change, 5, 1002–1006, doi:10.1038/nclimate2722, 2015.

Eichner, M., Rost, B., and Kranz, S. A.: Diversity of ocean acidification effects on marine $N_2$ fixers, Journal of Experimental Marine Biology and Ecology, 457, 199–207, doi:10.1016/j.jembe.2014.04.015, 2014.

Elmgren, R.: Understanding human impact on the Baltic ecosystem: Changing views in recent decades, AMBIO: A Journal of the Human Environment, 30, 222–231, doi:10.1579/0044-7447-30.4.222, 2001.

Eppley, R. and Peterson, B.: Particulate organic matter flux and planktonic new production in the deep ocean, Nature, 282, 677–680, doi:10.1038/282677a0, 1979.

Falkowski, P. G.: Evolution of the nitrogen cycle and its influence on the biological sequestration of $CO_2$ in the ocean, Nature, 387, 272–275, doi:10.1038/387272a0, 1997.

Farquhar, G. D., Caemmerer, S. v., and Berry, J. A.: A biochemical model of photosynthetic $CO_2$ assimilation in leaves of C3 species, Planta, 149, 78–90, doi:10.1007/BF00386231, 1980.

Fernandez, C., Farias, L., and Ulloa, O.: Nitrogen fixation in denitrified marine waters, PLoS ONE, 6, e20 539, doi:10.1371/journal.pone.0020539, 2011.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P.: Primary production of the biosphere: Integrating terrestrial and oceanic components, Science, 281, 237–240, doi:10.1126/science.281.5374.237, 1998.

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.

Fu, F.-X., Warner, M., Zhang, Y., Feng, Y., and Hutchins, D.: Effects of increased temperature and $CO_2$ on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria), Journal of Phycology, 43, 485–496, doi:10.1111/j.1529-8817.2007.00355.x, 2007.

Fu, F.-X., Mulholland, M., Garcia, N., Beck, A., Bernhardt, P., Warner, M., Sanudo-Wilhelmy, S., and Hutchins, D.: Interactions between changing $pCO_2$, $N_2$ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*, Limnology and Oceanography, 53, 2472–2484, doi: 10.4319/lo.2008.53.6.2472, 2008.

Fu, F.-X., Yu, E., Garcia, N. S., Gale, J., Luo, Y., Webb, E. A., and Hutchins, D. A.: Differing responses of marine $N_2$-fixers to warming and consequences for future diazotroph community structure, Aquatic Microbial Ecology, 72, 33–46, doi:10.3354/ame01683, 2014.

Garcia, N. S., Fu, F.-X., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A., and Hutchins, D. A.: Interactive effects of irradiance and $CO_2$ on $CO_2$ fixation and $N_2$ fixation in the diazotroph *Trichodesmium erythraeum* (cyanobacteria), Journal of Phycology, 47, 1292–1303, doi: 10.1111/j.1529-8817.2011.01078.x, 2011.

Garcia, N. S., Fu, F.-X., Breene, C. L., Yu, E. K., Bernhardt, P. W., Mulholland, M. R., and Hutchins, D. A.: Combined effects of $CO_2$ and light on large and small isolates of the unicellular $N_2$ -fixing cyanobacterium *Crocosphaera watsonii* from the western tropical Atlantic Ocean, European Journal of Phycology, 48, 128–139, 2013a.

Garcia, N. S., Fu, F.-X., and Hutchins, D. A.: Colimitation of the unicellular photosynthetic diazotroph *Crocosphaera watsonii* by phosphorus, light, and carbon dioxide, Limnology and Oceanography, 58, 1501–1512, doi:10.4319/1o.2013.58.4.1501, 2013b.

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

Gervais, F. and Riebesell, U.: Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different $CO_2$ concentrations, Limnology and Oceanography, 46, 497–504, doi:10.4319/lo.2001.46.3.0497, 2001.

Granéli, E., Wallström, K., Larsson, U., Granéli, W., and Elmgren, R.: Nutrient limitation of primary production in the Baltic Sea area, Ambio, 19, 142–151, 1990.

Gruber, N.: The Marine Nitrogen Cycle: Overview and Challenges, in: Nitrogen in the Marine Environment, edited by Capone, D. G., pp. 1–43, Academic Press, Elsevier, London, United Kingdom, 2nd edn., 2008.

Gunderson, A. R., Armstrong, E. J., and Stillman, J. H.: Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment, Annual Review of Marine Science, 8, doi:10.1146/annurev-marine-122414-033953, pMID: 26359817, 2016.

Hannerz, F. and Destouni, G.: Spatial characterization of the Baltic sea drainage basin and its unmonitored catchments, AMBIO, 35, 214–219, doi:10.1579/05-A-022R.1, 2006.

Havenhand, J. N.: How will ocean acidification affect Baltic Sea ecosystems? An assessment of plausible impacts on key functional groups, Ambio, 41, 637–644, doi:10.1007/s13280-012-0326-x, 2012.

HELCOM: Eutrophication in the Baltic Sea – An integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region, Tech. rep., 2009.

HELCOM: Climate change in the Baltic Sea Area: HELCOM thematic assessment in 2013, Tech. rep., Helsinki Commission, Helsinki, 2013.

Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R. C., Greene, S. E., Kiessling, W., Ries, J., Zachos, J. C., Royer, D. L., Barker, S., Marchitto, T. M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G. L., and Williams, B.: The geological record of ocean acidification, Science, 335, 1058–1063, doi:10.1126/science.1208277, 2012.

Howard, J. B. and Rees, D. C.: Structural basis of biological nitrogen fixation, Chemical Reviews, 96, 2965–2982, doi:10.1021/cr9500545, 1996.

Hunt, R. J. and Matveev, V. F.: The effects of nutrients and zooplankton community structure on phytoplankton growth in a subtropical Australian reservoir: An enclosure study, Limnologica - Ecology and Management of Inland Waters, 35, 90 – 101, doi:http://dx.doi.org/10.1016/j.limno.2005.01.004, 2005.

Hutchins, D., Fu, F., Zhang, Y., Warner, M., Feng, Y., Portune, K., Bernhardt, P., and Mulholland, M.: $CO_2$ control of *Trichodesmium* $N_2$ fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry, Limnology and Oceanography, 52, 1293–1304, doi:10.4319/lo.2007.52.4.1293, 2007.

Hutchins, D. A., Fu, F.-X., Webb, E. A., Walworth, N., and Tagliabue, A.: Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations, Nature Geoscience, 16, 790–795, doi:10.1038/ngeo1858, 2013.

Hutchins, D. A., Walworth, N. G., Webb, E. A., Saito, M. A., Moran, D., McIlvin, M. R., Gale, J., and Fu, F.-X.: Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide, Nature Communications, 6, 8155, doi:10.1038/ncomms9155, 2015.

Kahru, M., Håkansson, B., and Rud, O.: Distributions of the sea-surface temperature fronts in the Baltic Sea as derived from satellite imagery, Continental Shelf Research, 15, 663–679, doi:10.1016/0278-4343(94)E0030-P, 1995.

Karl, D., Michaels, A., Bergman, B., Capone, D., Carpenter, E., Letelier, R., Lipschultz, F., Paerl, H., Sigman, D., and Stal, L.: Dinitrogen fixation in the world's oceans, Biogeochemistry, 57-58, 47–98, doi:10.1023/A:1015798105851, 2002.

Karlberg, M. and Wulff, A.: Impact of temperature and species interaction on filamentous cyanobacteria may be more important than salinity and increased $pCO_2$ levels, Marine Biology, 160, 2063–2072, doi:10.1007/s00227-012-2078-3, 2013.

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/nature02454, 2004.

Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J., Pavelson, J., and Nõmmann, S.: Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea, Limnology and Oceanography, 41, 98–112, doi:10.4319/lo.1996.41.1.0098, 1996.

Kranz, S. A., Levitan, O., Richter, K.-U., Prasil, O., Berman-Frank, I., and Rost, B.: Combined effects of $CO_2$ and light on the $N_2$-fixing cyanobacterium *Trichodesmium* IMS101: Physiological responses, Plant Physiology, 154, 334–345, doi:10.1104/pp.110.159145, 2010.

Lam, P. and Kuypers, M. M.: Microbial nitrogen cycling processes in oxygen minimum zones, Annual Review of Marine Science, 3, 317–345, doi:10.1146/annurev-marine-120709-142814, 2011.

Lehmann, A., Getzlaff, K., and Harla, J.: Detailed assessment of climate variability in the Baltic Sea area for the period 1958 to 2009, Climate Research, 46, 185–196, doi:10.3354/cr00876, 2011.

Lehtimaki, J., Moisander, P., Sivonen, K., and Kononen, K.: Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria, Applied and Environmental Microbiology, 63, 1647–1656, 1997.

Leppäranta, M. and Myrberg, K.: The Baltic Sea: History and Geography, vol. Physical Oceanography of the Baltic Sea, pp. 1–54, Praxis Publishing Ltd., Chichiester, United Kingdom, 2009.

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., Prasil, O., and Berman-Frank, I.: Elevated $CO_2$ enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*, Global Change Biology, 13, 531–538, doi:10.1111/j.1365-2486.2006.01314.x, 2007.

Lignell, R., Heiskanen, A.-S., Kuosa, H., Gundersen, K., Kuuppo-Leinikki, P., Pajuniemi, R., and Uitto, A.: Fate of a phytoplankton spring bloom: sedimentation and carbon flow in the planktonic food web in the northern Baltic, Marine Ecology Progress Series, 94, 239–252, 1993.

Luo, Y.-W., Lima, I. D., Karl, D. M., Deutsch, C. A., and Doney, S. C.: Data-based assessment of environmental controls on global marine nitrogen fixation, Biogeosciences, 11, 691–708, doi:10.5194/bg-11-691-2014, 2014.

Malzahn, A. M., Aberle, N., Clemmesen, C., and Boersma, M.: Nutrient limitation of primary producers affects planktivorous fish condition, Limnology and Oceanography, 52, 2062–2071, doi:10.4319/lo.2007.52.5.2062, 2007.

Manahan, S. E.: Environmental Science and Technology: A Sustainable Approach to Green Science and Technology, Second Edition, CRC Press, Taylor & Francis Group,, Boca Raton, Florida, U.S.A., 2006.

Monod, J.: La technique de culture continue. Théorie et applications, Annales de l'Institut Pasteur, 79, 390–410, 1950.

Monteiro, F. M., Dutkiewicz, S., and Follows, M. J.: Biogeographical controls on the marine nitrogen fixers, Global Biogeochemical Cycles, 25, GB2003, doi:201110.1029/2010GB003902, 2011.

Mulholland, M. R. and Bernhardt, P. W.: The effect of growth rate, phosphorus concentration, and temperature on $N_2$ fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101, Limnology and Oceanography, 50, 839–849, doi:10.4319/lo.2005.50.3.0839, 2005.

Olli, K., Klais, R., and Tamminen, T.: Rehabilitating the cyanobacteria – niche partitioning, resource use efficiency, and phytoplankton community structure during diazotrophic cyanobacterial blooms, Journal of Ecology, 103, 1153–1164, doi:10.1111/1365-2745.12437, 2015.

Omstedt, A., Edman, M., Claremar, B., Frodin, P., Gustafsson, E., Humborg, C., Hägg, H., Mörth, M., Rutgersson, A., Schurgers, G., Smith, B., Wällstedt, T., and Yurova, A.: Future changes in the Baltic Sea acid–base (pH) and oxygen balances, Tellus B, 64, 19 586, doi:10.3402/tellusb.v64i0.19586, 2012.

Peng, Y., Stevens, P., De Vos, P., and De Ley, J.: Relation between pH, hydrogenase and nitrogenase activity, N-$NH_4^+$ concentration and hydrogen production in cultures of *Rhodobacter Sulfidophilus*, Microbiology, 133, 1243–1247, 1987.

Pham, D. N. and Burgess, B. K.: Nitrogenase reactivity: Effects of pH on substrate reduction and carbon monoxide inhibition, Biochemistry, 32, 13 725–13 731, doi:10.1021/bi00212a043, 1993.

Postgate, J.: Nitrogen Fixation, Cambridge University Press, Cambridge, United Kingdom, 3rd edn., 1998.

Pörtner, H.-O., Karl, D. M., Boyd, P. W., Cheung, W. W. L., Lluch-Cota, S. E., Nojiri, Y., Schmidt, D. N., and Zavialov, P. O.: Ocean systems, in: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. E., Chatterjee, M., Ebi, K. L., Estrada, Y. O., Genova, R. C., Girma, B., Kissel, E. S., Levy, A. N., MacCracken, S., Mastrandrea, P. R., and White, L. L., pp. 411 – 484, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2014.

Quéguiner, B., Hafsaoui, M., and Tréguer, P.: Simultaneous uptake of ammonium and nitrate by phytoplankton in coastal ecosystems, Estuarine, Coastal and Shelf Science, 23, 751–757, doi:10.1016/0272-7714(86)90072-7, 1986.

Raven, J. A.: Limits on growth rates, Nature, 361, 209–210, doi:10.1038/361209a0, 1993.

Raven, J. A. and Beardall, J.: Carbon Acquisition Mechanisms of Algae: Carbon Dioxide Diffusion and Carbon Dioxide Concentrating Mechanisms, in: Photosynthesis in Algae, edited by Larkum, A., Douglas, S., and Raven, J. A., pp. 226–241, Kluwer Academic Publishers, The Netherlands, 2003.

Redfield, A. C.: The biological control of chemical factors in the environment, American Scientist, 46, 205–221, 1958.

Reinfelder, J. R.: Carbon concentrating mechanisms in eukaryotic marine phytoplankton, Annual Review of Marine Science, 3, 291–315, doi:10.1146/annurev-marine-120709-142720, 2011.

Rhein, M., Rintoul, S. R., Aoki, S., Campos, E., Chambers, D., Feely, R. A., Gulev, S., Johnson, G. C., Josey, S. A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L. D., and Wang, F.: Observations: Ocean, in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by Stocker, T. F., Qin, D., Plattner, G.-K., M. Tignor, Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., pp. 255 – 316, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Riebesell, U. and Tortell, P. D.: Effects of ocean acidification on pelagic organisms and ecosystems, in: Ocean Acidification, edited by Gattuso, J.-P. and Hansson, L., p. 99, Oxford University Press, Oxford, United Kingdom, 2011.

Risgaard-Petersen, N., Meyer, R. L., and Revsbech, N. P.: Denitrification and anaerobic ammonium oxidation in sediments: effects of microphytobenthos and $NO_3^-$, Aquatic Microbial Ecology, 40, 67–76, doi:10.3354/ame040067, 2005.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C. S., Wallace, D. W. R., Tilbrook, B., Millero, F. J., Peng, T.-H., Kozyr, A., Ono, T., and Rios, A. F.: The oceanic sink for anthropogenic $CO_2$, Science, 305, 367–371, doi:10.1126/science.1097403, 2004.

Saito, M., Bertrand, E., Dutkiewicz, S., Bulygin, V., Moran, D., Monteiro, F., Follows, M., Valois, F., and Waterbury, J.: Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocosphaera watsonii*, Proceedings of the National Academy of Sciences of the United States of America, 108, 2184–2189, doi:10.1073/pnas.1006943108, 2011.

Sarmiento, J. L. and Gruber, N.: Ocean Biogeochemical Dynamics, Princeton University Press, Princeton, New Jersey, U.S.A., 2006.

Schiewer, U.: Ecology of Baltic Coastal Waters, Springer Science & Business Media, Berlin, Heidelberg, Germany, 2008.

Schneider, B., Eilola, K., Lukkari, K., Muller-Karulis, B., and Neumann, T.: Environmental Impacts—Marine Biogeochemistry, in: Second Assessment of Climate Change for the Baltic Sea Basin, edited by The BACC II Author Team, Regional Climate Studies, pp. 337–361, Springer International Publishing, Heidelberg, New York, Dordrecht, London, 2015.

Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater $pCO_2$, PLoS ONE, 9, e88 308, doi:10.1371/journal.pone.0088308, 2014.

Shi, D., Kranz, S. A., Kim, J.-M., and Morel, F. M. M.: Ocean acidification slows nitrogen fixation and growth in the dominant diazotroph *Trichodesmium* under low-iron conditions, Proceedings of the National Academy of Sciences, 109, E3094–E3100, doi:10.1073/pnas.1216012109, 2012.

Sohm, J. A., Webb, E. A., and Capone, D. G.: Emerging patterns of marine nitrogen fixation, Nature Reviews Microbiology, 9, 499–508, doi:10.1038/nrmicro2594, 2011.

Tans, P. and Keeling, R.: URL `www.esrl.noaa.gov/gmd/ccgg/trends/`, 2015.

Taylor, A. R., Chrachri, A., Wheeler, G., Goddard, H., and Brownlee, C.: A voltage-gated $H^+$ channel underlying pH homeostasis in calcifying coccolithophores, PLoS Biol, 9, e1001 085, doi:10.1371/journal.pbio.1001085, 2011.

The International Council for Exploration of the Sea: ICES Dataset on Ocean Hydrography, URL `http://ocean.ices.dk/helcom/Helcom.aspx?Mode=1`, 2014.

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

Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P., Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N., and Wulff, F.: Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea, AMBIO, 36, 186–194, doi:10.1579/0044-7447(2007)36[186:IEFENC]2.0.CO;2, 2007.

Van den Brink, F., Van Katwijk, M., and Van der Velde, G.: Impact of hydrology on phyto- and zooplankton community composition in floodplain lakes along the Lower Rhine and Meuse, Journal of Plankton Research, 16, 351–373, doi:10.1093/plankt/16.4.351, 1994.

Viitasalo, M., Blenckner, T., Gårdmark, A., Kaartokallio, H., Kautsky, L., Kuosa, H., Lindegren, M., Norkko, A., Olli, K., and Wikner, J.: Environmental Impacts—Marine Ecosystems, in: Second Assessment of Climate Change for the Baltic Sea Basin, edited by Team, T. B. I. A., Regional Climate Studies, pp. 363–380, Springer International Publishing, 2015.

Voipio, A.: The Baltic Sea, Elsevier Scientific Publishing Company, Amsterdam, The Netherlands, 1981.

Volk, T. and Hoffert, M. I.: Ocean carbon pumps: Analysis of relative strengths and efficiencies in ocean-drive atmospheric $CO_2$ changes, in: The Carbon Cycle and Atmospheric $CO_2$, edited by Sundquist, E. T. and Broecker, W. S., pp. 99–110, American Geophysical Union, Washington, D.C., U.S.A., 1985.

von Liebig, J.: Die Grundsätze der Agricultur-Chemie mit Rücksicht auf die in England angestellten Untersuchungen, Friedrich Vieweg und Sohn, Braunschweig, Germany, 1855.

Voss, M., Dippner, J. W., Humborg, C., Hürdler, J., Korth, F., Neumann, T., Schernewski, G., and Venohr, M.: History and scenarios of future development of Baltic Sea eutrophication, Estuarine, Coastal and Shelf Science, 92, 307 – 322, doi:10.1016/j.ecss.2010.12.037, 2011.

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of *Nodularia spumigena* to $pCO_2$ – Part I: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973–2988, doi:10.5194/bg-9-2973-2012, 2012.

Wasmund, N. and Siegel, H.: Phytoplankton, in: State and Evolution of the Baltic Sea, 1952–2005 : a detailed 50-year survey of meteorology and climate, physics, chemistry, biology and marine environment, edited by Feistel, R., Nausch, G., and Wasmund, N., pp. 441–481, John Wiley & Sons, Inc., Hoboken, New Jersey, U.S.A., 2008.

Wasmund, N., Nausch, G., and Matth, W.: Phytoplankton spring blooms in the southern Baltic Sea — spatio-temporal development and long–term trends, Journal of Plankton Research, 20, 1099–1117, doi:10.1093/plankt/20.6.1099, 1998.

Yamamoto, Y. and Nakahara, H.: The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length, Limnology, 6, 1–6, doi: 10.1007/s10201-004-0138-1, 2005.

# 2 | Manuscript I

# Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community

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# Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community

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**Abstract.** Ocean acidification is expected to influence plankton community structure and biogeochemical element cycles. To date, the response of plankton communities to elevated $CO_2$ has been studied primarily during nutrient-stimulated blooms. In this $CO_2$ manipulation study, we used large-volume ($\sim 55\,m^3$) pelagic in situ mesocosms to enclose a natural summer, post-spring-bloom plankton assemblage in the Baltic Sea to investigate the response of organic matter pools to ocean acidification. The carbonate system in the six mesocosms was manipulated to yield average $fCO_2$ ranging between 365 and $\sim 1230\,\mu atm$ with no adjustment of naturally available nutrient concentrations. Plankton community development and key biogeochemical element pools were subsequently followed in this nitrogen-limited ecosystem over a period of 7 weeks. We observed higher sustained chlorophyll *a* and particulate matter concentrations ($\sim 25\,\%$ higher) and lower inorganic phosphate concentrations in the water column in the highest $fCO_2$ treatment ($1231\,\mu atm$) during the final 2 weeks of the study period (Phase III), when there was low net change in particulate and dissolved matter pools. Size-fractionated phytoplankton pigment analyses indicated that these differences were driven by picophytoplankton ($< 2\,\mu m$) and were already established early in the experiment during an initial warm and more productive period with overall elevated chlorophyll *a* and particulate matter concentrations. However, the influence of picophytoplankton on bulk organic matter pools was masked by high biomass of larger plankton until Phase III, when the contribution of the small size fraction ($< 2\,\mu m$) increased to up to $90\,\%$ of chlorophyll *a*. In this phase, a $CO_2$-driven increase in water column particulate carbon did not lead to enhanced sinking material flux but was instead reflected in increased dissolved organic carbon concentrations. Hence ocean acidification may induce changes in organic matter partitioning in the upper water column during the low-nitrogen summer period in the Baltic Sea.

## 1 Introduction

The Baltic Sea is a semi-enclosed, brackish epicontinental sea with a substantial freshwater catchment area which is approximately 4 times larger than the water body itself. In addition, the Baltic Sea has limited and infrequent saline deep water inputs from the North Sea through the Danish Straits which form an important oxygen supply for the Baltic Sea bottom waters. Weak circulation, vertical mixing and water mass exchange in the Baltic Sea lead to strong horizontal and vertical salinity gradients from north ($< 5$) to south ($\sim 20$) and surface ($\sim 7$) to deep ($\sim 12$) in Gotland Deep (station BY15; International Council for the Exploration of the Sea, 2014). Consequently, the enclosed nature of the water body

and minimal water exchange mean that terrestrial and anthropogenic activities have a considerable influence on water quality, biogeochemistry and ecosystems in the Baltic Sea.

Global change is expected to have pronounced effects on the physical and chemical conditions in the Baltic Sea. Warming, decreasing pH, and increasing freshwater inputs are expected to affect primary productivity and decrease oxygen concentrations in the deeper basins (HELCOM, 2013). In combination with higher nutrient loads from changes in agricultural activity, this may lead to increased hypoxia or even anoxia in subsurface waters (Meier et al., 2011) with feedbacks on biogeochemical element cycles (Sutton et al., 2011) as well as ecosystem structure and functioning, particularly at higher trophic levels (Ekau et al., 2010; Turner, 2001; Wu, 2002). Changes in the Baltic Sea environment have already been detected. Regular monitoring of the Baltic Sea over the past 100 years has indicated higher rates of temperature increase (0.08 to 0.11 °C per decade) than the global average, along with a 20 % decrease in annual maximum ice extent (HELCOM, 2013). Observed shifts in the spring and summer phytoplankton community dynamics have been primarily associated with warming in northern Baltic Sea regions over the past three decades (Suikkanen et al., 2013).

Ocean acidification is another anthropogenic process of potential relevance for Baltic plankton communities. As $CO_2$ dissolves in seawater, the carbonate system shifts with an associated decrease in pH. Ocean acidification therefore adds to the decrease in seawater pH as a result of nitrogen and sulfate deposition in the form of acid rain (Doney et al., 2007). Between 1993 and 2012, pH in the Baltic proper decreased on the order of 0.1 pH units (International Council for the Exploration of the Sea, 2014), which is more than 2 times faster than observed in the Pacific Ocean ($\sim$ 0.04 pH decrease between 1992 and 2012 in surface 30 m, Station ALOHA, Hawaii Ocean Time-Series; Dore et al., 2009). Changes in $fCO_2$ and pH influence phytoplankton physiology, growth rates, and carbon fixation, with some phytoplankton functional groups such as calcifying organisms more sensitive than others such as diatoms (Riebesell and Tortell, 2011; Rost et al., 2008). Thus the relative fitness of each functional group determines the response of the plankton community as a whole. Changes in physiological processes in phytoplankton on a cellular level can cascade through trophic levels and induce shifts in the structure of the planktonic food web.

To date, the majority of ocean acidification experiments have utilised nutrient-replete starting conditions or added nutrients to investigate effects of high $CO_2$ on plankton communities and biogeochemical cycles (nutrient-replete/addition (e.g. Biswas et al., 2012; Engel et al., 2005, 2008, 2014; Feng et al., 2010; Hama et al., 2012; Hare et al., 2007; Hopkins et al., 2010; Hopkinson et al., 2010; Hoppe et al., 2013; Kim et al., 2006; Nielsen et al., 2010, 2011; Richier et al., 2014; Rossoll et al., 2013; Schulz et al., 2008, 2013; Tatters et al., 2013a, b; Yoshimura et al., 2010, 2013, 2014) vs. nutrient-depleted (e.g. Law et al., 2012; Lomas et al., 2012; Losh et al., 2012)). These studies mimic the productive spring bloom, where nutrient concentrations are relatively high and relatively low light levels initially limit phytoplankton growth. However, for considerable parts of the year, the opposite is the case. Growth is not limited by light but by nutrient concentrations and biomass tends to be low. This is also the case during summer in the Baltic Sea. Here, a diatom-dominated spring bloom in April/May usually draws down dissolved inorganic nutrients so that concentrations remain low from early summer. Diazotrophic filamentous cyanobacteria then commonly bloom in July and August, when surface water temperatures peak, calm weather conditions induce water column stratification and low nitrogen in a bioavailable form limits growth in the non-diazotrophic phytoplankton (Gasiūnaitė et al., 2005; Kanoshina et al., 2003; Stal et al., 1999).

We undertook a pelagic in situ mesocosm study on a summer Baltic Sea plankton community to investigate the response of this low-nutrient ecosystem to projected changes in $fCO_2$. Using this approach, many different trophic levels from bacteria and viruses through to zooplankton can be investigated over extended periods of time. Using the KOSMOS mesocosm system (Kiel Off-Shore Mesocosms for future Ocean Simulations; Riebesell et al., 2013), we were able to enclose large volumes containing whole plankton communities with a low level of disturbance and thereby utilising natural variability in light and temperature.

## 2 Methods

### 2.1 Study area, deployment site, and mesocosm setup

On 12 June 2012 (day $-10 = t-10$, 10 days before $CO_2$ manipulation), nine floating, pelagic mesocosms (Fig. 1, KOSMOS, volume $\sim 55\,m^3$) were deployed and moored at 59°51.5′ N, 23°15.5′ E in the Tvärminne Storfjärden, an open archipelago area on the eastern side of the Hanko peninsula on the south-west coast of Finland (Fig. 2). The water depth at the mooring site was approximately 30 m. The bottom ends of the mesocosm bags were lowered to a depth of 17 m below the surface to enclose the plankton community with minimal disturbance to the water column. A mesh of 3 mm was attached to the top, which was submerged $\sim$ 0.5 m below the surface, and bottom of the bag, at 17 m deep, to exclude any large organisms or particles with patchy distribution in the water column. Initially the mesocosm bags were kept open and covered with only the 3 mm nets at the top and bottom openings for 5 days to allow for rinsing of the mesocosm bags water and free exchange of plankton (< 3 mm). On $t-5$, the nets were removed, sediment traps (2 m long, Fig. 1) were then attached to close the bottom of the mesocosms and the top ends of the bags were pulled up to 1.5 m above the water surface, thereby isolating the water in the mesocosms from the surrounding Baltic Sea.

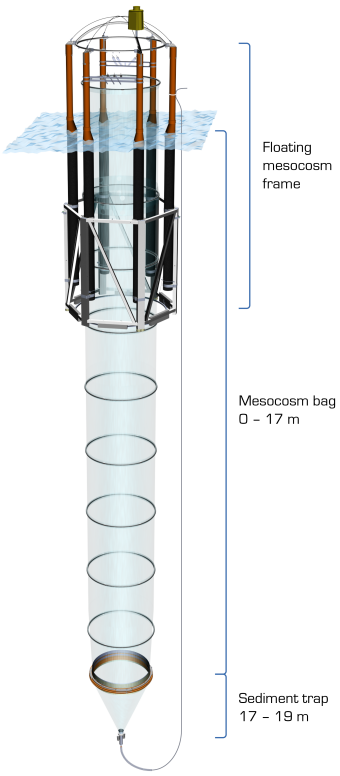


**Figure 1.** Diagram of Kiel Off-Shore Mesocosm for future Ocean Simulations showing floating frame, mesocosm bag and attached sediment trap. Source: GEOMAR.

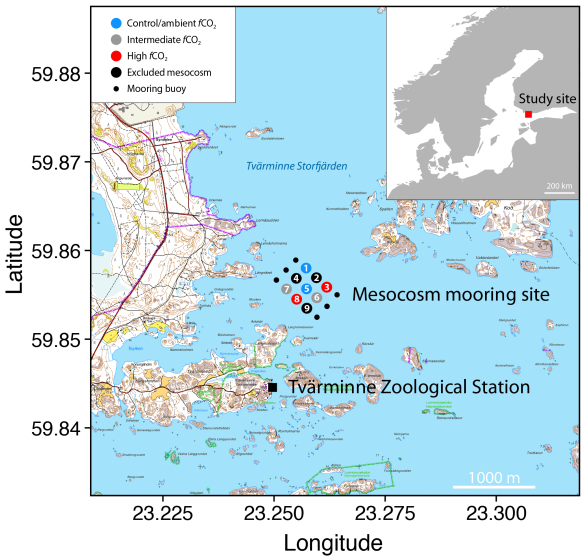


**Figure 2.** Map of study area (inset) and mesocosm mooring site in the Tvärminne Storfjärden, off the Hanko Peninsula close to the entrance to the Gulf of Finland in the Baltic Sea. Mesocosm representation is not to scale. Map contains data from the National Land Survey of Finland Topographic Database, accessed March 2015.

To ensure a homogeneous water column in each mesocosm at the start of the experiment, the halocline present was destroyed by bubbling each mesocosm with compressed air for 3.5 min on $t-5$. A video profile taken in one of the mesocosms on $t-4$ shows the plankton community present at the beginning of the study period (Boxhammer et al., 2015a). Figure 3 indicates the experiment timeline including important manipulations. Mesocosm bags were cleaned occasionally inside and outside throughout the experiment to minimise wall growth and keep the biofilm biomass at a minimum (see Fig. 3 and Riebesell et al., 2013, for further details). An isotope tracer ($^{15}N$-$N_2$ gas) specific to the nitrogen-fixing organisms present was injected in two additions ($t22$ and $t26$) into four mesocosm bags (M3, M5, M6, M8). Further details about the addition are described in Paul et al. (2015). No dissolved inorganic or organic nutrients were added to the mesocosms in this study. At the end of the experiment, the volume of each mesocosm (0–19 m) was determined through addition of a calibrated salt solution as described by Czerny et al. (2013). Final mesocosm volumes ranged between 53.1 and 55.1 $m^3$ with an estimated uncertainty of 2 %. Unfortunately, three mesocosms (M2, M4 and M9) were lost because of extensive and unquantifiable water exchange with the surrounding seawater due to a welding error on the mesocosm bags and were thus excluded from sampling and analyses.

### 2.2 $CO_2$ manipulations

$CO_2$ treatments were achieved by equally distributing filtered (50 µm), $CO_2$-saturated seawater into the mesocosm as described by Riebesell et al. (2013) in four separate additions (see Table 1 for details). The first addition of $CO_2$-enriched seawater defined the beginning of the experiment and took place on $t0$ following sampling activities. Seawater for the additions was collected from 10 m depth by a pipe connected to the laboratory in the research station. Different amounts of $CO_2$-saturated seawater were added to four mesocosms to set up an initial gradient in $fCO_2$ treatments from ambient ($\sim$ 240 µatm) up to $\sim$ 1650 µatm. On $t15$, $CO_2$ was manipulated in the upper 7 m to counteract pronounced outgassing in the mesocosm. Two mesocosms were selected as controls with no addition of $CO_2$-enriched seawater. Instead, unenriched filtered seawater (50 µm) was added for the initial manipulations. For the later smaller addition, the water distributor ("spider"; Riebesell et al., 2013) was pulled up and down in each mesocosm to simulate water column mixing and manipulation side effects caused by the device on $t15$.

**Table 1.** Volumes of $CO_2$-enriched seawater added for the $CO_2$ manipulation indicating day of addition and total manipulation volumes. Symbols and colours indicated here are used in all following figures.

| Mesocosm | | M1 | M5 | M7 | M6 | M3 | M8 | Baltic |
|---|---|---|---|---|---|---|---|---|
| Target $fCO_2$ (µatm) | | ambient/control | ambient/control | 600 | 950 | 1300 | 1650 | ambient |
| Average $fCO_2$ (µatm) $t1$–$t43$ | | 365 | 368 | 497 | 821 | 1007 | 1231 | 417 |
| Average $fCO_2$ (µatm) $t1$–$t30$ | | 346 | 348 | 494 | 868 | 1075 | 1333 | 343 |
| | Symbol | | | | | | | |
| Day | $t0$ | – | – | 20 L | 50 L | 65 L | 75 L | – |
| | $t1$ | – | – | 10 L | 40 L | 50 L | 65 L | – |
| | $t2$ | – | – | 10 L | 30 L | 45 L | 50 L | – |
| | $t3$ | – | – | 5 L | 8 L | 9 L | 10 L | – |
| | $t15$ | – | – | – | 9 L | 12 L | 18 L | – |
| | Total | – | – | 45 L | 137 L | 181 L | 218 L | – |

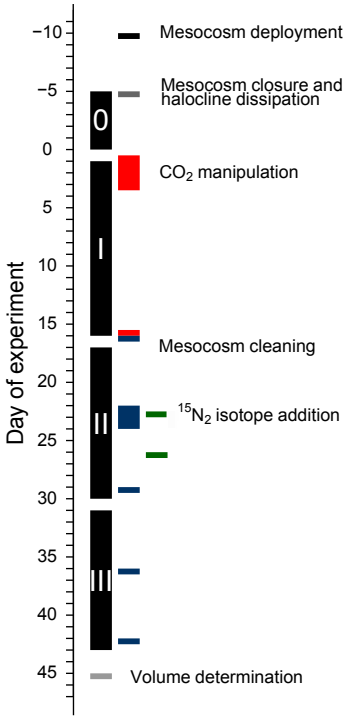


**Figure 3.** Experiment timeline indicating important activities such as $CO_2$ manipulations (red), cleaning (dark blue), phases (black, labelled with 0, I, II and III for Phases 0, I, II and III, respectively), volume determination (light grey) and isotope addition (dark green). Distinction of experimental phases is described in Sect. 3.1.

### 2.3 CTD and light measurements

CTD casts in each mesocosm and in the surrounding water were made with a hand-held self-logging CTD probe (CTD60M, Sea and Sun Technology) from 0.3 m down to ∼ 18 m (mesocosms) and to ∼ 30 m (surrounding water in archipelago from Baltic) between 13:30 and 14:30 local time (LT) daily until $t31$, and then every second day until $t46$. Temperature, pH, dissolved oxygen and PAR (photosynthetic active radiation) sensors were deployed on the CTD as well as a conductivity cell. Details on the sensors, their accuracy, precision, and corrections applied are described in Schulz and Riebesell (2013). The potentiometric CTD pH was corrected to spectrophotometric measurements (see Sect. 2.5.1). The depth of average water column light intensity in metres was calculated by averaging all water column PAR data and relating this to the depth where this intensity of PAR occurred.

A PAR sensor (LI-COR LI-192) was placed unobstructed at the end of a 2 m pole on the roof of Tvärminne Zoological Station (∼ 1 km from mesocosm mooring site) to record incoming PAR for the mesocosms. Incoming PAR was recorded from 14:43 LT on 14 June 2012 continuously as the mean of integrated 60 s intervals until the end of the experiment at 11:23 LT on 7 August 2012.

### 2.4 Sampling procedures

Water samples were collected regularly from each mesocosm and the surrounding water using depth-integrated water samplers (IWS, HYDRO-BIOS, Kiel). Unless otherwise reported, all samples are from the entire water column (0 to 17 m). For example, inorganic dissolved nutrient and fluorometric Chl *a* samples were also taken regularly for the upper water column (0 to 10 m). Full details of mesocosm sampling procedures and equipment are described in Riebesell et al. (2013) and Schulz et al. (2013). There were two intensive sampling periods where sampling took place every day ($t - 3$ to $t5$, $t29$ to $t31$), otherwise most variables were sampled every second day. Table 2 presents sampled variables, including sampling frequency and respective papers which report each data set. Samples for carbonate chemistry

variables and trace gas analyses were the first to be sampled and were taken from the IWS directly on board the sampling boat. Other samples (e.g. particulate matter, Chl *a*, phytoplankton pigments) were collected into 10 L carboys and stored in the dark. Carboys were stored at in situ temperature onshore and subsampling from these carboys was usually within 1 h and up to a maximum of 5 h after sampling. Care was taken to mix the water samples in the carboys well before taking subsamples to ensure homogeneous sampling for all parameters.

The sediment trap was emptied every second day using a manual vacuum pump system to acquire the settled material via a silicon tube reaching down to the collection cylinder of the sediment trap (Boxhammer et al., 2015b; Riebesell et al., 2013). This material was used to quantify and characterise particle sinking flux. Subsamples of the particle suspension (< 6 % in total) were taken before the material was concentrated. Particles and aggregates were allowed to settle down within 2 h at in situ temperature before separation of the supernatant. Collected particulate material was then centrifuged, while subsamples of the supernatant were filtered and analysed analogous to water column samples for particulate matter. Centrifuged material was subsequently frozen, lyophilised and ground to a fine powder of homogeneous composition. From this powder small subsamples of between 0.7 and 1.5 mg were weighed and analysed for carbon, nitrogen, phosphate and biogenic silica content as described in this paper for water column samples (see Sect. 2.5.3). Concentrations of particulate material were calculated based on total mesocosm volume (in litres). Mesocosm volume determined on *t*45 by salt addition in kilograms (Sect. 2.2) was converted using mean mesocosm temperature and salinity over 0–17 m between $t-3$ and *t*43 (mean temperature = 11.42 °C, mean salinity = 5.70) and the algorithms described by Fofonoff and Millard Jr. (1983). A more in-depth description of sampling and processing of particles collected in the sediment traps of the KOSMOS setup is presented in Boxhammer et al. (2015b).

## 2.5 Sample analyses

### 2.5.1 Carbonate system parameters (DIC, TA, $pH_T$)

Samples for total alkalinity (TA), dissolved inorganic carbon concentrations (DIC) and total pH (on the total pH scale: $pH_T$) were gently pressure-filtered (Sarstedt Filtropur PES, 0.2 µm pore size) using a membrane pump (Stepdos) to exclude calcareous particles and particulate organic material before analysis. Presence of particulate matter can influence precision of carbonate chemistry measurements. In addition, the sterile filtration eliminates the influence of biological processes on pH and DIC during sample storage by phytoplankton or bacteria.

Total pH was determined by spectrophotometry as described in Dickson et al. (2007). Samples were analysed on a Cary 100 (Varian) spectrophotometer in a temperature-controlled 10 cm cuvette using a low-ionic-strength *m*-cresol indicator dye matching the salinity of the sample water and an appropriate low-salinity pK (Mosley et al., 2004). CTD pH measurements were corrected to $pH_T$ by daily linear correlations of mean water column potentiometric pH measurements to spectrophotometric $pH_T$ measurements.

DIC concentrations were determined by infrared absorption using a LI-COR LI-7000 on an AIRICA system (MARIANDA, Kiel). Measurements were made on four replicates of 2 mL sample volume and DIC was calculated as the mean of the best three out of four measurements. The precision was typically better than 1.5 µmol $kg^{-1}$. Dissolved calcium concentrations in seawater were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a VARIAN 720-ES and quality-controlled with IAPSO reference material.

TA was analysed by potentiometric titration using a Metrohm 869 sample changer and a 907 Titrando dosing unit according to the open-cell method described in Dickson et al. (2007). Due to unaccounted contributions to TA in the range of 20 and 25 µmol $kg^{-1}$ by components such as organic acids and bases, spectrophotometric $pH_T$ and DIC were used to calculate carbonate chemistry speciation using the stoichiometric equilibrium constants for carbonic acid of Mehrbach et al. (1973) as refitted by Lueker et al. (2000). Buffering by organic compounds is not accounted for in the traditional TA definition (Dickson, 1981) and depends on unknown concentrations and acid–base equilibria of certain DOM components. Thus, using TA for carbonate chemistry speciation calculations would have resulted in errors (Koeve and Oschlies, 2012). Both TA and DIC measurements were calibrated using measurements of the certified reference material batch CRM 115 (Dickson, 2010).

### 2.5.2 Dissolved inorganic nutrients

Samples for nutrients were collected in acid-cleaned (1 mol $L^{-1}$ HCl) 60 mL low-density polyethylene bottles (Nalgene), stored at 4 °C in the dark following sampling and analysed within 12 h of collection. Dissolved silicate (DSi) concentrations were determined using standard colorimetric techniques (Grasshoff et al., 1983) at the micromolar level using a nutrient autoanalyser (Seal Analytical, Quattro). Nanomolar levels of dissolved nitrate + nitrite (hereafter nitrate) and dissolved inorganic phosphate (DIP) were determined with a colorimetric method using a 2 m liquid waveguide capillary cell (LWCC) (Patey et al., 2008; Zhang and Chi, 2002) with a miniaturised detector (Ocean Optics Ltd). Detection limits were 2 nmol $L^{-1}$ for nitrate and 1 nmol $L^{-1}$ for DIP, with a linear range up to 300 nmol $L^{-1}$. All samples for inorganic nutrient measurements were filtered using glass fibre filters (GF/F, nominal pore size of 0.7 µm, Fisher Scientific) prior to analysis. This was done to reduce the dissolution of nutrients from particulates during analysis,

**Table 2.** Summary of sampled variables for this study, including a brief description of method used, sampling frequency and corresponding papers in this special issue where data set and further details of methods used can be found.

| Variable | Method/instrument | Sampling frequency | Corresponding paper |
|---|---|---|---|
| ATP and phosphate uptake rates | $^{33}$P incorporation | Every 2nd day until *t* 29 | Nausch et al. (2015) |
| Bacteria and virus abundances | Flow cytometry | Daily until *t* 31, then every 2nd day until *t* 43 | Crawfurd et al. (2015) |
| Bacterial production | $^{14}$C-Leucine incorporation | $t-3$, *t*0, from *t*2 every 3rd day until *t*26, from *t*29 every 2nd day until *t*43 | Hornick et al. (2015); Nausch et al. (2015) |
| Biogenic silica | Spectrophotometry | Every 2nd day until *t*43 | This paper |
| Chlorophyll *a* | Fluorometry | Daily until *t*30, every 2nd day until *t*39 | This paper |
| Community respiration | $O_2$ consumption | Daily until *t*33, excluding *t*2, *t*14, *t*32 | Spilling et al. (2015) |
| Copepod (*Acartia bifilosa, Eurytemora affinis*) reproduction | Incubations, microscopy counts | Weekly (*t*3, *t*10, *t*17, *t*24 + *t*45 for *A. bifilosa*) | Almén et al. (2015); Vehmaa et al. (2015) |
| Copepod adult female size (*A. bifilosa*) | Microscopy measurements | Weekly (*t*3, *t*10, *t*17, *t*24, *t*45) | Vehmaa et al. (2015) |
| Copepod antioxidant capacity | ORAC | Weekly (*t*3, *t*10, *t*17, *t*31) | Almén et al. (2015); Vehmaa et al. (2015) |
| Dissolved inorganic carbon (DIC) | IR absorption | Daily until *t*30, every 2nd day until *t*43 | This paper |
| Dissolved organic carbon and nitrogen | Shimadzu TOC/TDN analyser | Every 2nd day until *t*43 | This paper |
| Dissolve organic phosphorus | Microwave digestion, spectrophotometry | Every 2nd day until *t*29 | This paper; Nausch et al. (2015) |
| Fatty acid concentrations (phytoplankton, copepods: *A. bifilosa, E. affinis*) | GC-MS | Phyto.: every 4th day until *t*29; copepods: weekly (*t*3, *t*10, *t*17, *t*24, *t*31, *t*38) | Almén et al. (2015); Bermúdez et al. (2015) |
| Fatty acid concentrations (*E. affinis* adults and eggs from reproduction incubations) | GC-MS | Weekly (*t*7, *t*14, *t*21, *t*28) | Almén et al. (2015) |
| Inorganic nutrient concentrations | Colorimetry (LWCC) | Every 2nd day until *t*43 | This paper |
| Light intensity (PAR) | LI-COR sensor | Daily between $t-5$ and *t*45 | This paper |
| Mesozooplankton abundances | Stereomicroscopy counts | $t-3$, $t-2$, $t-1$, *t*0, *t*3, *t*10, *t*17, *t*24, *t*31, *t*38, *t*43 | Lischka et al. (2015) |
| Microzooplankton abundances | Microscopy counts | $t-3$, *t*0, *t*2, *t*4, *t*7, *t*9, *t*11, *t*13, *t*15, *t*17, *t*21, *t*23, *t*25, *t*27, *t*29, *t*31, *t*33, *t*35, *t*37, *t*39, *t*41, *t*43 | Lischka et al. (2015) |
| $N_2$-fixation rates | $^{15}$N incorporation, EA-IRMS | Every 2nd day until *t*43 | Paul et al. (2015) |
| pH | Spectrophotometry and CTD sensor for mesocosm profiles | Daily until *t*30, every 2nd day until *t*43 | This paper |
| Phytoplankton abundances | Microscopy counts | Every 2nd day until *t*43 | Bermúdez et al. (2015); Paul et al. (2015) |
| Phytoplankton abundances | Flow cytometry | Daily until *t*31, then every 2nd day until *t*39 | Crawfurd et al. (2015) |
| Phytoplankton pigments | HPLC | Every 2nd day until *t*43, size fractions every 2nd sampling day excluding *t*37 and *t*39 | This paper |
| Primary production | $^{14}$C incorporation | Every 2nd day until *t*30, excluding *t*1, *t*2, *t*3, *t*6, *t*7, *t*8 | Spilling et al. (2015) |
| Salinity, temperature | CTD sensor | Daily until *t*30, every 2nd day until *t*43 | This paper |
| Sediment trap material – amount and elemental characterisation (C, N, P, BSi, pigment concentration) | EA-IRMS, HPLC, spectrophotometry | Every 2nd day until *t*43 | This paper; Paul et al. (2015) |
| Total alkalinity | Potentiometric titration | Daily until *t*30, every 2nd day until *t*43 | This paper |
| Total particulate carbon (including $\delta^{13}$C), particulate organic nitrogen (including $\delta^{15}$N), size fractions (total, < 55 µm, < 10 µm) | EA-IRMS | Every 2nd day until *t*43,except for < 10 µm fraction every 2nd day from *t*23 until *t*43 | This paper; Paul et al. (2015) ($\delta^{13}$C unpublished) |
| Total particulate phosphorus | Spectrophotometry | Every 2nd day until *t*43 | This paper |
| Trace gas concentration | GC-MS | Every 2nd day until *t*17 then daily until *t*30 | Webb et al. (2015) |
| Viral lysis and grazing of bacteria | Incubations, flow cytometry | $t-3$, *t*0, *t*4, *t*7, *t*11, *t*14, *t*18, *t*21 | Crawfurd et al. (2015) |
| Viral lysis and grazing of phytoplankton | Incubations, flow cytometry | *t*1, *t*3, *t*6, *t*10, *t*13, *t*17, *t*20, *t*24, *t*31 | Crawfurd et al. (2015) |

and also to avoid particles blocking the LWCCs and interfering with the spectrophotometric measurements. Ammonium ($NH_4^+$) measurements were undertaken following the method by Kérouel and Aminot (1997) with fluorimetric detection (Trilogy, Turner), and featuring a detection limit of 5 nmol $L^{-1}$.

### 2.5.3 Particulate material (C, N, P, Si)

Total particulate carbon, particulate organic nitrogen and total particulate phosphorus (TPC, PON, TPP) samples were collected onto combusted GF/F filters (Whatman, nominal pore size of 0.7 µm) using gentle vacuum filtration (< 200 mbar) and stored in glass Petri dishes at −20 °C directly after filtration until analysis. Filters and glass Petri dishes were combusted at 450 °C for 6 h before use. Filters were not acidified to distinguish between inorganic and organic particulate carbon before analyses; hence, we measured TPC. However, microscopy counts and total alkalinity drawdown indicated pelagic calcifying organisms were not abundant and there was no significant calcification; thus it was probably mostly particulate organic carbon. In addition to the total particulate matter fraction, gauze pre-filters were used to separate size-fractionated samples for C and N analyses (0.7 to 10 µm = $TPC/PON_{<10}$, 0.7 to 55 µm = $TPC/PON_{<55}$). Filtration volumes ranged from 500 mL for the total fraction ($POM_{tot}$) to up to 1500 mL for < 55 µm size fraction to ensure sufficient biomass on the filter for analyses. Sampling for $TPC_{<10}$ and $PON_{<10}$ only occurred after isotope tracer addition on $t23$ in the four mesocosms where tracer was added (M3, M5, M6, M8). This size fraction was sampled to exclude large filamentous diazotrophic cyanobacteria.

Filters for TPC/PON were dried at 60 °C, packed into tin capsules and stored in a dessicator until analysis. TPC and PON measurements were made on an elemental analyser (EuroEA) according to Sharp (1974), coupled by either a Conflo II to a Finnigan Delta$^{Plus}$ isotope ratio mass spectrometer or a Conflo III to a Thermo Finnigan Delta$^{Plus}$ XP isotope ratio mass spectrometer. Subsamples of sediment material powder (1–2 mg) were weighed directly into tin capsules using an electronic microbalance (Sartorius M2P) with an accuracy of 0.001 mg. In addition to the standard calibration at the beginning of each run, standard materials (caffeine, peptone, acetanilide, nicotinamide, glutamic acid) were also included within runs to identify any drift and ensure accuracy and full combustion of the samples during analysis. Selected samples for sediment material TPC and PON were reanalysed on an elemental analyser (EuroEA) not coupled to a mass spectrometer, using the same method and standard materials. Total sinking particle flux is the sum of both the particulate matter concentrations determined in sediment powder and supernatant.

Filters for total particulate phosphorus (TPP) were placed in 40 mL of deionised water (Milli-Q, Millipore) with oxidising decomposition reagent (MERCK, catalogue no. 112936) and autoclaved for 30 min in a pressure cooker to oxidise the organic phosphorus to orthophosphate. Samples were allowed to cool before concentrations were determined by spectrophotometric analysis as for dissolved inorganic phosphate concentrations according to Hansen and Koroleff (1999).

For biogenic silica (BSi), samples were collected on cellulose acetate filters (0.65 µm, Whatman) as described above for TPC, PON and TPP. Particulate silicate was leached from filtered material using 0.1 mol $L^{-1}$ NaOH at 85 °C for 2 h and 15 min, neutralised with $H_2SO_4$ (0.05 mol $L^{-1}$, Titrisol) and analysed as dissolved silicate by spectrophotometry according to Hansen and Koroleff (1999).

Content of TPP and BSi in finely ground sediment trap samples was determined from subsamples and analysed according to methods described for water column samples.

### 2.5.4 Dissolved organic matter (C, N, P)

For dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analyses, 35 mL of sample was filtered through pre-combusted GF/F filters (450 °C, 6 h) and collected in acid-cleaned and combusted glass vials (450 °C, 6 h), acidified with HCl to pH 1.9, and then flame-sealed and dark-stored in a fridge (4 °C) for subsequent analysis. DOC and TDN concentrations were determined using a high-temperature catalytic combustion technique with a Shimadzu TOC-TN V analyser following Badr et al. (2003). Acidified deep Sargasso Sea water, preserved in glass ampoules and provided by D. Hansell (University of Miami), served as a certified reference material. Our analytical precision, based on the coefficient of variation (SD/mean) of consecutive measurements of a single sample (generally between three and five injections), was typically < 1 %. Dissolved organic nitrogen (DON) concentrations were calculated from TDN by the subtraction of the inorganic nitrogen concentrations.

Dissolved organic phosphorus (DOP) samples were collected as for DOC and TDN but stored at −20 °C in acid-rinsed, high-density polyethylene (HDPE) bottles. Total dissolved phosphate was decomposed to inorganic phosphate using an oxidising solution and microwave radiation (MARS 5X microwave, CEM) before analysis according to Hansen and Koroleff (1983). DOP concentrations were calculated from total dissolved phosphate by subtracting dissolved inorganic phosphate concentrations. Samples for DOP were only taken until $t30$. For further details, please refer to Nausch et al. (2015).

### 2.5.5 Phytoplankton pigments

Samples for fluorometric chlorophyll *a* (Chl *a*) determination and for phytoplankton pigment analyses by reverse-phase high-performance liquid chromatography (HPLC)

were collected as described for POM with care taken to minimise exposure to light. Size fractionation for HPLC samples was achieved by pre-filtration using a 20 µm mesh and 2 µm membrane filters (Nuclepore) and sampling was undertaken every fourth day, except for between $t31$ and $t39$, where sampling occurred only on $t31$, $t33$ and $t39$ (Table 2). Filtration volume for the total and < 2 µm fraction as well as for Chl *a* was 500 mL, whereas for the large fraction (> 20 µm) volume ranged between 3000 and 5000 mL. All HPLC samples were stored at −80 °C for under 6 months and Chl *a* samples at −20 °C overnight until analysis.

Pigments from both fluorometric and HPLC analyses were extracted in acetone (90 %) in plastic vials by homogenisation of the filters using glass beads in a cell mill. After centrifugation (10 min, 800 × *g*, 4 °C) the supernatant was analysed on a fluorometer (TURNER 10-AU) to determine Chl *a* concentrations (Welschmeyer, 1994). Samples for phytoplankton pigment analyses were also centrifuged (10 min, 5200 rpm, 4 °C) and the supernatant was filtered through 0.2 µm PTFE filters (VWR International). Phytoplankton pigment concentrations were determined in the supernatant by reverse-phase high-performance liquid chromatography (HPLC; WATERS HPLC with a Varian Microsorb-MV 100-3 C8 column; Barlow et al., 1997; Derenbach, 1969) and peaks were calibrated with the help of a library of pre-measured commercial standards. Relative contributions of phytoplankton groups to total Chl *a* were calculated using the CHEMTAX matrix factorisation program (Mackey et al., 1996). Pigment ratios were adapted accordingly to those reported for Baltic Sea phytoplankton (Eker-Develi et al., 2008; Schluter et al., 2000; Zapata et al., 2000). The size fraction 2–20 µm was calculated as < 2 and > 20 µm subtracted from the total size fraction.

### 2.6 Statistical data treatment

As in previous mesocosm experiments, an $fCO_2$ gradient was chosen for reasons as outlined in Schulz et al. (2013). Linear regression analyses were used to determine the relationship between average $fCO_2$ and average response of the variables during each experimental phase. Outliers were detected based on Grubb's test ($p < 0.05$). This test was applied to all treatments by experiment phase to account for temporal development of each variable. Detected outliers were not included in the calculation of experiment phase average. Exceptions to outlier exclusion include biogenic silicate concentrations in M8 on $t23$ because all data were higher on this particular sampling day, and C : N in total POM on $t19$ in M8 because the C : N in this treatment was also markedly higher than other treatments on the following sampling day ($t21$). The same line of reasoning for the latter also applies to the contribution of cryptophytes to total Ch *a* M8 on $t17$ and all five outliers in contribution of euglenophytes to total Chl *a* detected in Phase III for the same line of reasoning as (b). All data points are included in the figures, with excluded outliers clearly marked. Linear regression analyses and outlier detection and exclusion were undertaken using R software (http://www.r-project.org/).

## 3 Results

### 3.1 Variations in temperature, salinity and oceanographic conditions

Conditions in the Tvärminne Storfjärden at the beginning of the experiment and during mesocosm closure were typical for the early summer season. Daily solar irradiance was at the annual peak (summer solstice) and surface water temperatures were ∼ 10 °C. Daily average water column temperature was highly variable over the experiment ranging from 8.0 to 8.5 °C at the beginning of the experiment to 16 °C on $t16$ (Fig. 4). Temperature variations as well as the first $CO_2$ manipulation on $t0$ were used to define different experimental phases (Phase $0 = t-5$ to $t0$, Phase I $= t1$ to $t16$, Phase II $= t17$ to $t30$, Phase III $= t31$ to $t43$). Warming occurred over the first 15 days and average water column temperatures peaked at 16 °C (Phase I). A cooling phase (Phase II) occurred until $t31$ (∼ 8 °C), followed by a second warming period (Phase III) which continued until the end of the experiment, reaching around 12 °C on average in the water column (Fig. 4 and 5c). The cooling in Phase II occurred around the same time as a period of lower incoming PAR between $t15$ and $t25$ (land-based PAR measurements, Fig. 6a). Surface water temperatures reached a maximum of 18 °C with a surface-to-depth gradient of 6 °C. The water column in the mesocosms remained thermally stratified throughout the study according to daily CTD profiles. Stratification strength, defined here as the potential density anomaly ($\sigma_T$) difference between the surface 10 m and bottom 7 m above the sediment trap in each mesocosm, was variable but lower in Phase I than in II and III. Detected changes in density over time were largely driven by changes in temperature within the mesocosms as there was only a minimal increase in salinity during the experiment probably due to evaporation (Fig. 5). Here, M8 was arbitrarily selected as representative of all mesocosms in Figs. 5 and 6. A typical daily difference in measured average water column temperature and salinity between mesocosms was 0.04 °C and 0.01, respectively. The increase in salinity on $t45$ is from addition of a calibrated salt solution for mesocosm volume determination. A notable decrease in temperature and increase in salinity in the archipelago between $t15$ and $t31$ coincided with a period of stormy weather and a change in wind direction from north-easterly to a more westerly direction, indicating a period of upwelling. During this period, there was slightly lower incoming PAR, indicating higher cloud cover (Fig. 6). The depth of average light intensity was relatively stable between 3.7 and 4.7 m inside the mesocosms and very similar between treatments over time (Fig. 6).

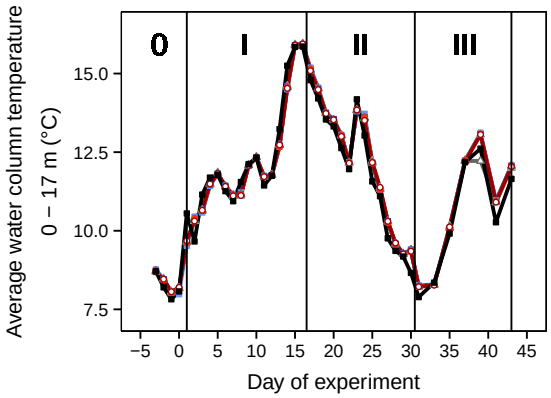


**Figure 4.** Variation in average water column temperature for all mesocosms and surrounding water during the study period. $CO_2$ enrichment (after $t0$) and temperature variations defined experimental phases. Phase 0: no $CO_2$ treatments; Phase I: warming; Phase II: cooling; Phase III: second warming phase until end of the experiment at $t43$. Colours and symbols are described in Table 1.

### 3.2 Temporal variations in carbonate system

All mesocosms had a similar $pH_T$ of around 8.0 prior to $CO_2$ perturbations. Initial $CO_2$ enrichment reached target values on $t4$ ranging from $\sim 240$ µatm in the two ambient control mesocosms up to $\sim 1650$ µatm in the highest treatment, corresponding to a $pH_T$ range of $\sim 7.45$ to 8.2 (Fig. 7). Aside from the $CO_2$ addition on $t15$, $fCO_2$ was allowed to vary naturally and treatments remained well separated over the entire experiment. The decrease in $fCO_2$ over time in the high $CO_2$ treatment mesocosms was mostly driven by outgassing rather than biological uptake as productive biomass remained relatively low in this experiment (see Sect. 3.3). The effect of outgassing is evident in the rapid increase in surface $pH_T$ in all treatment mesocosms (Fig. 8). Surrounding water $pH_T$ (0–17 m) ranged from 8.30 initially to 7.75 during the experiment. The profound $pH_T$ variability outside the mesocosms was due to upwelling of deeper, $CO_2$-rich seawater. Within each mesocosm, $CO_2$ manipulations over the entire depth were relatively homogeneous initially. However, a decrease in pH in the ambient control mesocosms below 5 m depth was detected from around $t15$ onwards, suggesting heterotrophic activity at depth involving respiration of organic matter to $CO_2$ (Fig. 8). DIC increased in the control mesocosms due to gas exchange. This counteracted losses through uptake by the plankton community leaving the water column undersaturated in $CO_2$ compared to the overlying atmosphere ($\sim 230$ µatm in control mesocosms vs. $\sim 400$ µatm in atmosphere; Schernewski, 2011). Undersaturation of $CO_2$ is typical for post-spring-bloom conditions such as those in the Tvärminne Storfjärden before the first $CO_2$ enrichment in this study on $t0$.

Calcium concentration was 2.17 mmol $kg^{-1}$, which was higher than calculated from a typical mean ocean salinity relationship of 1.67 mmol $kg^{-1}$ (Dickson et al., 2007), because of high riverine calcium carbonate inputs in the Baltic Sea (Feistel et al., 2010). We accounted for this in the calculation of the calcium carbonate saturation state in the water (Fig. 7d). All mesocosms apart from the two ambient controls during Phase 0 and I were undersaturated with respect to aragonite (Fig. 7d), and the highest three $fCO_2$ treatments were also undersaturated with respect to calcite (data not shown) during the entire experiment.

### 3.3 Effects of elevated $CO_2$

Out of 105 linear regressions applied to particulate and dissolved material from the water column and the accumulated sediment trap material to analyse the effect of $CO_2$, we detected a significant correlation in 18. These are summarised in Table 3 and highlighted in the following sections. The majority of detected responses (14) indicated a positive effect of $CO_2$, whereas only 4 indicated a negative effect of $CO_2$.

In this study, the low number of $fCO_2$ treatments (six) due to the exclusion of three mesocosms limited the statistical power of our conclusions. However the effect of $CO_2$ was consistent across biogeochemical element pools with higher sustained particulate matter concentrations and lower dissolved phosphate under high $CO_2$. This gives us confidence that the results of our study are indicative of the response of this particular plankton community in the Baltic Sea to ocean acidification.

### 3.4 Chlorophyll *a* dynamics

Chl *a* concentrations were low but typical of a post-spring bloom period. An increase in Chl *a* began after $t1$ and signified a phase characterised by higher Chl *a* concentrations ($\sim 2$ µg $L^{-1}$) until $t16$ (Fig. 9; Phase I: $t1$ to $t16$). Chl *a* concentrations decreased by $\sim 0.8$ µg $L^{-1}$ in the mesocosms during Phase II and remained low and relatively stable in Phase III ($\sim 0.9$ to 1.2 µg $L^{-1}$). Between 50 and 80 % of Chl *a* was in the upper water column (IWS samples 0–10 m, Fig. 9c). Chl *a* concentrations were in general lower (0.9 to 2.5 µg $L^{-1}$) in the mesocosms than in the surrounding water (1.2 to 5.5 µg $L^{-1}$, Fig. 9). $CO_2$-related differences first developed during Phase II and remained stable during Phase III, with 24 % higher Chl *a* in the highest $fCO_2$ treatment in Phase III (Table 3).

### 3.5 Dissolved inorganic and organic matter dynamics

No dissolved inorganic or organic nutrients were added to the mesocosms in this study, and nutrient concentrations remained relatively stable with low inorganic nitrogen concentrations throughout the entire experiment. There was low inorganic nitrogen ($\sim 50$ nmol $L^{-1}$ nitrate and $\sim 200$ nmol $L^{-1}$ ammonium) relative to phosphate ($\sim 150$ nmol $L^{-1}$) in all

**Table 3.** Summary of linear regression analyses of $CO_2$ effects on particulate and dissolved matter and sediment trap material including elemental stoichiometry in different size fractions for each experimental phase. $fCO_2$ and the parameter were averaged for each phase and using a linear model, a regression analysis was done to test for statistical significance of a potential $CO_2$ effect. Significant positive effects detected are in bold; significant negative effects of $CO_2$ are in italics. Degrees of freedom = 4, apart from particulate matter size fraction < 10 µm, where $n = 2$.

| | Particulate matter | | | | Dissolved matter and Chl *a* | | | | Sediment material | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Parameter | *p* | Multiple $R^2$ | F statistic | Parameter | *p* | Multiple $R^2$ | F statistic | Parameter | *p* | Multiple $R^2$ | F statistic |
| Phase I | TPC total | 0.152 | 0.438 | 3.113 | Nitrate (0–17 m) | 0.547 | 0.098 | 0.433 | Total accumulated material | 0.265 | 0.296 | 1.680 |
| Phase II | | 0.902 | 0.761 | 12.760 | | 0.602 | 0.074 | 0.320 | | 0.593 | 0.078 | 0.336 |
| Phase III | | **0.011** | **0.834** | **20.070** | | 0.768 | 0.034 | 0.105 | | 0.945 | 0.001 | 0.005 |
| Phase I | TPC < 55 µm | 0.580 | 0.083 | 0.363 | Nitrate (0–10 m) | 0.709 | 0.085 | 0.185 | Total accumulated material in phase | 0.265 | 0.296 | 1.680 |
| Phase II | | 0.536 | 0.103 | 0.458 | | *0.033* | *0.718* | *10.170* | | 0.799 | 0.018 | 0.074 |
| Phase III | | 0.759 | 0.026 | 0.108 | | 0.540 | 0.101 | 0.448 | | 0.372 | 0.202 | 1.010 |
| Phase I | TPC < 10 µm | – | – | – | DIP (0–17 m) | 0.486 | 0.128 | 0.589 | Cumulative TPC in phase | 0.752 | 0.028 | 0.115 |
| Phase II | | **0.036** | **0.929** | **26.120** | | 0.076 | 0.587 | 0.679 | | 0.902 | 0.004 | 0.017 |
| Phase III | | 0.187 | 0.661 | 3.899 | | *0.003* | *0.910* | *40.170* | | 0.386 | 0.191 | 0.947 |
| Phase I | PON total | 0.668 | 0.051 | 0.214 | DIP (0–10 m) | 0.651 | 0.056 | 0.239 | Cumulative PON in phase | 0.848 | 0.010 | 0.042 |
| Phase II | | 0.490 | 0.126 | 0.576 | | 0.075 | 0.589 | 5.737 | | 0.662 | 0.052 | 0.222 |
| Phase III | | **0.001** | **0.940** | **62.890** | | *0.030* | *0.732* | *10.950* | | 0.309 | 0.253 | 1.357 |
| Phase I | PON < 55 µm | 0.640 | 0.060 | 0.255 | $NH_4^+$ (0–17 m) | 0.225 | 0.340 | 2.058 | Cumulative TPP in phase | 0.621 | 0.067 | 0.286 |
| Phase II | | 0.516 | 0.113 | 0.508 | | 0.297 | 0.265 | 1.439 | | 0.749 | 0.028 | 0.117 |
| Phase III | | 0.381 | 0.195 | 0.968 | | 0.217 | 0.349 | 2.147 | | 0.358 | 0.212 | 1.079 |
| Phase I | PON < 10 µm | – | – | – | Dissolved silicate | 0.389 | 0.189 | 0.930 | Cumulative BSi in phase | 0.950 | 0.001 | 0.005 |
| Phase II | | 0.207 | 0.630 | 3.401 | | 0.272 | 0.288 | 1.617 | | 0.850 | 0.010 | 0.041 |
| Phase III | | 0.098 | 0.813 | 8.703 | | 0.642 | 0.059 | 0.252 | | 0.108 | 0.515 | 4.255 |
| Phase I | TPP | 0.084 | 0.567 | 5.240 | P* | 0.554 | 0.094 | 0.416 | | | | |
| Phase II | | 0.363 | 0.208 | 1.050 | | 0.549 | 0.096 | 0.427 | | | | |
| Phase III | | **0.004** | **0.897** | **34.690** | | *0.003* | *0.918* | *44.470* | | | | |
| Phase I | Biogenic silica (BSi) | 0.070 | 0.601 | 6.032 | DOC | 0.324 | 0.240 | 1.262 | | | | |
| Phase II | | **0.034** | **0.717** | **10.120** | | 0.230 | 0.334 | 2.006 | | | | |
| Phase III | | 0.553 | 0.095 | 0.419 | | **0.005** | **0.882** | **29.920** | | | | |
| Phase I | C : N in total POM | 0.653 | 0.056 | 0.236 | DON | 0.652 | 0.056 | 0.236 | | | | |
| Phase II | | **0.020** | **0.779** | **14.080** | | 0.358 | 0.212 | 1.079 | | | | |
| Phase III | | **0.050** | **0.659** | **7.716** | | 0.926 | 0.002 | 0.010 | | | | |
| Phase I | C : N in POM < 55 µm | 0.487 | 0.128 | 0.587 | DOP | 0.914 | 0.003 | 0.013 | | | | |
| Phase II | | 0.208 | 0.360 | 2.249 | | 0.391 | 0.188 | 0.924 | | | | |
| Phase III | | **0.037** | **0.704** | **9.516** | | 0.812 | 0.016 | 0.065 | | | | |
| Phase I | C : N in POM < 10 µm | – | – | – | Chl *a* (0–17 m) | 0.796 | 0.019 | 0.076 | | | | |
| Phase II | | **0.009** | **0.982** | **105.800** | | **0.020** | **0.780** | **14.180** | | | | |
| Phase III | | 0.164 | 0.699 | 4.643 | | **0.022** | **0.766** | **13.070** | | | | |
| Phase I | N : P in total POM | 0.707 | 0.039 | 0.163 | Chl *a* (0–10 m) | 0.227 | 0.337 | 2.037 | | | | |
| Phase II | | 0.848 | 0.010 | 0.042 | | **0.034** | **0.714** | **9.995** | | | | |
| Phase III | | 0.397 | 0.184 | 0.900 | | **0.008** | **0.859** | **24.320** | | | | |
| Phase I | C : P in total POM | 0.507 | 0.117 | 0.529 | | | | | | | | |
| Phase II | | 0.582 | 0.082 | 0.358 | | | | | | | | |
| Phase III | | 0.056 | 0.641 | 7.133 | | | | | | | | |
| Phase I | C : BSi in total POM | 0.989 | 0.000 | 0.000 | | | | | | | | |
| Phase II | | 0.127 | 0.480 | 3.695 | | | | | | | | |
| Phase III | | 0.307 | 0.255 | 1.370 | | | | | | | | |

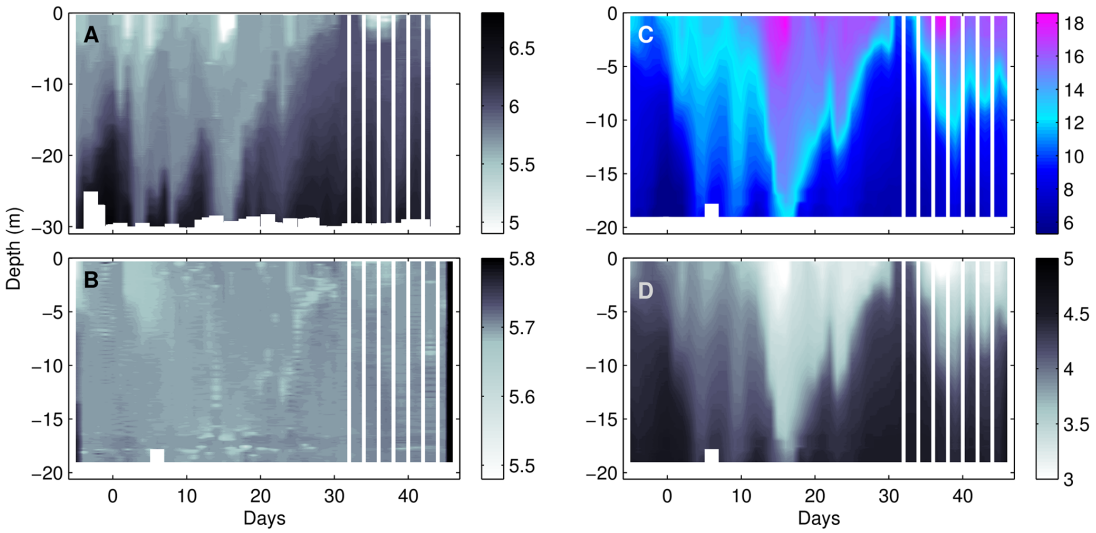


**Figure 5.** CTD profiles taken between $t-5$ and $t46$ for **(a)** salinity of surrounding water (Baltic), and **(b)** salinity, **(c)** temperature (°C), and **(d)** density anomaly of M8 ($\sigma_T$ in kg m$^{-3}$). M8 profiles are representative of all mesocosms. White vertical lines indicate CTD profiles were taken every second day after $t31$.

mesocosms at the start of the study period compared to the canonical Redfield nutrient stoichiometry (Fig. 10, C : N : P = 106 : 16 : 1; Redfield, 1958). These concentrations are within the natural range for this region in a post-spring/early summer bloom phase (Fig. 10). Fixed nitrogen availability primarily limited the development of phytoplankton biomass in this system. This is common in the Baltic Sea following the spring bloom (Matthäus et al., 1999). Temporal dynamics between phosphate and nitrate showed decoupling. Nitrate concentrations increased from ∼ 20 up to ∼ 80 nmol L$^{-1}$ from $t1$ until the end of the experiment ($t43$), whereas phosphate concentrations were slightly more dynamic, decreasing in Phase I and increasing in Phases II and III (Fig. 11). Around $t30$, differences in phosphate concentrations between $fCO_2$ treatments became visible with a significant negative relationship between $fCO_2$ and phosphate concentration in Phase III (Table 3). For further details and discussion on phosphorus pool sizes, uptake rates and cycling, see Nausch et al. (2015).

Ammonium concentrations decreased from between ∼ 170 and ∼ 280 nmol L$^{-1}$ on $t-3$ to between 40 and 150 nmol L$^{-1}$ on $t39$, with a small increase until $t43$ in all mesocosms (Fig. 10c). Samples for $NH_4^+$ concentration were lost on $t27$ and $t29$ for all mesocosms. The strongest decrease occurred during Phase I and concentrations remained relatively stable in Phase II and III. No significant $fCO_2$ effect was detected during any experimental phase above the variability in the data. Inside the mesocosms, dissolved silicate concentrations decreased minimally from around 6.2 µmol L$^{-1}$ on $t-1$ to between 5.5 and 5.8 µmol L$^{-1}$ at the end of the initial productive Phase I on $t16$ (Fig. 10d). Thereafter, dissolved silicate remained relatively constant until the end of the experiment. No significant effect of $fCO_2$ on dissolved silicate concentrations was detected in any phase.

DOC concentrations ranged between 410 and 420 µmol L$^{-1}$ on $t2$ and increased by ∼ 30 µmol L$^{-1}$ up to between 440 and 450 µmol L$^{-1}$ on $t43$ (Fig. 11a). In Phase III, DOC positively correlated with $fCO_2$ (Table 3). There was no statistically significant correlation of $fCO_2$ with DON or DOP concentrations in any experimental phase. No clear temporal trends were distinguished in DOP concentrations, although DON decreased during Phase I (Fig. 11). Where data points are missing, DON could not be corrected for $NH_4^+$ concentrations; hence, they are excluded from the data set.

### 3.6 Particulate matter dynamics

Particulate C, N and P concentrations were higher in Phase I than in Phase II and III (Fig. 12), as also observed for Chl *a* (Fig. 9a). The importance of small particles was even more pronounced in Phase III, where up to ∼ 90 % of total particulate organic matter was attributed to the fraction $TPC_{<10}$ in the four mesocosms sampled for this size fraction (M3, M5, M6, M8; Fig. 12). In Phase III, there was a significant positive correlation between $fCO_2$ and average total TPC, PON and TPP (Table 3).

C : N and C : P ratios in $POM_{tot}$ (Fig. 13) were above the Redfield ratio ($C : N : P_{tot} = 106 : 16 : 1$) during the productive phase, peaked at the beginning of Phase I ($C : N_{tot} = 7$–8.5, $C : P_{tot} = 110$–160) then decreased and became stable

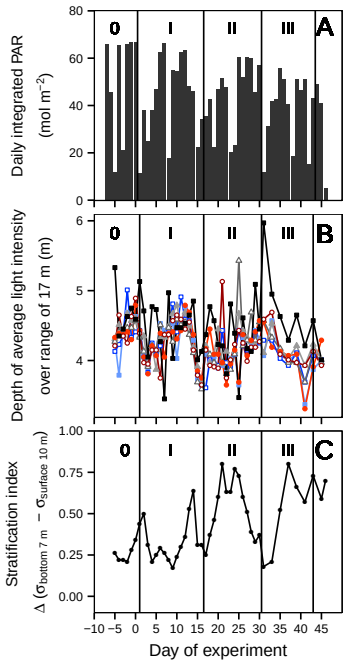


**Figure 6. (a)** Daily integrated incoming photosynthetically active radiation (PAR) measured by a unobstructed sensor on land during the study period, **(b)** depth of average water column light intensity calculated from CTD PAR sensor profiles between 0 and 17 m deep, and **(c)** stratification index calculated from $\sigma_T$ difference between the top 10 m and bottom 7 m in M8 as representative of all mesocosms. Symbols and colours are described in Table 1.

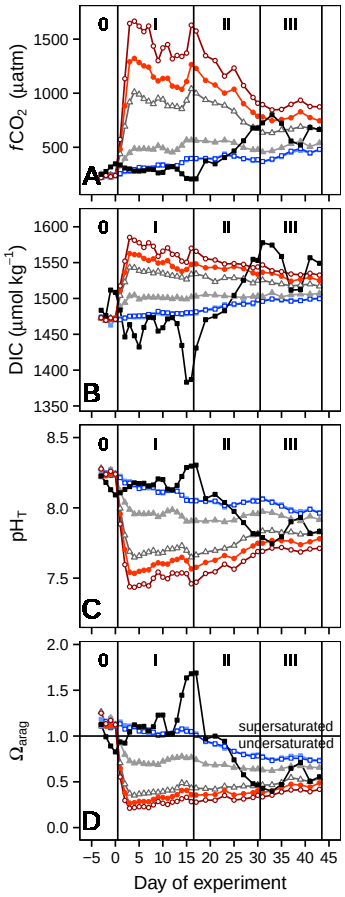


**Figure 7.** Dynamics in carbonate chemistry speciation with **(a)** calculated fugacity of $CO_2$, **(b)** measured dissolved inorganic carbon concentrations, **(c)** measured pH on total scale and calculated for in situ temperatures, and **(d)** calculated saturation state ($\Omega$) of calcium carbonate (aragonite). $\Omega_{arag}$ and $fCO_2$ were calculated from DIC and TA using the stoichiometric equilibrium constants for carbonic acid of Mehrbach et al. (1973) as refitted by Lueker et al. (2000). Colours and symbols are described in Table 1.

during Phase II ($C:N_{tot} = 5.8$–$7.0$, $C:P_{tot} = 80$–$140$). Differences between $fCO_2$ treatments were first observed in Phase III with higher $C:N_{tot}$ in the highest $fCO_2$ treatment (Table 3). No significant effect of $fCO_2$ on N : P or C : P was detected in any experiment phase or in any size fraction.

BSi decreased from around 1.0 µmol L$^{-1}$ at the beginning to $\sim 0.3$ µmol L$^{-1}$ at the end of the experiment (Fig. 12). During Phase II, there was a statistically significant correlation of BSi with $fCO_2$; however, this was absent in Phases I and III (Table 3).

### 3.7 Phytoplankton succession

The contribution to Chl *a* by different phytoplankton groups varied over time, although the temporal trends in all mesocosms appeared remarkably similar (Fig. 14). Results from CHEMTAX analyses of the phytoplankton community present indicate that cryptophytes and chlorophytes had the highest contribution to total Chl *a* during Phase I and Phase II/III, respectively. The total abundances of cryptophytes decreased from $t-3$ to $t17$ in all mesocosms, succeeded by a brief euglenophyte peak around $t15$, with chlorophytes being the dominant contributor to Chl *a* from $t17$ on (Fig. 14). Total abundances of cyanobacteria, probably non-diazotrophic *Synechococcus*, were highest during both Phase II and III. Diatoms made up a relatively small proportion of the plankton assemblage and contributed to less than 10 % of Chl *a* in Phases I and II and between 10 and 25 % in Phase III. Other key groups detected included dinoflagellates and prasinophytes; however, they made up minor proportions (below 15 % of total Chl *a*) of the plankton community throughout the entire experiment (dinoflagellate data not shown).

We analysed the relationship between $fCO_2$ and the contribution of phytoplankton groups to Chl *a* by linear regression for each experimental phase (Table 4). These analyses indicated small differences in plankton community compo-

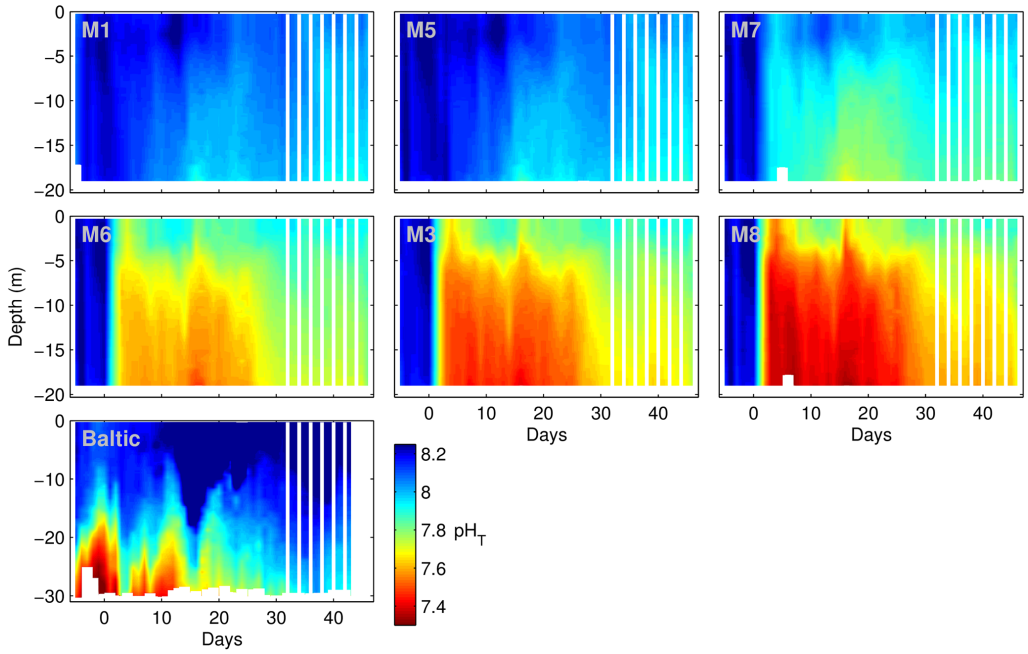


**Figure 8.** Vertical $pH_T$ profiles taken using a pH sensor on a hand-operated CTD during the experiment in the mesocosms and in the surrounding water, here named "Baltic". For details of CTD operations and $pH_T$ calculations, see Sect. 2.5.1. White vertical lines indicate CTD profiles were taken every second day after $t31$.

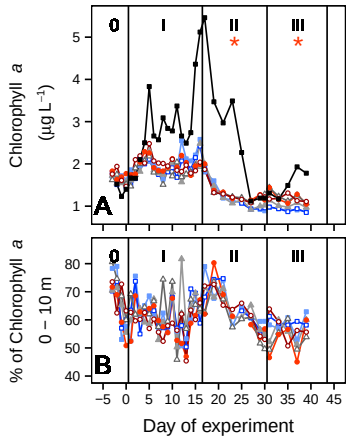


**Figure 9.** Temporal dynamics in **(a)** chlorophyll $a$ (0–17 m) including surrounding water and **(b)** percent of total chlorophyll $a$ in the upper 10 m. Colours and symbols are described in Table 1. Red asterisks denote significant positive effect of $CO_2$ (* = $p < 0.05$).

sition between $CO_2$ treatments. There was a significant negative correlation between $CO_2$ and total diatom contribution to Chl $a$ in Phase III. In Phase III, $fCO_2$ was also negatively correlated to the contribution of cryptophytes to Chl $a$ and a significant positive effect on the contribution of prasinophytes to Chl $a$.

Linear regression of the absolute concentrations of a number of phytoplankton pigments in the size fraction < 2 µm indicated primarily a positive correlation to $fCO_2$ during Phase I (i.e. Chl $a$, violaxanthin, neoxanthin), although a statistically significant effect was not detected in all pigments (Table 5). In Phase III, where the highest Chl $a$ concentrations were in the size fraction < 2 µm, mass balance calculations indicated more than 100 % of total Chl $a$ in this size range, which is not physically possible. These unbalanced Chl $a$ measurements are the result of measurement uncertainties at such low absolute concentrations, particularly in the > 20 µm size fraction and of mass balance calculations between three independent filtrations. As the increase and decline in Chl $a$ < 2 µm and 2–20 µm fractions, respectively, are supported by flow cytometry data for picoeukaryote and nanoeukaryote abundances, we still consider the observed temporal variations to be robust. A positive correlation between picoeukaryote abundance and $CO_2$ treatment was also already detected in Phase I (Crawfurd et al., 2015). Absolute concentrations of Chl $a$, Chl $b$, prasinoxanthin, violaxanthin

**Table 4.** Results of linear regression analyses of $CO_2$ and percentage contribution of phytoplankton groups to chlorophyll *a*. Bold indicates a significant positive effect, and italic indicates a significant negative effect of $CO_2$.

| Phytoplankton group | Phase I | | | Phase II | | | Phase III | | |
|---|---|---|---|---|---|---|---|---|---|
| | *p* | Multiple $R^2$ | F statistic | *p* | Multiple $R^2$ | F statistic | *p* | Multiple $R^2$ | F statistic |
| Prasinophytes | 0.645 | 0.058 | 0.248 | 0.095 | 0.543 | 4.751 | **0.025** | **0.754** | **12.270** |
| Cryptophytes | 0.995 | 0.001 | 0.004 | 0.463 | 0.141 | 0.657 | *0.041* | *0.687* | *8.789* |
| Chlorophytes | 0.631 | 0.063 | 0.269 | 0.244 | 0.317 | 1.860 | **0.008** | **0.857** | **24.020** |
| Cyanobacteria | 0.224 | 0.341 | 2.067 | 0.421 | 0.167 | 0.803 | 0.153 | 0.437 | 3.110 |
| Diatoms | 0.866 | 0.008 | 0.324 | 0.515 | 0.113 | 0.508 | *0.009* | *0.849* | *22.560* |
| Euglenophytes | 0.962 | 0.001 | 0.003 | 0.438 | 0.156 | 0.741 | 0.976 | 0.000 | 0.001 |

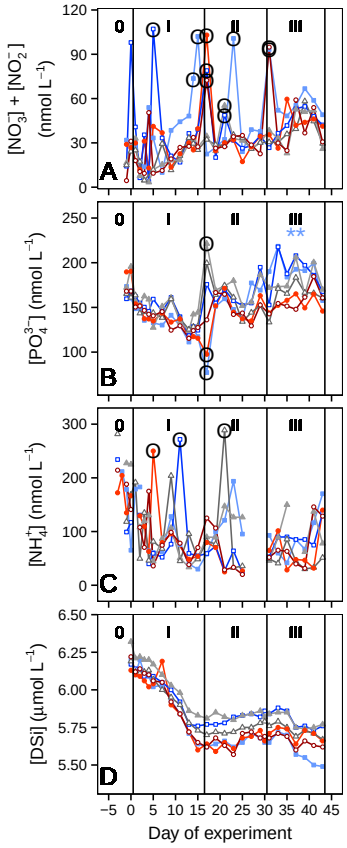


**Figure 10.** Temporal variation in concentrations of **(a)** dissolved nitrate + nitrite, **(b)** dissolved inorganic phosphate, **(c)** ammonium, and **(d)** dissolved silicate. Colours and symbols are described in Table 1. Blue asterisks denote a statistically significant negative effect of $CO_2$ (** = $p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

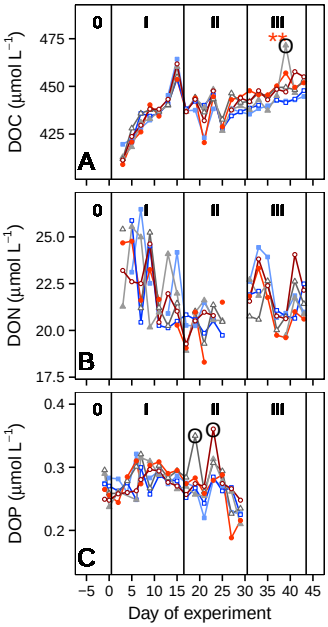


**Figure 11.** Temporal variation in concentrations of **(a)** dissolved organic carbon, **(b)** dissolved organic nitrogen, and **(c)** dissolved organic phosphorus. $CO_2$ treatments are indicated by colours and symbols described in Table 1. Red asterisks denote a statistically significant positive effect of $CO_2$ (** = $p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

and neoxanthin in the total fraction had a statistically significant positive correlation with $f\,CO_2$ during Phase III (see Table 5). Fucoxanthin concentrations (key pigment in diatoms but also present in dinoflagellates) and $f\,CO_2$ were also positively correlated in the fraction > 20 µm during Phase III. Size fractionation of HPLC pigment analyses indicated a higher proportion of Chl *a* in all treatments in biomass < 2 µm during Phases II and III (Fig. 15).

**Table 5.** Summary of linear regression analyses done on absolute concentrations of phytoplankton pigments for the three experiment phases in different size fractions. Bold indicates significant positive effect and italic indicates significant negative effect of $CO_2$ concentration. ND indicates pigment was not detected. Where no pigment was detected in any phase in any size fraction, results were not included in this table.

| Pigment | Size fraction | Phase I | | | Phase II | | | Phase III | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $p$ | Multiple $R^2$ | F statistic | $p$ | Multiple $R^2$ | F statistic | $p$ | Multiple $R^2$ | F statistic |
| Chlorophyll *a* | total | 0.470 | 0.137 | 0.636 | **0.008** | **0.854** | **23.440** | 0.081 | 0.573 | 5.377 |
| | < 2 µm | **0.014** | **0.815** | **17.650** | 0.658 | 0.053 | 0.228 | 0.659 | 0.057 | 0.227 |
| | > 20 µm | **0.009** | **0.850** | **22.720** | **0.011** | **0.836** | **20.440** | 0.273 | 0.288 | 1.616 |
| Chlorophyll *b* | total | 0.143 | 0.454 | 3.321 | **0.034** | **0.713** | **9.920** | 0.885 | 0.006 | 0.024 |
| | < 2 µm | 0.815 | 0.015 | 0.063 | 0.726 | 0.034 | 0.141 | 0.369 | 0.204 | 1.025 |
| | > 20 µm | **0.001** | **0.944** | **66.940** | **0.004** | **0.896** | **34.320** | ND | ND | ND |
| Chlorophyll C2 | total | 0.283 | 0.278 | 1.538 | *0.026* | *0.750* | *12.010* | 0.371 | 0.202 | 1.015 |
| | < 2 µm | 0.877 | 0.007 | 0.027 | 0.437 | 0.157 | 0.745 | 0.876 | 0.007 | 0.028 |
| | > 20 µm | ND | ND | ND | 0.094 | 0.544 | 4.765 | ND | ND | ND |
| Canthaxanthin | total | **0.031** | **0.726** | **10.590** | ND | ND | ND | ND | ND | ND |
| | < 2 µm | 0.078 | 0.582 | 5.576 | ND | ND | ND | 0.973 | ND | 0.001 |
| | > 20 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fucoxanthin | total | 0.876 | 0.007 | 0.028 | 0.420 | 0.168 | 0.807 | 0.371 | 0.202 | 1.012 |
| | < 2 µm | 0.131 | 0.472 | 3.581 | 0.374 | 0.200 | 1.000 | 0.257 | 0.304 | 1.743 |
| | > 20 µm | 0.649 | 0.057 | 0.242 | 0.370 | 0.201 | 1.020 | **0.037** | **0.705** | **9.560** |
| Myxoxanthophyll | total | 0.056 | 0.642 | 7.157 | 0.755 | 0.027 | 0.112 | ND | ND | ND |
| | < 2 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | >20 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Neoxanthin | total | 0.940 | 0.002 | 0.007 | **0.006** | **0.880** | **29.310** | 0.089 | 0.555 | 4.986 |
| | < 2 µm | **0.030** | **0.730** | **10.820** | 0.660 | 0.053 | 0.225 | 0.820 | 0.015 | 0.059 |
| | >20 µm | **0.005** | **0.890** | **32.470** | **0.003** | **0.907** | **39.090** | ND | ND | ND |
| Prasinoxanthin | total | **0.040** | **0.691** | **8.947** | **0.001** | **0.945** | **68.540** | ND | ND | ND |
| | < 2 µm | 0.517 | 0.112 | 0.504 | 0.072 | 0.595 | 5.883 | 0.503 | 0.119 | 0.539 |
| | >20 µm | **0.001** | **0.951** | **77.440** | **0.003** | **0.917** | **44.360** | ND | ND | ND |
| Violaxanthin | total | **0.030** | **0.731** | **10.840** | **0.002** | **0.929** | **52.580** | **0.035** | **0.711** | **9.839** |
| | < 2 µm | **0.017** | **0.797** | **15.710** | 0.854 | 0.010 | 0.038 | 0.882 | 0.006 | 0.025 |
| | >20 µm | **0.002** | **0.926** | **49.770** | **0.002** | **0.925** | **49.480** | 0.982 | ND | 0.001 |

### 3.8 Sinking material flux

The amount of material collected in the sediment traps in each phase reflected biomass (here POM and Chl *a*) build-up from the water column. We calculated that > 84 % of total carbon sinking into the sediment trap was collected during Phases I and II and less than 16 % during Phase III (Fig. 16). This corresponds to average accumulation rates (± SD) of $0.303 \pm 0.011$, $0.203 \pm 0.033$ and $0.094 \pm 0.029$ µmol C $L^{-1}$ $day^{-1}$ across all mesocosms in Phases I, II and III, respectively. No significant $CO_2$ trends were detected during any phase with regard to the total amount of C, N, P and BSi in the sediment trap material.

## 4 Discussion

### 4.1 Phase I: productive phase with high organic matter turnover

Phase I ($t1$ to $t16$) was characterised by the highest sustained Chl *a* and particulate matter concentrations in the water column. Relatively high light availability, particularly between $t6$ and $t15$ (Fig. 6a), accompanied by increasing water column temperatures likely supported autotrophic growth. However, no increase in particulate matter pool size was observed in any treatment during this productive phase. Instead carbon was diverted into the sinking particle flux and DOC pool (Fig. 11) with a net daily accumulation of DOC of between 10 and 15 % of the total TPC pool between $t3$ and $t13$. As inorganic nitrogen availability was very low, we assume this is due to carbon overconsumption (Toggweiler, 1993). Thus, organic matter turnover in the system appeared to be high during this period, although overall phytoplankton biomass production was limited by low inorganic nitrogen availability.

Although phytoplankton carbon fixation is expected to be stimulated by increased $CO_2$ availability (Hein and Sand-Jensen, 1997; Losh et al., 2012; Riebesell et al., 2007), previous $CO_2$ enrichment experiments using natural plankton assemblages under various conditions of nutrient repletion in different regions have shown no consistent response of primary production to elevated $CO_2$ (Engel et al., 2005; Hopkins et al., 2010; Hopkinson et al., 2010; Nielsen et al., 2011; Riebesell et al., 2007; G. K. Schulz, personal communication, 2015; Yoshimura et al., 2013). During high organic matter turnover in Phase I, we detected no statistically significant differences in bulk organic matter concentrations or el-

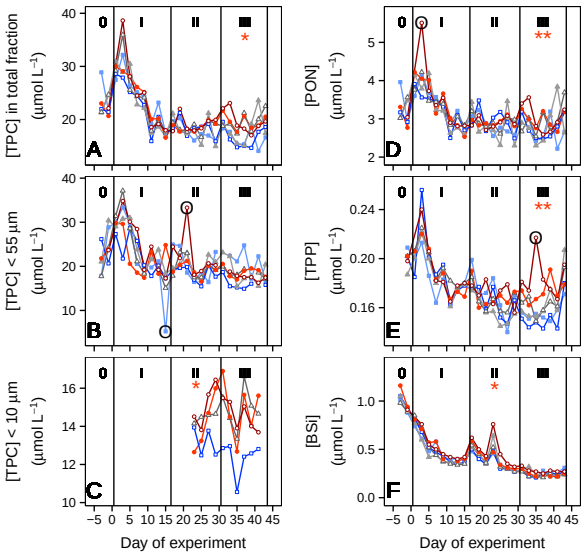


**Figure 12.** Temporal dynamics in concentrations of **(a)** total particulate carbon, **(b)** particulate carbon < 55 µm, **(c)** particulate carbon < 10 µm, **(d)** particulate organic nitrogen, **(e)** total particulate phosphorus, and **(f)** particulate biogenic silica. Colours and symbols are described in Table 1. Red asterisks denote significant positive effect of $CO_2$ ($* = p < 0.05$, $** = p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

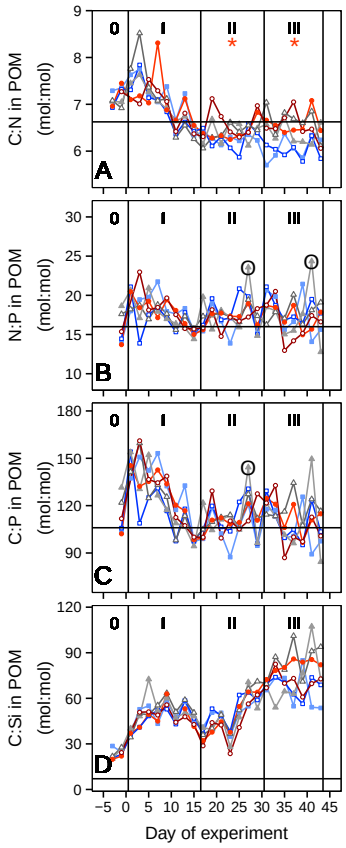


**Figure 13.** Temporal dynamics of elemental stoichiometry in particulate organic matter: **(a)** carbon to nitrogen, **(b)** nitrogen to phosphorus, **(c)** carbon to phosphorus, **(d)** carbon to biogenic silica. Horizontal lines indicate Redfield stoichiometry ($C:N:P:Si = 106:16:1:15$; Redfield, 1958). Colours and symbols for different treatments are described in Table 1. Red asterisks denote significant positive effect of $CO_2$ ($* = p < 0.05$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

emental stoichiometry between $CO_2$ treatments. No effect of $CO_2$ treatment could be detected in the most abundant, and presumably most productive, phytoplankton size class (2–20 µm, Fig. 15). Instead, detected differences between $fCO_2$ treatments in particulate matter in Phase I were mostly confined to pigment concentrations in the smallest size fraction (< 2 µm). Here, pigment concentrations were generally higher in the highest $CO_2$ treatment (Table 5). This is in line with flow cytometry counts which revealed a positive effect of $CO_2$ on the abundance of picoeukaryotes (Crawfurd et al., 2015.) and is in agreement with studies in the Arctic (Brussaard et al., 2013), the subarctic North Pacific (Endo et al., 2013), and North Atlantic Ocean (Newbold et al., 2012) but contrasts the results from Richier et al. (2014) from shelf seas in the north-east Atlantic Ocean. The positive influence of $CO_2$ on phytoplankton pigment concentrations was also detected in the largest size fraction (> 20 µm) in Phase I; however, this size class made up only a small portion of total Chl *a* (< 10 % Fig. 15, size fractionated pigment analyses). Thus, small $CO_2$-driven differences in plankton community structure in the smallest and largest phytoplankton were not relevant for biogeochemical element cycling in this plankton assemblage during this productive phase.

### 4.2 Phase II: decline in autotrophic biomass and organic matter turnover

The distinct changes in the phytoplankton communities in the mesocosms coincided with the decrease in temperature during the upwelling even in the archipelago in Phase II ($t17$ to $t30$). Temperature decreases of greater than 10 °C in surface water, as observed in this study, have been reported for upwelling events during periods of thermal stratification (Lehmann and Myrberg, 2008) with considerable influence on the ecosystem productivity (Nômmann et al., 1991). Here we assume that the combination of higher grazing pressure, lower PAR and cooler temperatures likely slowed down phytoplankton productivity and contributed to decreased phyto-

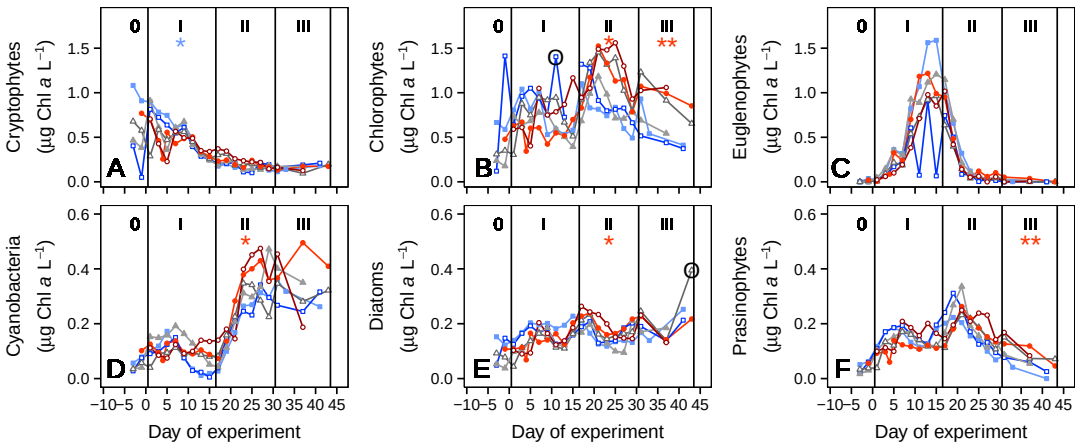


**Figure 14.** Contribution to total chlorophyll *a* by different phytoplankton groups as calculated by CHEMTAX from HPLC pigment analyses: **(a)** cryptophytes, **(b)** chlorophytes, **(c)** euglenophytes, **(d)** cyanobacteria, **(e)** diatoms, and **(f)** prasinophytes. Colours and symbols for each $CO_2$ treatment are described in Table 1. Red asterisks denote significant positive effect and blue asterisk a significant negative effect of $CO_2$ ($* = p < 0.05$, $** = p < 0.01$). Outliers are indicated by black circles and were excluded from linear regression analyses.

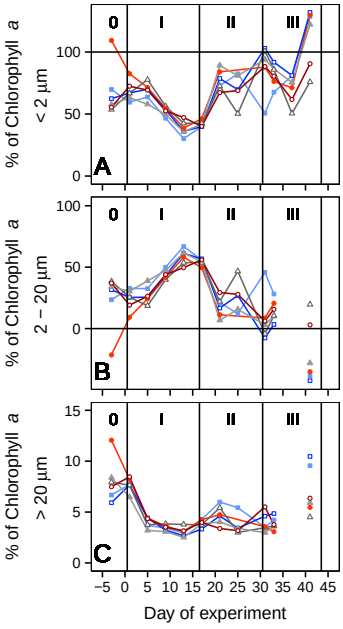


**Figure 15.** Relative contribution of different size fractions to total chlorophyll *a*. Size fraction 2–20 µm was calculated as a mass balance from total fraction and the two size fractions < 2 µm and > 20 µm. Colours and symbols for different treatments are described in Table 1. Values larger than 100 % or smaller than 0 % are due to errors in mass balance calculation.

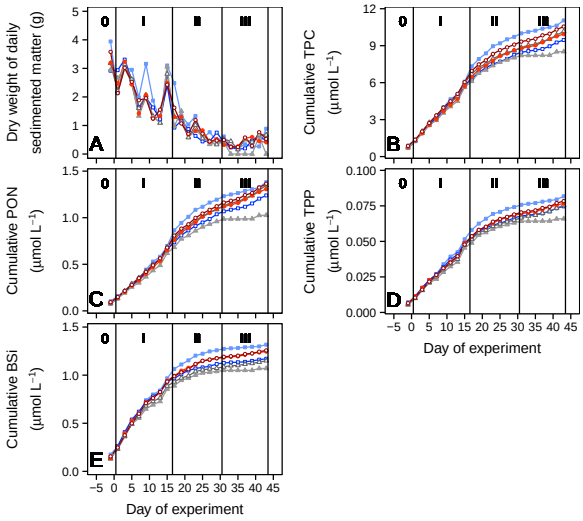


**Figure 16.** Temporal dynamics in **(a)** collected sediment trap material mass and cumulative **(b)** total particulate carbon, **(c)** particulate organic nitrogen, **(d)** total particulate phosphorus, and **(e)** particulate biogenic silica. Concentrations in (**b**–**e**) were calculated based on individual mesocosm volumes determined at the end of the study. Colours and symbols for different treatments are described in Table 1.

plankton biomass, observed here as a decrease in Chl *a*, during this period (Fig. 9).

An increase in $TPC_{tot}$ : Chl *a* from ∼ 10 µmol µg$^{-1}$ on *t*17 to over 15 µmol µg$^{-1}$ on *t*29 indicates that carbon was being shifted from autotrophic to heterotrophic organisms, assum-

ing that the Chl *a* content of the autotrophs remained constant. CTD profiles showed a decrease in $pH_T$ below 10 m in both control mesocosms (Fig. 8) at the same time as surface Chl *a* (0 to 10 m) decreased between *t*18 and *t*30. This pH decrease (i.e. $CO_2$ increase) could indicate a possible change in the equilibrium between dominance of autotrophic ($CO_2$ uptake) and heterotrophic ($CO_2$ release) processes during a phase of strong cooling in the lower water column. Higher organic material availability seemed to stimulate bacterial activity up until *t*23 (Hornick et al., 2015). Furthermore, higher zooplankton abundances after *t*17 (Lischka et al., 2015), as well as a peak in abundance of a potential mixotroph around *t*17 (Euglenophycaea), also likely contributed to higher organic matter remineralisation and $CO_2$ release. Hence Phase II is defined by increased heterotrophy and organic matter remineralisation. Carbon was primarily channelled into sinking material flux and higher trophic levels rather than accumulating in the DOC pool, mediated by increased zooplankton grazing pressure on primary producers.

Differences between $CO_2$ treatments in the dissolved and particulate matter pools developed during the Chl *a* decrease and apparent increase in net heterotrophy in Phase II. In addition, size-fractionated pigment analyses indicated a shift in phytoplankton community size to smaller organisms with up to ∼ 90 % of Chl *a* in phytoplankton < 2 µm at the end of Phase II. This was not caused by a remarkable gain in Chl *a* in the smaller size class but instead due to Chl *a* loss in the larger size class, which we think was driven by high grazing pressure from abundant zooplankton at this time (Lischka et al., 2015). This removal of larger phytoplankton unmasked the underlying positive $CO_2$ response of picoplankton that was already present since Phase I but now became clearly visible. In other words, a positive $CO_2$ effect on picophytoplankton seemed to be present throughout the entire experiment. However, their ecological and biogeochemical relevance within the plankton community was too small initially, so that the $CO_2$ effect was not detectable in the other bulk biogeochemical element pools.

Interestingly, measured carbon fixation rates did not show any fertilising effect of $CO_2$ (Spilling et al., 2015), whereas both respiration (Spilling et al., 2015) and bacterial production rates between *t*14 and *t*23 (Hornick et al., 2015; Nausch et al., 2015) were lower at higher $CO_2$. This suggests slower net particulate matter loss rather than increased production under ocean acidification (see Hornick et al., 2015, and Spilling et al., 2015) in this issue for more on this topic).

### 4.3 Phase III: inactive plankton community

While temperature increased again during Phase III, there did not seem to be any recovery of phytoplankton biomass to the same level as in Phase I. In Phase II autotrophic growth was apparently dampened so severely that it could not recover within the duration of this study and was likely strongly controlled by high zooplankton grazing pressure. There was very little change in the amount or stoichiometry of the particulate or dissolved matter pools, suggesting that production and loss of particulate matter in the water column were either very low or relatively well balanced in Phase III. Only a small amount of TPC (∼ 1 µmol $L^{-1}$, ∼ 16 % of total suspended TPC) was collected in the sediment traps, implying low particulate matter sinking flux strength in this phase. The positive (picoplankton-mediated) effect of $CO_2$ on particulate and dissolved pools unmasked in Phase II was sustained throughout Phase III in Chl *a*, TPC, PON, TPP and DIP. Thus, in this study, higher autotrophic biomass was sustained under elevated $CO_2$ in this plankton community during the post-bloom phase and had a significant influence on biogeochemical pool sizes.

Variations in water column particulate matter concentrations did not translate into statistically significant differences in the amount of accumulated sediment trap material between $CO_2$ treatments. This may be because the response of $CO_2$ was the strongest in phytoplankton < 2 µm, which taxonomically were likely to be chlorophytes and prasinophytes (Fig. 14b and f, Table 4). The unicellular organisms are, however, too small to sink as individual cells. Instead picoplankton contribute indirectly to carbon export through secondary processing of sinking picoplankton material (Richardson and Jackson, 2007). The positive effect of $CO_2$ on particulate matter pools was reflected positively in the DOC pool, suggesting that a higher proportion of freshly produced organic matter was directed into the microbial food web, rather than being exported during the period of low organic matter turnover in Phase III. A similar channelling of carbon and the positive $CO_2$ response in the DOC pool was observed during nutrient-depleted conditions in an Arctic $CO_2$ enrichment mesocosm study (Engel et al., 2013). Here, this could be a consequence of continued reduced organic matter remineralisation at elevated $CO_2$ (Spilling et al., 2015), as hypothesised for Phase II (see also Sect. 4.2), although unfortunately no respiration data for Phase III are available.

Based on our results, we hypothesise that, under future ocean acidification the Baltic Sea in low nitrogen, summer periods may shift towards a system where more organic matter is retained for longer time periods in the upper water column but may not result in increased particulate matter sinking flux.

### 4.4 Potential ecosystem resilience under elevated $CO_2$

Although a significant, but small, response to $CO_2$ was detected in a number of particulate and dissolved matter pools, in numerous others no significant effect of $CO_2$ was detected in any phase (e.g. DON and DOP concentration, N : P and C : P in POM). The muted response of the plankton community and biogeochemistry to elevated $CO_2$ observed in this experiment might be linked to higher tolerance or resilience of the plankton community. The Baltic Sea is a highly dynamic system with much larger annual temperature, light pe-

riod, inorganic nutrient, pH, and salinity fluctuations than in many other major water bodies and the open ocean. Thus the community present in this study may have considerable physiological plasticity through exposure to large natural diurnal and annual fluctuations in carbonate chemistry speciation and pH (see also Joint et al., 2011, and Nielsen et al., 2011). Low nitrogen availability in this study may have dampened underlying trends particularly in larger phytoplankton size classes. In past $CO_2$ enrichment experiments, nutrient addition amplified the existing effect of $CO_2$ between treatments (for example Schulz et al., 2013). This is one of few plankton community experiments where nutrient concentrations were very low initially and concentrations and nutrient ratios were not manipulated. Such conditions are representative of a steady-state stratified water column present in many ecosystems for most of the year.

## 5 Conclusions

We observed higher post-bloom Chl *a*, particulate organic matter and DOC concentrations under elevated $fCO_2$ in this low nitrogen plankton community. No effect of $CO_2$ was identified in larger organisms (2 to 20 µm) which were dominant in the phytoplankton community during the period of higher productivity in Phase I. Hence their dominance masked the $CO_2$ signal from picophytoplankton in bulk particulate and dissolved pools. As a result of the shift in phytoplankton community size structure towards dominance of smaller phytoplankton size classes around 3 weeks after initial $CO_2$ enrichment, the underlying positive effect of $CO_2$ present on picophytoplankton (< 2 µm) biomass since Phase I was revealed in particulate and dissolved matter pools. This signal could not be explained by a detectable increase in carbon fixation in this study (Spilling et al., 2015).

Differences in water column biomass did not directly translate into increased particle sinking flux at higher $fCO_2$. Instead, higher organic matter concentrations are more likely due to decreased net respiration at higher $fCO_2$ with the positive $CO_2$ effect on biomass channelled into the DOC pool. Alternatively, secondary processing of sinking material may have removed the $CO_2$ signal present in the water column particulate matter, driven by picophytoplankton so that it was not reflected in the collected sinking material during the study period. Hence we suggest $CO_2$-induced changes in productivity in the upper water column may be decoupled from particle sinking flux.

In this study, it took almost 4 weeks until we first observed $CO_2$-related differences in the size and stoichiometry of some bulk biogeochemical pools. In many other variables, simulated ocean acidification did not have any significant effect at all. This slow response or lack of detected effect to ocean acidification may have been modulated by overall low inorganic nitrogen availability and high natural pH variability in the ecosystem. Therefore we recommend running future experiments for as long as practically feasible, focusing on the vast oligotrophic regions and avoiding nutrient additions. Changes in the abundance of key phytoplankton groups in steady-state systems due to higher $CO_2$ may underpin sustained fundamental changes in biogeochemical cycling in these regions.

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## References

Almén, A. K., Vehmaa, A., Brutemark, A., Bach, L., Lischka, S., Stuhr, A., Furuhagen, S., Paul, A., Bermudez, J. R., Riebesell, U., and Engström-Öst, J.: Negligible effects of ocean acidification on Eurytemora affinis (Copepoda) offspring production, Biogeosciences Discuss., in press, 2015.

Badr, E.-S. A., Achterberg, E. P., Tappin, A. D., Hill, S. J., and Braungardt, C. B.: Determination of dissolved organic nitrogen in natural waters using high-temperature catalytic oxidation, TrAC-Trend, Anal. Chem., 22, 819–827, doi:10.1016/S0165-9936(03)01202-0, 2003.

Barlow, R. G., Cummings, D. G., and Gibb, S. W.: Improved resolution of mono- and divinyl chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC, Mar. Ecol.-Prog. Ser., 161, 303–307, doi:10.3354/meps161303, 1997.

Bermúdez, J. R., Winder, M., Stuhr, A., Almén, A.-K., Engström-Öst, J., and Riebesell, U.: Effect of ocean acidification on the structure and fatty acid composition of a natural plankton community in the Baltic Sea, Biogeosciences Discuss., in preparation, 2015.

Biswas, H., Gadi, S. D., Ramana, V. V., Bharathi, M. D., Priyan, R. K., Manjari, D. T., and Kumar, M. D.: Enhanced abundance of tintinnids under elevated $CO_2$ level from coastal Bay of Bengal, Biodivers. Conserv., 21, 1309–1326, doi:10.1007/s10531-011-0209-7, 2012.

Boxhammer, T., Sswat, M., Paul, A. J., Nicolai, M., and Riebesell, U.: Video of a plankton community enclosed in a "Kiel Off-Shore Mesocosms for future Ocean Simulations" (KOSMOS) during a study in Tvärminne Storfjärden (Finland) 2012, doi:10.3289/KOSMOS_PLANKTON_FINLAND_2012, 2015a.

Boxhammer, T., Bach, L. T., Czerny, J., and Riebesell, U.: Technical note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis, Biogeosciences Discuss., in preparation, 2015b.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated $CO_2$ levels, Biogeosciences, 10, 719–731, doi:10.5194/bg-10-719-2013, 2013.

Crawfurd, K. J., Riebesell, U., and Brussaard, C. P. D.: Shifts in the microbial community in the Baltic Sea with increasing $CO_2$, Biogeosciences Discuss., in preparation, 2015.

Czerny, J., Schulz, K. G., Krug, S. A., Ludwig, A., and Riebesell, U.: Technical Note: The determination of enclosed water volume in large flexible-wall mesocosms "KOSMOS", Biogeosciences, 10, 1937–1941, doi:10.5194/bg-10-1937-2013, 2013.

Derenbach, J.: Zur Homogenisation des Phytoplanktons für die Chlorophyllbestimmung, Kieler Meeresforschungen, 25, 166–171, 1969.

Dickson, A. G.: An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, Deep-Sea Res., 28, 609–623, 1981.

Dickson, A. G.: Standards for ocean measurements, Oceanography, 23, 34–47, doi:10.5670/oceanog.2010.22, 2010.

Dickson, A. G., Sabine, C., and Christian, J. (Eds.): Guide to best practices for ocean $CO_2$ measurements, PICES Special Publication 3, 191 pp., http://aquaticcommons.org/1443/ (last access: 16 October 2012), 2007.

Doney, S. C., Mahowald, N., Lima, I., Feely, R. A., Mackenzie, F. T., Lamarque, J.-F., and Rasch, P. J.: Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system, P. Natl. Acad. Sci. USA, 104, 14580–14585, doi:10.1073/pnas.0702218104, 2007.

Dore, J. E., Lukas, R., Sadler, D. W., Church, M. J., and Karl, D. M.: Physical and biogeochemical modulation of ocean acidification in the central North Pacific, P. Natl. Acad. Sci. USA, 106, 12235–12240, doi:10.1073/pnas.0906044106, 2009.

Ekau, W., Auel, H., Pörtner, H.-O., and Gilbert, D.: Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish), Biogeosciences, 7, 1669–1699, doi:10.5194/bg-7-1669-2010, 2010.

Eker-Develi, E., Berthon, J.-F., and Van der Linde, D.: Phytoplankton class determination by microscopic and HPLC-CHEMTAX analyses in the southern Baltic Sea, Mar. Ecol.-Prog. Ser., 359, 69–87, doi:10.3354/meps07319, 2008.

Endo, H., Yoshimura, T., Kataoka, T., and Suzuki, K.: Effects of $CO_2$ and iron availability on phytoplankton and eubacterial community compositions in the northwest subarctic Pacific, J. Exp. Mar. Biol. Ecol., 439, 160–175, doi:10.1016/j.jembe.2012.11.003, 2013.

Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J.-P., Harlay, J., and Heemann, C.: Testing the direct effect of $CO_2$ concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments, Limnol. Oceanogr., 50, 493–507, doi:10.4319/lo.2005.50.2.0493, 2005.

Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R., Delille, B., and Schartau, M.: Effects of $CO_2$ on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), Biogeosciences, 5, 509–521, doi:10.5194/bg-5-509-2008, 2008.

Engel, A., Borchard, C., Piontek, J., Schulz, K. G., Riebesell, U., and Bellerby, R.: $CO_2$ increases $^{14}C$ primary production in an Arctic plankton community, Biogeosciences, 10, 1291–1308, doi:10.5194/bg-10-1291-2013, 2013.

Engel, A., Piontek, J., Grossart, H.-P., Riebesell, U., Schulz, K. G., and Sperling, M.: Impact of $CO_2$ enrichment on organic matter dynamics during nutrient induced coastal phytoplankton blooms, J. Plankton Res., 36, 641–657, doi:10.1093/plankt/fbt125, 2014.

Feistel, R., Weinreben, S., Wolf, H., Seitz, S., Spitzer, P., Adel, B., Nausch, G., Schneider, B., and Wright, D. G.: Density and Absolute Salinity of the Baltic Sea 2006–2009, Ocean Sci., 6, 3–24, doi:10.5194/os-6-3-2010, 2010.

Feng, Y., Hare, C. E., Rose, J. M., Handy, S. M., DiTullio, G. R., Lee, P. A., Smith Jr., W. O., Peloquin, J., Tozzi, S., Sun, J., Zhang, Y., Dunbar, R. B., Long, M. C., Sohst, B., Lohan, M., and Hutchins, D. A.: Interactive effects of iron, irradiance and $CO_2$ on Ross Sea phytoplankton, Deep-Sea Res. Pt. I, 57, 368–383, doi:10.1016/j.dsr.2009.10.013, 2010.

Fofonoff, N. P. and Millard Jr., R. C.: Algorithms for computation of fundamental properties of seawater, UNESCO Technical Papers in Marine Science, 44, 56 pp., 1983.

Gasiūnaitė, Z. R., Cardoso, A. C., Heiskanen, A.-S., Henriksen, P., Kauppila, P., Olenina, I., Pilkaitytė, R., Purina, I., Razinkovas, A., Sagert, S., Schubert, H. and Wasmund, N.: Seasonality of coastal phytoplankton in the Baltic Sea: Influence of salinity and eutrophication, Estuar. Coast. Shelf. S., 65, 239–252, doi:10.1016/j.ecss.2005.05.018, 2005.

Grasshoff, K., Ehrhardt, M., Kremling, K., and Almgren, T.: Methods of seawater analysis, Wiley Verlag Chemie GmbH, Weinheim, Germany, 1983.

Hama, T., Kawashima, S., Shimotori, K., Satoh, Y., Omori, Y., Wada, S., Adachi, T., Hasegawa, S., Midorikawa, T., Ishii, M., Saito, S., Sasano, D., Endo, H., Nakayama, T., and Inouye, I.: Effect of ocean acidification on coastal phytoplankton composition and accompanying organic nitrogen production, J. Oceanogr., 68, 183–194, doi:10.1007/s10872-011-0084-6, 2012.

Hansen, H. P. and Koroleff, F.: Determination of nutrients, in Methods of Seawater Analysis, edited by: Grasshoff, K., Kremling, K., and Ehrhardt, M., Wiley Verlag Chemie GmbH, Weinheim, Germany, 159–228, 1983.

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

Hare, C. E., Leblanc, K., DiTullio, G. R., Kudela, R. M., Zhang, Y., Lee, P. A., Riseman, S., and Hutchins, D. A.: Consequences

of increased temperature and $CO_2$ for phytoplankton community structure in the Bering Sea, Mar. Ecol.-Prog. Ser., 352, 9–16, 2007.

Hein, M. and Sand-Jensen, K.: $CO_2$ increases oceanic primary production, Nature, 388, 526–527, doi:10.1038/41457, 1997.

HELCOM: Climate change in the Baltic Sea Area: HELCOM thematic assessment in 2013, Helsinki Commission, Helsinki, Finland, 2013.

Hopkins, F. E., Turner, S. M., Nightingale, P. D., Steinke, M., Bakker, D., and Liss, P. S.: Ocean acidification and marine trace gas emissions, P. Natl. Acad. Sci. USA, 107, 760–765, doi:10.1073/pnas.0907163107, 2010.

Hopkinson, B. M., Xu, Y., Shi, D., McGinn, P. J., and Morel, F. M. M.: The effect of $CO_2$ on the photosynthetic physiology of phytoplankton in the Gulf of Alaska, Limnol. Oceanogr., 55, 2011–2024, doi:10.4319/lo.2010.55.5.2011, 2010.

Hoppe, C. J. M., Hassler, C. S., Payne, C. D., Tortell, P. D., Rost, B., and Trimborn, S.: Iron limitation modulates ocean acidification effects on Southern Ocean phytoplankton communities, PLoS ONE, 8, e79890, doi:10.1371/journal.pone.0079890, 2013.

Hornick, T., Bach, L. T., Spilling, K., Crawfurd, K., Riebesell, U., and Grossart, H. P.: Effect of ocean acidification on bacterial dynamics during a low productive late summer situation in the Baltic Sea, Biogeosciences Discuss., in preparation, 2015.

International Council for the Exploration of the Sea: ICES Dataset on Ocean Hydrography, ICES Oceanography Baltic Sea Monitoring Data, available at: http://ocean.ices.dk/helcom/Helcom.aspx?Mode=1, last access: 7 August 2014.

Joint, I., Doney, S., and Karl, D.: Will ocean acidification affect marine microbes?, ISME J., 5, 1–7, doi:10.1038/ismej.2010.79, 2011.

Kanoshina, I., Lips, U., and Leppänen, J.-M.: The influence of weather conditions (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic Sea), Harmful Algae, 2, 29–41, doi:10.1016/S1568-9883(02)00085-9, 2003.

Kérouel, R. and Aminot, A.: Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis, Mar. Chem., 57, 265–275, doi:10.1016/S0304-4203(97)00040-6, 1997.

Kim, J.-M., Lee, K., Shin, K., Kang, J.-H., Lee, H.-W., Kim, M., Jang, P.-G., and Jang, M.-C.: The effect of seawater $CO_2$ concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment, Limnol. Oceanogr., 51, 1629–1636, 2006.

Koeve, W. and Oschlies, A.: Potential impact of DOM accumulation on $fCO_2$ and carbonate ion computations in ocean acidification experiments, Biogeosciences, 9, 3787–3798, doi:10.5194/bg-9-3787-2012, 2012.

Law, C. S., Breitbarth, E., Hoffmann, L. J., McGraw, C. M., Langlois, R. J., LaRoche, J., Marriner, A., and Safi, K. A.: No stimulation of nitrogen fixation by non-filamentous diazotrophs under elevated $CO_2$ in the South Pacific, Glob. Change Biol., 18, 3004–3014, doi:10.1111/j.1365-2486.2012.02777.x, 2012.

Lehmann, A. and Myrberg, K.: Upwelling in the Baltic Sea – A review, J. Marine Syst., 74, S3–S12, doi:10.1016/j.jmarsys.2008.02.010, 2008.

Lischka, S., Bach, L. T., Schulz, K.-G., and Riebesell, U.: Micro- and mesozooplankton community response to increasing levels of $CO_2$ in the Baltic Sea: insights from a large-scale mesocosm experiment, Biogeosciences Discuss., in preparation, 2015.

Lomas, M. W., Hopkinson, B. M., Ryan, J. L. L. D. E., Shi, D. L., Xu, Y., and Morel, F. M. M.: Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic, Aquat. Microb. Ecol., 66, 211–222, doi:10.3354/ame01576, 2012.

Losh, J. L., Morel, F. M. M., and Hopkinson, B. M.: Modest increase in the C:N ratio of N-limited phytoplankton in the California Current in response to high $CO_2$, Mar. Ecol.-Prog. Ser., 468, 31–42, doi:10.3354/meps09981, 2012.

Lueker, T. J., Dickson, A. G., and Keeling, C. D.: Ocean $pCO_2$ calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of $CO_2$ in gas and seawater at equilibrium, Mar. Chem., 70, 105–119, doi:10.1016/S0304-4203(00)00022-0, 2000.

Mackey, M. D., Mackey, D. J., Higgins, H. W., and Wright, S. W.: CHEMTAX – A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton, Mar. Ecol.-Prog. Ser., 144, 265–283, doi:10.3354/meps144265, 1996.

Matthäus, W., Nausch, G., Lass, H. U., Nagel, K., and Siegel, H.: The Baltic Sea in 1998 – characteristic features of the current stagnation period, nutrient conditions in the surface layer and exceptionally high deep water temperatures, Deutsche Hydrographische Zeitschrift, 51, 67–84, doi:10.1007/BF02763957, 1999.

Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M.: Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, Limnol. Oceanogr., 18, 897–907, 1973.

Meier, H. E. M., Andersson, H. C., Eilola, K., Gustafsson, B. G., Kuznetsov, I., Müller-Karulis, B., Neumann, T., and Savchuk, O. P.: Hypoxia in future climates: A model ensemble study for the Baltic Sea, Geophys. Res. Lett., 38, L24608, doi:10.1029/2011GL049929, 2011.

Mosley, L. M., Husheer, S. L. G., and Hunter, K. A.: Spectrophotometric pH measurement in estuaries using thymol blue and m-cresol purple, Mar. Chem., 91, 175–186, doi:10.1016/j.marchem.2004.06.008, 2004.

Nausch, M., Bach, L., Czerny, J., Goldstein, J., Grossart, H. P., Hellemann, D., Hornick, T., Achterberg, E., Schulz, K., and Riebesell, U.: Effect of $CO_2$ perturbation on phosphorus pool sizes and uptake in a mesocosm experiment during a low productive summer season in the northern Baltic Sea, Biogeosciences Discuss., in press, 2015.

Newbold, L. K., Oliver, A. E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M., Andersen, G., Van der Gast, C. J., and Whiteley, A. S.: The response of marine picoplankton to ocean acidification, Environ. Microbiol., 14, 2293–2307, doi:10.1111/j.1462-2920.2012.02762.x, 2012.

Nielsen, L. T., Jakobsen, H. H., and Hansen, P. J.: High resilience of two coastal plankton communities to twenty-first century seawater acidification: Evidence from microcosm studies, Mar. Biol. Res., 6, 542–555, doi:10.1080/17451000903476941, 2010.

Nielsen, L. T., Hallegraeff, G. M., Wright, S. W., and Hansen, P. J.: Effects of experimental seawater acidification on an estuarine plankton community, Aquat. Microb. Ecol., 65, 271–285, 2011.

Nômmann, S., Sildam, J., Nôges, T., and Kahru, M.: Plankton distribution during a coastal upwelling event off Hiiumaa, Baltic Sea:

impact of short-term flow field variability, Cont. Shelf Res., 11, 95–108, doi:10.1016/0278-4343(91)90037-7, 1991.

Patey, M. D., Rijkenberg, M. J. A., Statham, P. J., Stinchcombe, M. C., Achterberg, E. P., and Mowlem, M.: Determination of nitrate and phosphate in seawater at nanomolar concentrations, TrAC-Trend, Anal. Chem., 27, 169–182, doi:10.1016/j.trac.2007.12.006, 2008.

Paul, A. J., Achterberg, E. P., Bach, L. T., Boxhammer, T., Czerny, J., Haunost, M., Schulz, K.-G., Stuhr, A., and Riebesell, U.: No observed effect of ocean acidification on nitrogen biogeochemistry in a summer Baltic Sea plankton community, Biogeosciences Discuss., submitted, 2015.

Redfield, A. C.: The biological control of chemical factors in the environment, Am. Sci., 46, 205–221, 1958.

Richardson, T. L. and Jackson, G. A.: Small phytoplankton and carbon export from the surface ocean, Science, 315, 838–840, doi:10.1126/science.1133471, 2007.

Richier, S., Achterberg, E. P., Dumousseaud, C., Poulton, A. J., Suggett, D. J., Tyrrell, T., Zubkov, M. V., and Moore, C. M.: Phytoplankton responses and associated carbon cycling during shipboard carbonate chemistry manipulation experiments conducted around Northwest European shelf seas, Biogeosciences, 11, 4733–4752, doi:10.5194/bg-11-4733-2014, 2014.

Riebesell, U. and Tortell, P. D.: Effects of ocean acidification on pelagic organisms and ecosystems, in: Ocean Acidification, edited by: Gattuso, J.-P. and Hansson, L., p. 99, Oxford University Press, Oxford, United Kingdom, 2011.

Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J., and Zöllner, E.: Enhanced biological carbon consumption in a high $CO_2$ ocean, Nature, 450, 545–548, doi:10.1038/nature06267, 2007.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R., and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean change research, Biogeosciences, 10, 1835–1847, doi:10.5194/bg-10-1835-2013, 2013.

Rossoll, D., Sommer, U., and Winder, M.: Community interactions dampen acidification effects in a coastal plankton system, Mar. Ecol.-Prog. Ser., 486, 37–46, doi:10.3354/meps10352, 2013.

Rost, B., Zondervan, I., and Wolf-Gladrow, D.: Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions, Mar. Ecol.-Prog. Ser., 373, 227–237, doi:10.3354/meps07776, 2008.

Schernewski, G.: Global Change and Baltic Coastal Zones, Springer Science & Business Media, Dordrecht, Heidelberg, London, New York, 2011.

Schluter, L., Mohlenberg, F., Havskum, H., and Larsen, S.: The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: testing the influence of light and nutrients on pigment/chlorophyll a ratios, Mar. Ecol.-Prog. Ser., 192, 49–63, doi:10.3354/meps192049, 2000.

Schulz, K. G. and Riebesell, U.: Diurnal changes in seawater carbonate chemistry speciation at increasing atmospheric carbon dioxide, Mar. Biol., 160, 1889–1899, doi:10.1007/s00227-012-1965-y, 2013.

Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyerhöfer, M., Müller, M. N., Egge, J. K., Nejstgaard, J. C., Neill, C., Wohlers, J., and Zöllner, E.: Build-up and decline of organic matter during PeECE III, Biogeosciences, 5, 707–718, doi:10.5194/bg-5-707-2008, 2008.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences, 10, 161–180, doi:10.5194/bg-10-161-2013, 2013.

Sharp, J.: Improved analysis for particulate organic carbon and nitrogen from seawater, Limnol. Oceanogr., 19, 984–989, 1974.

Spilling, K., Paul, A. J., Virkkala, N., Hastings, T., Lischka, S., Stuhr, A., Bermúdez, R., Czerny, J., Schulz, K., Ludwig, A., and Riebesell, U.: Ocean acidification may decrease plankton respiration: evidence from a mesocosm experiment, Biogeosciences Discuss., in preparation, 2015.

Stal, L. J., Staal, M., and Villbrandt, M.: Nutrient control of cyanobacterial blooms in the Baltic Sea, Aquat. Microb. Ecol., 18, 165–173, doi:10.3354/ame018165, 1999.

Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., and Brutemark, A.: Climate change and eutrophication induced shifts in northern summer plankton communities, PLoS ONE, 8, e66475, doi:10.1371/journal.pone.0066475, 2013.

Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., Grinsven, H., and Grizzetti, B.: The European Nitrogen Assessment: Sources, Effects and Policy Perspectives, Cambridge University Press, Cambridge, United Kingdom, 2011.

Tatters, A. O., Roleda, M. Y., Schnetzer, A., Fu, F., Hurd, C. L., Boyd, P. W., Caron, D. A., Lie, A. A. Y., Hoffmann, L. J., and Hutchins, D. A.: Short- and long-term conditioning of a temperate marine diatom community to acidification and warming, Philos. T. R. Soc. B, 368, 20120437, doi:10.1098/rstb.2012.0437, 2013a.

Tatters, A. O., Schnetzer, A., Fu, F., Lie, A. Y. A., Caron, D. A., and Hutchins, D. A.: Short- versus long-term responses to changing $CO_2$ in a coastal dinoflagellate bloom: Implications for interspecific competitive interactions and community structure, Evolution, 67, 1879–1891, doi:10.1111/evo.12029, 2013b.

Toggweiler, J. R.: Carbon overconsumption, Nature, 363, 210–211, doi:10.1038/363210a0, 1993.

Turner, R. E.: Some Effects of Eutrophication on Pelagic and Demersal Marine Food Webs, in Coastal Hypoxia: Consequences for Living Resources and Ecosystems, edited by: Rabalais, N. N. and Turner, R. E., 371–398, American Geophysical Union, Washington D.C., USA, 2001.

Vehmaa, A., Almén, A.-K., Brutemark, A., Paul, A. J., Riebesell, U., Furuhagen, S., and Engström-Öst, J.: Ocean acidification challenges copepod reproductive plasticity, Biogeosciences Discuss., in preparation, 2015.

Webb, A. L., Leedham-Elvidge, E., Hughes, C., Hopkins, F. E., Malin, G., Riebesell, U., Schulz, K., Bach, L. T., Crawfurd, K., Brussaard, C., and Liss, P. S.: Effect of ocean acidification and elevated $fCO_2$ on trace gas production by a Baltic Sea summer phytoplankton community, Biogeosciences Discuss., in preparation, 2015.

Welschmeyer, N. A.: Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments, Limnol. Oceanogr., 39, 1985–1992, doi:10.4319/lo.1994.39.8.1985, 1994.

Wu, R. S. S.: Hypoxia: from molecular responses to ecosystem responses, Mar. Pollut. Bull., 45, 35–45, doi:10.1016/S0025-326X(02)00061-9, 2002.

Yoshimura, T., Nishioka, J., Suzuki, K., Hattori, H., Kiyosawa, H., and Watanabe, Y. W.: Impacts of elevated $CO_2$ on organic carbon dynamics in nutrient depleted Okhotsk Sea surface waters, J. Exp. Mar. Biol. Ecol., 395, 191–198, doi:10.1016/j.jembe.2010.09.001, 2010.

Yoshimura, T., Suzuki, K., Kiyosawa, H., Ono, T., Hattori, H., Kuma, K., and Nishioka, J.: Impacts of elevated $CO_2$ on particulate and dissolved organic matter production: microcosm experiments using iron-deficient plankton communities in open subarctic waters, J. Oceanogr., 69, 601–618, doi:10.1007/s10872-013-0196-2, 2013.

Yoshimura, T., Sugie, K., Endo, H., Suzuki, K., Nishioka, J., and Ono, T.: Organic matter production response to $CO_2$ increase in open subarctic plankton communities: Comparison of six microcosm experiments under iron-limited and -enriched bloom conditions, Deep-Sea Res. Pt. I, 94, 1–14, doi:10.1016/j.dsr.2014.08.004, 2014.

Zapata, M., Rodriguez, F., and Garrido, J. L.: Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase $^{C}8$ column and pyridine-containing mobile phases, Mar. Ecol.-Prog. Ser., 195, 29–45, doi:10.3354/meps195029, 2000.

Zhang, J.-Z. and Chi, J.: Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide, Environ. Sci. Technol., 36, 1048–1053, doi:10.1021/es011094v, 2002.

# 3 | Manuscript II

# No observed effect of ocean acidification on nitrogen biogeochemistry in a summer Baltic Sea plankton community

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# Abstract

Nitrogen fixation by filamentous cyanobacteria supplies significant amounts of new nitrogen (N) to the Baltic Sea. This balances N loss processes such as denitrification and anammox and forms an important N source supporting primary and secondary production in N-limited post-spring bloom plankton communities. Laboratory studies suggest that filamentous diazotrophic cyanobacteria growth and $N_2$-fixation rates are sensitive to ocean acidification with potential implications for new N supply to the Baltic Sea. In this study, our aim was to assess the effect of ocean acidification on diazotroph growth and activity as well as the contribution of diazotrophically-fixed N to N supply in a natural plankton assemblage. We enclosed a natural plankton community in a summer season in the Baltic Sea near the entrance to the Gulf of Finland in six large-scale mesocosms (volume $\sim$55 $\mathrm{m}^3$) and manipulated $fCO_2$ over a range relevant for projected ocean acidification by the end of this century (average treatment $fCO_2$ range of 365 – 1231 µatm). The direct response of diazotroph growth and activity was followed in the mesocosms over a 47 day study period during N-limited growth in the summer plankton community. Diazotrophic filamentous cyanobacteria abundance throughout the study period and $N_2$-fixation rates (determined only until day 21 due to subsequent use of contaminated commercial $^{15}N$-$N_2$ gas stocks) remained low. Thus estimated new N inputs from diazotrophy were too low to relieve N limitation and stimulate a summer phytoplankton bloom. Instead, regeneration of organic N sources likely sustained growth in the plankton community. We could not detect significant $CO_2$-related differences in inorganic or organic N pools sizes, or particulate matter N:P stoichiometry. Additionally, no significant effect of elevated $CO_2$ on diazotroph activity was observed. Therefore, ocean acidification had no observable impact on N cycling or biogeochemistry in this N-limited, post-spring bloom plankton assemblage in the Baltic Sea.

## 3.1 Introduction

Nitrogen (N) is an essential element for cell functioning in the biosphere due to its presence in many important biomolecules such as nucleic acids and proteins. However, in many marine ecosystems N is considered the limiting nutrient for important cellular processes in phytoplankton (Vitousek and Howarth, 1991), as indicated through a stimulation in carbon fixation and pigment synthesis through addition of inorganic N (e.g. Moore et al., 2008, 2013). This low N availability also prevails in post-spring bloom plankton communities in the Baltic Sea, as the nitrate pool is exhausted during the spring-bloom leaving behind an excess of dissolved inorganic phosphorus (Wasmund et al., 2001). Consequently, filamentous diazotrophic ($N_2$-fixing) cyanobacteria, in particular heterocystous *Nodularia spumigena* and *Aphanizomenon flos-aquae*, capitalise on this excess phosphate and increasing water column temperatures in summer months (Kononen et al., 1996; Pliński and Jóźwiak, 1999; Wasmund, 1997) and commonly form extensive blooms and surface aggregations (e.g. Kahru and Elmgren, 2014).

The atmospheric nitrogen gas ($N_2$) fixed by these heterocystous cyanobacteria during the summer months forms a key N source for the wider plankton community in the Baltic Sea, since a significant fraction of the fixed N can be released as ammonium (Ohlendieck et al., 2000; Ploug et al., 2010; Stal et al., 2003; Wannicke et al., 2013) and dissolved organic N compounds (Ohlendieck et al., 2007, 2000; Wannicke et al., 2013). Thus in addition to N in diazotroph biomass, newly fixed N is also available for direct assimilation by phytoplankton and bacteria and is estimated to support up to 20 – 45% of annual primary production in the Baltic Sea (Gustafsson et al., 2013). This new N input partly replenishes N loss processes such as anammox and denitrification in the deep anoxic basins (Vahtera et al., 2007). Furthermore, this fixed N can also be directly transferred to higher trophic levels through grazing by zooplankton (Engström-Öst et al., 2011; Hogfors et al., 2014; Wannicke et al., 2013).

Changes in seawater carbonate chemistry due to increased atmospheric $CO_2$ concentrations are expected to induce changes in phytoplankton physiology. The associated decrease in seawater pH is called ocean acidification. Numerous single-strain culture studies have investigated the physiological responses of a variety of diazotrophic organisms and generally indicated increased $N_2$-fixation and diazotroph growth rates under elevated $CO_2$ (Barcelos e Ramos et al., 2007; Fu et al., 2008; Hutchins et al., 2007; Kranz et al., 2010; Levitan et al., 2007), with contrasting evidence under iron limitation (Shi et al., 2012) and with freshwater strains of *A. flos-aquae* (Yamamoto and Nakahara, 2005). Three studies on the common Baltic Sea species, *N. spumigena*, produced contrasting results with two studies under phosphate repletion suggesting a negative effect (Czerny et al., 2009; Eichner et al., 2014), and one study under low inorganic phosphate availability, indicating a positive effect (Wannicke et al., 2012) of increased $CO_2$ on growth and $N_2$-fixation rates. This discrepancy may, however, be due to differences in phosphate availability (Eichner et al., 2014). Considering the contribution of diazotrophs to the N budget and primary productivity in the Baltic Sea, it is vital to understand the influence of future changes in $CO_2$ on new N inputs by diazotrophs.

In this mesocosm study, our aim was to assess diazotroph growth and rates of $N_2$-fixation under a range of $CO_2$ concentrations in a natural plankton community. N limitation of phytoplankton growth was reported in the study area in the Finland Archipelago Sea (Kirkkala et al.,

1997; Tamminen and Andersen, 2007). By utilizing the naturally occurring low N conditions in the Baltic Sea we wanted to examine the importance of new N inputs by diazotrophic organisms to the wider plankton community N supply under projected future ocean acidification scenarios.

## 3.2 Materials and methods

### 3.2.1 Experimental set-up and sampling

The study took place in the period between June and August 2012 in Tvärminne Storfjärden which is situated in the Archipelago Sea on the southwestern tip of Finland. Six pelagic mesocosms (total volume ∼55 $\mathrm{m}^3$ , KOSMOS, Riebesell et al. 2013) were deployed on 12 June 2012 (day of experiment -10 = *t*-10, i.e. 10 days before $CO_2$ manipulation) and moored at 59° 51.5' N, 23° 15.5' E. The cylindrical mesocosm bags of 2 m in diameter extended from 1.5 m above to 19 m below the water surface and were closed at the bottom by a 2 m long sediment trap funnel on *t*-5. A 3 mm net was used to exclude larger organisms or particles before mesocosm closure.

A gradient of $CO_2$ treatments across the mesocosms was established over a four day period by additions of filtered (50 µm), $CO_2$-saturated seawater evenly distributed in the water column, as described by Riebesell et al. (2013). $CO_2$ additions were carried out in the afternoons of *t*0 – *t*4 not to interfere with the daily sampling. A $CO_2$ addition was also made in the upper 7 m on *t*15 to counter strong outgassing in the upper water column. Initial $fCO_2$ ranged from ∼240 µatm in the two ambient control mesocosms to up to 1650 µatm (Fig. 3.1). Unenriched filtered (50 µm) seawater was added to the two control mesocosms (M1, M5). The seawater used for the additions to the mesocosms was collected from the Tvärminne Storfjärden from a depth of 10 m by a pipe connected to the laboratory at the research station.

Depth-integrating water samplers (IWS, HYDRO-BIOS, Kiel) were used to collect water from 0 – 17 m depth in each mesocosm for analysis of particulate matter, dissolved inorganic and organic matter, phytoplankton pigments, phytoplankton abundances, carbonate chemistry variables. Samples for carbonate chemistry variables were taken directly from the IWS on board the sampling boat whereas all other samples were pooled in 10 L plastic carboys and stored on board in the dark until sub-sampling on shore (Paul et al., 2015). Particulate matter collected in the sediment trap was pumped to the surface and collected in sampling bottles (Boxhammer et al., submitted).

Particulate matter (C, N, P) was collected onto GF/F filters (nominal pore size of 0.7 µm, 25 mm diameter, Whatman) by gentle vacuum filtration (pressure <200 mbar). Filters and glass petri dishes were combusted at 450°C for 6 hours before use. Collected particulate sediment material was concentrated, freeze-dried and ground to a homogenous powder, while supernatant subsamples were filtered and subsequently analysed as for water column material. Total particulate carbon and nitrogen (TPC and PON) content and isotopic composition were analysed according to Sharp (1974) using an elemental analyser (EuroEA) coupled by either a Conflo II to a Finnigan DeltaPlus isotope ratio mass spectrometer or by a Conflo III to a Thermo Finnigan DeltaPlus XP isotope ratio mass spectrometer. Stable N isotope composition of particulate N is reported in permil (‰) relative to the atmospheric $N_2$ standard (AIR). Total particulate

phosphorus (TPP) concentrations were determined spectrophotometrically following sample digestion as described in Hansen and Koroleff (1999). Samples for biogenic silica (BSi) analyses were collected on cellulose acetate filters (pore size of 0.65 µm, 25 mm diameter, Whatman) by filtration as described above for particulate matter. Concentrations were determined spectrophotometrically following sample digestion according to Hansen and Koroleff (1999). Samples for determination of nanomolar concentrations of dissolved inorganic nutrients were filtered (GF/F, nominal pore size of 0.7 µm, Fisher Scientific). Nitrate and nitrite (hereafter nitrate) and dissolved inorganic phosphate concentrations were then analysed colorimetrically using a 2 m liquid waveguide capillary cell (Patey et al., 2008; Zhang and Chi, 2002) and a miniaturised detector (Ocean Optics Ltd). Concentrations of ammonium ($NH_4^+$) were determined fluorimetrically (Trilogy, Turner) according to Kérouel and Aminot (1997). Total dissolved nitrogen (TDN) was analysed using a high-temperature catalytic combustion technique with a Shimadzu TOC-TN V analyser as described by Badr et al. (2003). Samples were filtered (GF/F, nominal pore size of 0.7 µm, Fisher Scientific) to remove particulate material and collected in clean glass vials, acidified with HCl to pH 1.9 and flame sealed. Filters and vials were combusted for 6 hours at 450°C before use. Dissolved organic nitrogen (DON) concentrations were calculated by subtracting the inorganic N concentrations from TDN. Phytoplankton pigments were extracted in acetone (90%) and after homogenisation and centrifugation, the supernatant was filtered (0.2 µm PTFE filters, VWR International) and concentrations were determined by reverse phase high performance liquid chromatography (HPLC; WATERS HPLC with a Varian Microsorb-MV 100-3 C8 column; Barlow et al. 1997, Derenbach 1969). A library of pre-measured commercial standards was used to calibrate peaks.

Phosphate excess (P*, Deutsch et al. 2007) was calculated from the dissolved inorganic phosphate ($PO_4^{3-}$), nitrate ($NO_3^-$) and ammonium ($NH_4^+$) concentrations according to:

$$P^* = [PO_4^{3-}] - \frac{[NO_3^-] + [NH_4^+]}{16} \tag{3.1}$$

Dissolved silicate (DSi) drawdown was calculated as the difference in DSi concentration on a given sampling day (tx) and t1:

$$\text{DSi drawdown} = [\text{DSi}]_{t1} - [\text{DSi}]_{tx} \tag{3.2}$$

A comprehensive description of mesocosm deployment, set-up and sampling procedures including sample collection, handling and analyses for particulate matter, dissolved inorganic and organic matter, phytoplankton pigments, and sediment trap particulate matter is covered in (Paul et al., 2015), also in this special issue. An overview table of sampled variables for the entire experiment, including sampling frequency, is also presented in this accompanying manuscript.

### 3.2.2 $N_2$-fixation rate incubations

Incubations for determination of $N_2$-fixation rates were carried out according to Mohr et al. (2010). Seawater used for $^{15}N$-$N_2$ enrichments was filtered (polycarbonate Isopore™ filter, pore size of 0.22 µm, 47 mm diameter) before being pumped through a degassing membrane (Membrana Mini Module G542) attached to a water-jet pump to remove ambient $N_2$. The degassing system was cleaned with 5% HCl before and after use, followed by cycling with deionised water

(MilliQ, Millipore) to remove any traces of acid. Seawater from the Tvärminne Storfjärden was collected from a depth of 10 m and cycled once through the degassing system before collection in an air-tight, acid-cleaned bag with septum (SKC Tedlar® Bag with single polypropylene fitting) without exposure to the atmosphere. 1 mL of $^{15}N$-$N_2$ gas (98 atom % $^{15}N$, Sigma Aldrich, Lot no.: CX0937 until *t*21, SZ1670V after *t*21) was injected through the septum into the bag for every 100 mL of sample. The resulting bubble was dissolved and the $^{15}NN_2$-enriched seawater was stored at in situ temperature of the mesocosms until addition to incubation bottles. Seawater for the blank incubations was prepared in a separate bag using the same process however ambient air was added instead of isotopically labelled $^{15}N$-$N_2$ gas.

Water samples for $N_2$-fixation rate incubations were directly transferred in a gentle manner from the integrating water sampler into 2.3 L polycarbonate bottles on board the sampling boat using silicon tubing. The bottles were stored in a closed cool box to control temperature and to block sunlight until return to the on shore laboratory. Each bottle was weighed and homogenised by gentle rotation before 70 – 90 mL of water was removed to make space for the $^{15}N$-$N_2$-enriched seawater. Enriched or 'blank' seawater was transferred from the Tedlar® bags to the respective bottles through Tygon™ tubing, immersed in the sample bottle, using a peristaltic pump to minimise tracer loss through exposure to atmosphere. Incubation bottles were filled with no headspace. After addition, the caps were immediately screwed on to seal the bottles air tight. During these procedures, the bottles were reweighed at each step in order to determine the exact amount of isotope label inside each bottle. The final $^{15}N$-enrichment of dissolved $N_2$ gas in each bottle was between 1.0 – 3.5 atom %. The bottles were then mixed by gentle rotation and placed in a climate chamber at in situ temperature and under controlled light conditions (73 ± 1 µmol photons $\mathrm{m^{-2}\,s^{-1}}$, mean ± S.D). Light was measured using a LI-COR LI-1000 DataLogger light meter. Measured light intensities were within the range of average depth-integrated (0 – 17 m) intensities in the mesocosms taken from daily CTD profiles at between 13:30 and 14:30 LT (20 to 300 photons $\mathrm{m^{-2}\,s^{-1}}$). The light-dark cycle followed the natural sunrise-sunset variation which on the summer solstice (21 June 2012, *t*-1) was 19:5 hours (L:D). Climate chamber temperature was programmed to follow the daily integrated water column temperature as recorded by the afternoon CTD sampling and thus is reported as in situ temperature. Identical light conditions at each bottle position were achieved by a rotation regime. Bottles were rotated gently to mix and the bottle position rotated systematically approximately every three hours during the light cycle. Time of rotation was recorded allowing the calculation of average light conditions between each individual bottle.

Incubations were terminated after 24 hours by filtration through a combusted (6 h at 450°C) and acid rinsed (1% HCl) GF/F filter (0.7 µm pore size, 25 mm diameter, Whatman) under reduced vacuum (<200 mbar). Filters were placed in glass petri dishes (combusted 6 h, 450°C), frozen immediately and stored at -20°C until analysis on a mass spectrometer as described for particulate C and N analyses above and also in Paul et al. (2015). Rates were calculated according to Montoya et al. (1996). Estimated internal analytical uncertainty in calculated $N_2$–fixation rates was less than ±10% when rates were above the detection limit. The detection limit was determined as a difference in $\delta^{15}N$ between initial and final values of larger than 1.0‰. This corresponded to a calculated rate of more than 0.15 nmol N $\mathrm{L^{-1}\,d^{-1}}$.

### 3.2.3 Enrichment of mesocosms with $^{15}N$-$N_2$

Four of six mesocosms spanning the range of treatments were enriched with the isotopically labelled $^{15}N$-$N_2$ gas to investigate the fate of newly fixed N in this plankton community under future ocean acidification conditions. A similar approach to Mohr et al. (2010), as described for the $N_2$-fixation incubations (see Section 3.2.2), was employed on a larger scale. A total of approximately 1500 L of unfiltered seawater was collected from the Baltic at ca. 10 m depth and pumped into the laboratory building at Tvärminne Zoological Station. Mesocosm enrichment occurred in two pulses on $t22$ and $t26$. We added this in two steps because of the limited number of bags available for preparing the $^{15}N$-$N_2$ enriched seawater. For the first step, seawater was filtered and collected as for the $N_2$-fixation incubations in bags (thermoplastic polyurethane, ∼30 L capacity) with a tap and a crimp sealed septum (N20 grey butyl rubber plugs, Macherey and Nagel) on opposite ends of the bag. The large physical effort required to dissolve the gas by 'bag-slapping', as commonly done for small volumes using the method described by Mohr et al. (2010), led to a modification of the enrichment method for the second enrichment step. Water was collected and degassed as previously described through the degassing membrane. Instead of collecting the water directly after this step, the water then passed through a second membrane that was flooded with $^{15}N$-$N_2$ gas and was connected to an overflow system which allowed monitoring of gas dissolution (Fig. 3.2). The high surface area in the membrane enhanced the labelled gas dissolution. This enriched water was then pumped directly into the empty collection bags using a peristaltic pump without contact with the atmosphere. One complete cartridge of gas (500 mL, nitrogen - $^{15}N$-$N_2$, 98 atom % $^{15}N$ Sigma Aldrich, Lot no.: SZ1670V, SZ1423V, CX0937) was added per bag through the septum. A total of 150 L of enriched seawater prepared was added to four mesocosms (M3, M5, M6, M8), and 100 L unenriched filtered seawater was added to the other two mesocosms (M1, M7) as isotope label controls on $t22$ and $t26$.

### 3.2.4 Phytoplankton counts

Counts of phytoplankton cells >20 µm were made from 50 mL samples fixed with acidic Lugol's iodine solution (1% final concentration). Samples were concentrated using gravitational settling and counted under an inverted microscope (ZEISS Axiovert 100) after Utermöhl (1958) and following the guidelines for determination of phytoplankton species composition, abundance and biomass for the COMBINE programme provided by HELCOM (Annex C-6). The cells were counted either on half of the chamber at 100 fold or on 3 to 4 strips at 200 fold magnification. Filamentous cyanobacteria were counted in 50 µm length units. Plankton were identified where possible to the species level according to Tomas (1997), Hoppenrath et al. (2009), and Kraberg et al. (2010). Biovolumes of counted plankton cells were calculated according to Olenina et al. (2006) and converted to cellular organic carbon quotas by the equations of Menden-Deuer and Lessard (2000).

### 3.2.5 Statistical analyses

A linear regression analysis was applied to determine the relationship between mean $fCO_2$ and the mean response of each variable for the three experimental phases (Phase I, II and III),

as described in Paul et al. (2015). Linear regression analyses were undertaken using R (R Core Team, 2014).

## 3.3 Results

Three experimental phases after initial $CO_2$ manipulation on $t0$ were defined in Paul et al. (2015) using temperature and chlorophyll *a* (Chl *a*) fluctuations: Phase I ($t1$ – $t16$), Phase II ($t17$ – $t30$) and Phase III ($t31$ – $t43$). These phases are also used to assist with data interpretation in this manuscript.

### 3.3.1 Inorganic nutrient availability and nutrient limitation

There were low concentrations of inorganic N present throughout the study period with inorganic nitrate concentrations in the range of 3 – 107 nmol $L^{-1}$ (Fig. 3.1). Ammonium ($NH_4^+$) was the dominant source of inorganic N with concentrations ranging between 20 and 289 nmol $L^{-1}$. Hence $NH_4^+$ was also included in the calculations of P* (excess phosphate) and inorganic nutrient elemental stoichiometry according to the Redfield ratio (Fig. 3.3, Eqn. 3.1).

There was an excess of inorganic phosphate to inorganic N in all mesocosms (P*> 0 nmol $L^{-1}$, Fig. 3A) and the surrounding waters throughout the study period, with phosphate concentrations ranging between 72 and 214 nmol $L^{-1}$ in the mesocosms and up to 410 nmol $L^{-1}$ outside the mesocosms in the surrounding Archipelago Sea. Inorganic phosphate concentrations decreased during Phase I, followed by an increase during Phase II with more stable concentrations in Phase III. Nitrate concentrations increased throughout the experiment with a possible small drawdown after $t39$ in all treatments, whereas $NH_4^+$ concentrations were variable. Samples for $NH_4^+$ analyses were lost on $t27$ and $t29$. There did not appear to be any remarkable relationship linking accumulated precipitation (between sampling days), and the increase in nitrate (Fig. 3.1), indicating that wet atmospheric deposition of nitrate into the mesocosms was effectively prevented by the mesocosm roofs and did not affect the nitrate pool. Precipitation data for the Hanko weather station (ID no.: GHCND:FIE00142025, latitude: 59.8439, longitude: 23.2517) were obtained from the National Oceanographic Data Center (NOAA).

### 3.3.2 Diatom abundance, silicate dynamics and dissolved N utilisation

Diatoms were mostly abundant at the beginning of the experiment with the species *Chaetoceros* sp. and *Skeletonema marinoi* present in the large size class (>20 µm, Fig. 4). Fucoxanthin marker pigment concentrations in this size class and suspended BSi concentrations (>0.65 µm) declined markedly during the first few days in Phase I and the dynamics fitted well to the microscopy counts of both *Chaetoceros* sp. and *S. marinoi*. Dissolved silicate (DSi) concentrations continued to decrease up until $t13$. No statistically significant difference between $CO_2$ treatments was detected for diatom abundance (microscopy counts), DSi drawdown or BSi concentrations (Table 3.1, Fig. 3.4), apart from BSi in Phase II where a positive effect was detected ($p = 0.034$, see Paul et al. (2015) for statistical analyses).

Dissolved organic nitrogen (DON) concentrations ranged between 20 and 25 µmol $L^{-1}$ (Fig. 3.4). DON concentrations appeared to decrease during Phase I, however considerable variability in the data meant this DON drawdown could not be accurately quantified.

### 3.3.3 Diazotroph abundance, $N_2$-fixation rates, and $\delta^{15}N$ in particulate N

The abundance of filamentous diazotrophic cyanobacteria remained low throughout the experiment with no significant bloom development (<6 µg C $L^{-1}$ , Fig. 3.5). The most dominant species, *A. flos-aquae*, had a maximum biomass of 4.9 µg C $L^{-1}$ in the mesocosms (M1, *t*27), whereas the next most abundant species, *Anabaena* sp., had a maximum biomass in the water column of 0.18 µg C $L^{-1}$ (M1, *t*17). Aphanizophyll, a pigment present in *A. flos-aquae* and *Anabaena* sp. (Schluter et al., 2004), was detected in both suspended material in the water column, and in the sinking material collected in the sediment trap. Concentrations of this pigment increased at the end of Phase I concurrent with an increase in $N_2$-fixation rates (Figs. 3.5 C and B respectively). Although numbers in the mesocosms remained generally low, *A. flos-aquae* abundances based on microscopy counts and phytoplankton pigment analyses, were highest in Phases II/III and lowest in Phase I (Fig. 3.5). *A. flos-aquae* biomass outside the mesocosms was up to 30 µg C $L^{-1}$ on *t*15 and is supported by high Aphanizophyll pigment concentrations of 109 ng (mg TPC)$^{-1}$ also on *t*15 (data not shown).

Rates of $N_2$-fixation until *t*21 ranged from below the detection limit at the beginning of the experiment, up to 4.4 nmol $L^{-1}\,d^{-1}$ inside the mesocosms and up to 37.9 nmol $L^{-1}\,d^{-1}$ in the waters outside. We observed a substantial increase in the $N_2$-fixation rates from 2.6 to 4.4 nmol $L^{-1}\,d^{-1}$ up to 50 to 60 nmol $L^{-1}\,d^{-1}$ between *t*21 and *t*23 without any remarkable change in diazotroph abundance of the same magnitude (Fig. 3.5). This is also evident in *A. flos-aquae* biomass-related $N_2$-fixation rates (Fig. 3.6). This increase coincided with the use of a new $^{15}N$-$N_2$ gas bottle with a lot number (Sigma Aldrich, Lot no. SZ1670V) which was reported two years later as contaminated with $^{15}N$-labelled $NH_4^+$ and $NO_3^-$ by Dabundo et al. (2014). The measured rates from *t*23 on are therefore not exclusively $N_2$-fixation and are not reliable thus they were excluded from analyses. In addition to the bottle assays, the $^{15}N$-$N_2$ isotope tracer was also added directly to all mesocosms except for M1 (control) and M7. Therefore these two mesocosms were not affected by this contamination issue. Hence, the natural abundance $\delta^{15}N$ data from the suspended material in the water column and the sinking material from the sediment trap is reported for the entire experiment (*t*-3 until *t*43) for M1 and M7 mesocosms (Figs. 3.5 E and F) but only until *t*21 for M3, M5, M6 and M8. Any $NH_4^+$ or nitrate added to the four mesocosms with the isotope tracer was highly isotopically enriched in $^{15}N$ but was in very low concentration and so was insignificant for the nutrient budget.

The natural abundance $\delta^{15}N$ in suspended particulate N in the mesocosms decreased during the period of higher Chl *a* in Phase I from 6.0 ± 0.5‰ on *t*1 to 2.6 ± 0.5‰ on *t*15 (mean ± S.D.). This indicated potential input of atmospheric N with a low $\delta^{15}N$ into particulate matter via $N_2$-fixation during this period. A sharp decrease in $\delta^{15}N$ in the sinking particulate material occurred on *t*17, the same day that considerable amounts of Aphanizophyll and Fucoxanthin were found in the sediment trap material (Figs. 3.5 D and F, Fucoxanthin not shown). This was one day after the mesocosm walls were cleaned indicating that there were likely diazotrophic species and diatoms attached to the mesocosm walls. Identification from microscope photos revealed the presence of

filamentous cyanobacteria with heterocysts in the collected sediment trap material. Aside from this, there were no remarkable fluctuations in $\delta^{15}$N in either the suspended or sinking particulate matter pools, including after *t*21 in M1 and M7 (Figs. 3.5 E and F).

Assessment of in situ $N_2$-fixation rates based on $^{15}$N-uptake from the combined dissolved N pool of $NO_3^-$, $NH_4^+$ and $N_2$ was abandoned due to high uncertainty in initial $^{15}$N enrichment and concentrations of the combined dissolved N pool, and fast saturation of label uptake after ca. four days (two successive sampling days). To assess the contribution of diazotrophy to N supply in the mesocosms, we calculated a theoretical cumulative diazotrophic N input using measured $N_2$-fixation rates from bioassays up until *t*21 (M1 = 20 nmol $L^{-1}$), and then assumed a constant $N_2$-fixation rate of 4 nmol $L^{-1}$ $d^{-1}$ into particulate N between *t*23 and *t*43 (total = 80 nmol $L^{-1}$). The assessment for between *t*23 and *t*43 is based on the premise of continued elevated *A. flos-aquae* biomass and assuming 50% exudation of fixed N as DON or $NH_4^+$ (<*t*21 = 20 nmol $L^{-1}$, >*t*21 = 80 nmol $L^{-1}$, total = 100 nmol $L^{-1}$). This yielded a theoretical new N input from *A. flos-aquae* of only 200 nmol $L^{-1}$, amounting to $\sim$ 5% of mean PON pool standing stock (3 µmol $L^{-1}$) and is clearly at the higher end of estimations. We calculated corresponding N requirement of the plankton community of 27.2 nmol $L^{-1}$ $d^{-1}$ from the average phosphorus uptake rate across all treatments of 1.7 nmol $PO_4^{3-}$ $L^{-1}$ $d^{-1}$ from *t*1 – *t*30 as reported by (Nausch et al., 2015), by assuming Redfield nutrient uptake stoichiometry (16N:1P). This is almost seven times larger than estimated daily diazotrophic N inputs of $\sim$ 4 nmol $L^{-1}$ $d^{-1}$, corresponding to 14% of calculated community N requirement.

Low filamentous diazotrophic cyanobacteria abundances exacerbated the inherent sampling error in both microscopy and pigment analyses due to patchy distribution and the tendency of filaments to aggregate. Hence, unfortunately no reliable statistical analyses on the effect of higher $fCO_2$ on diazotroph abundance or marker pigment concentration could be undertaken, for any phase of the experiment. Any potential $CO_2$ effect on diazotroph abundance was also not obvious on visual data inspection, and no effect could be detected on $N_2$-fixation rates or $\delta^{15}$N natural abundance in suspended particulate matter from the water column or sediment trap particulate matter up until *t*21 (Table 3.1), when rates were reliable and there was data from a sufficient number of $CO_2$ treatments.

## 3.4 Discussion

### 3.4.1 Effects of elevated $CO_2$ on diazotrophic N inputs

Bioavailable N was present in low concentrations and was probably the limiting macronutrient in the plankton community. Hence, higher phytoplankton biomass and lower phosphate concentrations at higher $CO_2$ observed in this same mesocosm study (Paul et al., 2015), may have suggested relief of N limited growth by potentially increased $N_2$-fixation. However we have no strong evidence to support this hypothesis based on N pool standing stocks and estimated diazotrophic N inputs. The only statistically significant, but very minor, correlation was a positive relationship between $CO_2$ and PON concentrations (Fig. 3.3B, 0.08 µmol $L^{-1}$, 3% difference in PON, slope = $1.75 \times 10^{-4}$ µmol $L^{-1}$ µatm$^{-1}$, data from Paul et al., 2015).

No significant difference in $N_2$-fixation rates (until *t*21) or *A. flos-aquae* abundance at elevated $CO_2$ compared to the ambient treatments was detected (Table 3.1, Fig. 3.5). Phosphate turnover rates, a potential indicator of P demand for $N_2$-fixation, were also unaffected by $CO_2$ in Phases I or II (Nausch et al., 2015). These variables $N_2$-fixation and phosphate uptake rates) provide a more sensitive measure of turnover rates of N and P than assessing changes in N pool standing stocks in this tightly-coupled regenerative plankton community. Unfortunately, we only have reliable $N_2$-fixation rates from incubations until *t*21 due to contamination of $^{15}N$-$N_2$ gas with bioavailable N compounds (Dabundo et al., 2014) and not after $\sim$ *t*25 when significant $CO_2$-related differences in C and P pools were apparent. Hence, in the later stages of the experiment (Phase II and III), it is possible that there was a divergence in $N_2$-fixation rates between treatments that was missed, despite low abundances of *A. flos-aquae*, the dominant filamentous diazotrophic cyanobacterium present. Nonetheless we estimate that the contribution of diazotrophy to N supply in the mesocosms over the study duration of 43 days was small ($\sim$200 nmol $L^{-1}$).

Maximum measured $N_2$-fixation rates of 4.4 nmol $L^{-1}\,d^{-1}$ were low compared to reported for the Baltic Sea in mid-summer which range from 1.7 up to 550 nmol $L^{-1}\,d^{-1}$ (Farnelid et al., 2013; Ohlendieck et al., 2000, 2007; Wasmund et al., 2001). This is due to the rather low *A. flos-aquae* biomass in the mesocosms compared to literature values (this study: maximum biomass = 5 µg C $L^{-1}$ integrated over 0 – 17 m; Gulf of Finland: 22 – 26 µg C $L^{-1}$ in the surface 5 m, 6 – 7 µg C $L^{-1}$ at 20 m deep in July, Laamanen and Kuosa, 2005). Thus even if all newly-fixed N by diazotrophs was transferred to diazotroph and plankton biomass (i.e. PON pool), this small accumulation would most likely remain below the detection limits in the suspended PON pool ($\sim$10% = 0.3 µmol $L^{-1}$). On top of this, any $CO_2$–related differences in $N_2$-fixation would be near impossible to resolve in this small contribution by diazotrophs.

The absence of any detectable effect may of course be influenced by the relatively low abundances of filamentous diazotrophic cyanobacteria in this study, as temperatures were mostly below temperatures thought to stimulate bloom development (16°C, (Wasmund, 1997); this study 8 – 16°C, (Paul et al., 2015)). Nevertheless our results from this $CO_2$ manipulation study are in agreement with studies from both the marine (Böttjer et al., 2014; Law et al., 2012) and freshwater (Shapiro, 1997; Yamamoto, 2009) realms which detected no significant effect of decreased pH/increased $CO_2$ on diazotroph abundance and/or activity in natural plankton communities. These four independent studies all contradict physiological investigations in single-strain culture experiments where diazotroph growth and activity was modulated by $CO_2$ availability (e.g. Barcelos e Ramos et al., 2007; Czerny et al., 2009; Eichner et al., 2014; Fu et al., 2008; Hutchins et al., 2013; Wannicke et al., 2012). Diazotrophic organisms typically have slower growth rates than other organisms. Hence any potential influence of ocean acidification on their physiology may take longer to become apparent in biogeochemical parameters sampled in larger-scale field studies. However to the best of our knowledge, there are no direct $N_2$-fixation rate measurements from $CO_2$-manipulation studies with *A. flos-aquae* in the field which could shed light on any underlying physiological response of this diazotroph and confirm laboratory findings in the field. Furthermore, high grazing pressure, hence top-down control, particularly after *t*17 (Lischka et al., in preparation) may have overridden any potential $CO_2$ effect of bottom-up control on diazotroph growth.

In addition to these highly visible filamentous $N_2$-fixers, there is growing evidence to support the role of heterotrophic and non-phototrophic $N_2$-fixation by smaller unicellular organisms in diverse ecosystems (Halm et al., 2012; Loescher et al., 2014; Moisander et al., 2010; Zehr et al., 2008) including in the Baltic Sea and Kattegat (Bentzon-Tilia et al., 2015; Farnelid et al., 2009), which cannot be quantified by common microscopic methods used in this experiment. Hence, while there appeared to be a good correlation between *A. flos-aquae* abundance and $N_2$-fixation rates until *t*21 in this study, we cannot rule out the contribution of heterotrophic organisms to the measured rates. However, regardless of the diazotroph community present, $N_2$-fixation rates were low and diazotrophy made only a small contribution (< 200 nmol $L^{-1}$) to the N cycle in this study. Thus we have no direct evidence from observations in this study that $N_2$-fixation or diazotroph abundance (Fig. 3.5) were significantly influenced by $CO_2$ nor that this could explain the observed higher particulate matter concentrations or lower phosphate concentrations in the higher $CO_2$ treatments (Paul et al., 2015) based on hypothesised relief of N-limitation.

In this area of the Baltic Sea, plankton communities, containing filamentous diazotrophic cyanobacteria, are exposed to large diurnal and seasonal changes in pH (Almén et al., 2014; Brutemark et al., 2011). In addition, filamentous cyanobacteria form characteristic surface aggregations, similar to the tufts and puffs formed by *Trichodesmium*. Inside these aggregations, microenvironments can create substantially different conditions compared to the surrounding water with large diurnal fluctuations in pH (7.4 vs 9.0) and $O_2$ concentrations (∼150 – 450 µmol $O_2$ $L^{-1}$) and thus also inorganic carbon availability (Ploug, 2008). Hence natural exposure to highly variable carbonate chemistry conditions may have also played a role in dampening any potential influence of ocean acidification in this plankton community.

### 3.4.2 Evidence from N pools of the importance of regenerative production and effects of $CO_2$

Productivity in this plankton community appeared to be dominated by regenerative production (sensu Dugdale and Goering 1967) under low nitrate availability during Phase I, as has been observed in summer plankton communities in the Baltic Sea (Kuparinen, 1987; Sahlsten and Sörensson, 1989; Tamminen, 1995). DON appeared to be a more important N source than N derived from $N_2$-fixation. Any relatively fresh and labile N-rich dissolved organic matter (DOM) present after the decline of the spring bloom was likely remineralised by the bacterial community. Here, simultaneous drawdown of DSi and DON between *t*-1 and *t*15 suggests that in particular diatoms, also persisting from the spring bloom, were beneficiaries of this organic N turnover.

Available $NH_4^+$ (∼100 nmol $L^{-1}$) could not have supported the DSi uptake (∼0.4 µmol $L^{-1}$) as the sole N source based on ∼1:1 molar Si:N requirement by diatoms, thus suggesting instead potential rapid resupply of $NH_4^+$ through remineralisation of organic N by the heterotrophic community particularly in Phase I and Phase II. Although there is no indication of a high level of $NH_4^+$ production above the variability in the data set, we presume this bioavailable $NH_4^+$ would have been very quickly assimilated into particulate N in the N-limited plankton community. This rate of N regeneration probably limited net phytoplankton growth such that significant phytoplankton biomass could not accumulate in the water column. Nevertheless, neither the readily available $NH_4^+$ nor the nitrate pool were fully exploited by the plankton assemblage with up to 50 nmol

$L^{-1}$ of nitrate and 170 nmol $L^{-1}$ of $NH_4^+$ remaining at the end of the study period on *t*43. In fact, nitrate concentrations continually increased throughout the experiment at an average net rate of 1 nmol $L^{-1}\,d^{-1}$ (Fig. 3.1 C) despite proportionally high phosphate availability. This suggests a small net imbalance in N cycle processes and may be connected to ammonium inhibition of nitrate uptake during spring-bloom decline and post-bloom period in the study area (Tamminen, 1995), leading to this small accumulation of nitrate in the water column.

No significant effect of $CO_2$ was detected on the DON pool, nor DSi drawdown, or PON or BSi cumulative sinking fluxes (see also Paul et al. 2015 in this special issue). Likewise, if there was any difference in uptake of N from the N-rich DOM pool (N:P ∼80:1) between $CO_2$ treatments, we could not detect the small signal (nmol $L^{-1}$) outside of the analytical precision (µmol $L^{-1}$) of the DON measurements. Thus this organic N drawdown via regenerative production in diatoms in this study appeared to be either unaffected or immeasurable by simulated ocean acidification.

# Summary

Plankton biomass build-up in this study was limited by low inorganic N availability therefore organic N pools were utilised supporting regenerative production during the more productive period in Phase I, with diatoms benefitting from this N turnover. Estimated $N_2$-fixation rates and abundances of the most dominant filamentous diazotroph, *A. flos-aquae* remained very low, therefore diazotrophs probably made only a minor contribution to overall N supply in this plankton community. Hence we did not observe relief of N limitation and stimulation of a summer plankton bloom by non-diazotrophic organisms. Indeed, dissolved inorganic nitrate present increased throughout the experiment indicating higher supply than consumption, despite a considerable phosphate excess present.

We detected no significant differences in N pool sizes between $CO_2$ treatments apart from the PON pool. However, the detected positive effect of $CO_2$ on PON standing stocks was minor (<3% difference in PON concentration). Thus N uptake rates were well balanced with supply or any net differences were too small to be detected in N pool sizes across the range of simulated ocean acidification scenarios. In addition, we found no conclusive evidence from our data until *t*21 ($N_2$-fixation rates, *A. flos-aquae* abundances, natural $\delta^{15}N$ abundances) that $CO_2$ had a measurable impact on N inputs via diazotrophy. The absence of any detectable effect may have been influenced by the low abundances of filamentous diazotrophic cyanobacteria in this study. However, the lack of response was consistent with other studies of diazotrophic organisms in natural plankton communities where resource competition with other plankton functional groups and top-down control may also play important roles in mediating the physiological response of $N_2$-fixing organisms.

Nonetheless, it appears that increased $CO_2$ may have slightly enhanced the ability of the N-limited plankton community in the Baltic Sea to exploit the low N sources available thereby potentially explaining lower phosphate concentrations, higher particulate matter concentrations and Chl *a* observed under higher $CO_2$ (Paul et al., 2015). However, we have no direct evidence of increased new N inputs via diazotrophy or changed N biogeochemistry within the first three

weeks and no conclusive indirect evidence from N pool sizes up to six weeks after $CO_2$ manipulation. Therefore we conclude that elevated $CO_2$ had no observable impact on the N cycle in this summer Baltic Sea plankton community.

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# References


Almén, A.-K., Vehmaa, A., Brutemark, A., and Engström-Öst, J.: Coping with climate change? Copepods experience drastic variations in their physicochemical environment on a diurnal basis, Journal of Experimental Marine Biology and Ecology, 460, 120–128, doi:10.1016/j.jembe.2014.07.001, 2014.

Badr, E.-S. A., Achterberg, E. P., Tappin, A. D., Hill, S. J., and Braungardt, C. B.: Determination of dissolved organic nitrogen in natural waters using high-temperature catalytic oxidation, TrAC Trends in Analytical Chemistry, 22, 819–827, doi:10.1016/S0165-9936(03)01202-0, 2003.

Barcelos e Ramos, J., Biswas, H., Schulz, K., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochemical Cycles, 21, GB2028, doi:10.1029/2006GB002898, 2007.

Barlow, R. G., Cummings, D. G., and Gibb, S. W.: Improved resolution of mono- and divinyl chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC, Marine Ecology Progress Series, 161, 303–307, doi:10.3354/meps161303, 1997.

Bentzon-Tilia, M., Traving, S., Mantikci, M., Knudsen-Leerbeck, H., Hansen, J., Markager, S., and Riemann, L.: Significant $N_2$ fixation by heterotrophs, photoheterotrophs and heterocystous cyanobacteria in two temperate estuaries, ISME Journal, 9, 273–285, doi:10.1038/ismej.2014.119, 2015.

Boxhammer, T., Bach, L. T., Czerny, J., and Riebesell, U.: Technical note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis, Biogeosciences Discussions, submitted.

Brutemark, A., Engström-Öst, J., and Vehmaa, A.: Long-term monitoring data reveal pH dynamics, trends and variability in the western Gulf of Finland, Oceanological and Hydrobiological Studies, 40, 91–94, doi:10.2478/s13545-011-0034-3, 2011.

Böttjer, D., Karl, D. M., Letelier, R. M., Viviani, D. A., and Church, M. J.: Experimental assessment of diazotroph responses to elevated seawater $pCO_2$ in the North Pacific Subtropical Gyre, Global Biogeochemical Cycles, 28, 601–616, doi:10.1002/2013GB004690, 2014.

Czerny, J., Ramos, J., and Riebesell, U.: Influence of elevated $CO_2$ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, Biogeosciences, 6, 1865–1875, doi:10.5194/bg-6-1865-2009, 2009.

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., and Granger, J.: The contamination of commercial $^{15}N_2$ gas stocks with $^{15}N$-labeled nitrate and ammonium and consequences for nitrogen fixation measurements, PLoS ONE, 9, e110 335, doi:10.1371/journal.pone.0110335, 2014.

Derenbach, J.: Zur Homogenisation des Phytoplanktons für die Chlorophyllbestimmung, Kieler Meeresforschungen, 25, 166–171, 1969.

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.

Dugdale, R. and Goering, J.: Uptake of new and regenerated forms of nitrogen in primary productivity, Limnology and Oceanography, 12, 196–206, doi:10.4319/lo.1967.12.2.0196, 1967.

Eichner, M., Rost, B., and Kranz, S. A.: Diversity of ocean acidification effects on marine $N_2$ fixers, Journal of Experimental Marine Biology and Ecology, 457, 199–207, doi:10.1016/j.jembe.2014.04.015, 2014.

Engström-Öst, J., Hogfors, H., El-Shehawy, R., De Stasio, B., Vehmaa, A., and Gorokhova, E.: Toxin-producing cyanobacterium *Nodularia spumigena*, potential competitors and grazers: testing mechanisms of reciprocal interactions, Aquatic Microbial Ecology, 62, 39–48, doi:10.3354/ame01456, 2011.

Farnelid, H., Oberg, T., and Riemann, L.: Identity and dynamics of putative $N_2$-fixing picoplankton in the Baltic Sea proper suggest complex patterns of regulation, Environmental Microbiology Reports, 1, 145–154, doi:10.1111/j.1758-2229.2009.00021.x, 2009.

Farnelid, H., Bentzon-Tilia, M., Andersson, A. F., Bertilsson, S., Jost, G., Labrenz, M., Jürgens, K., and Riemann, L.: Active nitrogen-fixing heterotrophic bacteria at and below the chemocline of the central Baltic Sea, The ISME Journal, 7, 1413–1423, doi:10.1038/ismej.2013.26, 2013.

Fu, F.-X., Mulholland, M., Garcia, N., Beck, A., Bernhardt, P., Warner, M., Sanudo-Wilhelmy, S., and Hutchins, D.: Interactions between changing $pCO_2$, $N_2$ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*, Limnology and Oceanography, 53, 2472–2484, doi:10.4319/lo.2008.53.6.2472, 2008.

Gustafsson, ., Gelting, J., Andersson, P., Larsson, U., and Roos, P.: An assessment of upper ocean carbon and nitrogen export fluxes on the boreal continental shelf: A 3-year study in the open Baltic Sea comparing sediment traps, $^{234}$Th proxy, nutrient, and oxygen budgets, Limnology and Oceanography: Methods, 11, 495–510, doi:10.4319/lom.2013.11.495, 2013.

Halm, H., Lam, P., Ferdelman, T. G., Lavik, G., Dittmar, T., LaRoche, J., D'Hondt, S., and Kuypers, M. M. M.: Heterotrophic organisms dominate nitrogen fixation in the South Pacific Gyre, The ISME journal, 6, 1238–1249, doi:10.1038/ismej.2011.182, 2012.

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

HELCOM: Manual for Marine Monitoring in the COMBINE Programme of HELCOM, Tech. rep., Helsinki Commission, Helsinki, URL `http://helcom.fi/Documents/Action%20areas/Monitoring%20and%20assessment/Manuals%20and%20Guidelines/Manual%20for%20Marine%20Monitoring%20in%20the%20COMBINE%20Programme%20of%20HELCOM.pdf`, last accessed: 20 June 2012, 2012.

Hogfors, H., Motwani, N. H., Hajdu, S., El-Shehawy, R., Holmborn, T., Vehmaa, A., Engström-Öst, J., Brutemark, A., and Gorokhova, E.: Bloom-forming cyanobacteria support copepod reproduction and development in the Baltic Sea, Plos One, 9, e112 692, doi:10.1371/journal.pone.0112692, 2014.

Hoppenrath, M., Elbrächter, M., and Drebes, G., eds.: Marine phytoplankton, Schweizerbart Science Publishers, Stuttgart, Germany, 2009.

Hutchins, D., Fu, F., Zhang, Y., Warner, M., Feng, Y., Portune, K., Bernhardt, P., and Mulholland, M.: $CO_2$ control of *Trichodesmium* $N_2$ fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry, Limnology and Oceanography, 52, 1293–1304, doi:10.4319/lo.2007.52.4.1293, 2007.

Hutchins, D. A., Fu, F.-X., Webb, E. A., Walworth, N., and Tagliabue, A.: Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations, Nature Geoscience, 16, 790–795, doi:10.1038/ngeo1858, 2013.

Kahru, M. and Elmgren, R.: Multidecadal time series of satellite-detected accumulations of cyanobacteria in the Baltic Sea, Biogeosciences, 11, 3619–3633, doi:10.5194/bg-11-3619-2014, wOS:000339265800011, 2014.

Kirkkala, T., Helminen, H., and Erkkilä, A.: Variability of nutrient limitation in the Archipelago Sea, SW Finland, Hydrobiologia, 363, 117–126, doi:10.1023/A:1003192831321, 1997.

Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J., Pavelson, J., and Nõmmann, S.: Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea, Limnology and Oceanography, 41, 98–112, doi:10.4319/lo.1996.41.1.0098, 1996.

Kraberg, A., Baumann, M., and Durselen, C.-D.: Coastal phytoplankton: Photo guide for northern European seas, Pfeil, Munich, Germany, 2010.

Kranz, S. A., Levitan, O., Richter, K.-U., Prasil, O., Berman-Frank, I., and Rost, B.: Combined effects of $CO_2$ and light on the $N_2$-fixing cyanobacterium *Trichodesmium* IMS101: Physiological responses, Plant Physiology, 154, 334–345, doi:10.1104/pp.110.159145, 2010.

Kérouel, R. and Aminot, A.: Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis, Marine Chemistry, 57, 265–275, doi:10.1016/S0304-4203(97)00040-6, 1997.

Kuparinen, J.: Production and respiration of overall plankton and ultraplankton communities at the entrance to the Gulf of Finland in the Baltic Sea, Marine Biology, 93, 591–607, doi:10.1007/BF00392797, 1987.

Laamanen, M. and Kuosa, H.: Annual variability of biomass and heterocysts of the $N_2$-fixing cyanobacterium *Aphanizomenon flos-aquae* in the Baltic Sea with reference to *Anabaena* spp. and *Nodularia spumigena*, Boreal Environment Research, 10, 19–30, 2005.

Law, C. S., Breitbarth, E., Hoffmann, L. J., McGraw, C. M., Langlois, R. J., LaRoche, J., Marriner, A., and Safi, K. A.: No stimulation of nitrogen fixation by non-filamentous diazotrophs under elevated $CO_2$ in the South Pacific, Global Change Biology, 18, 3004–3014, doi:10.1111/j.1365-2486.2012.02777.x, 2012.

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., Prasil, O., and Berman-Frank, I.: Elevated $CO_2$ enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*, Global Change Biology, 13, 531–538, doi:10.1111/j.1365-2486.2006.01314.x, 2007.

Lischka, S., Bach, L. T., Schulz, K. G., and Riebesell, U.: Micro- and mesozooplankton community response to increasing levels of $CO_2$ in the Baltic Sea: insights from a large-scale mesocosm experiment, Biogeosciences Discussions, in preparation.

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, The ISME Journal, 8, 2180–2192, doi: 10.1038/ismej.2014.71, 2014.

Menden-Deuer, S. and Lessard, E. J.: Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton, Limnology and Oceanography, 45, 569–579, doi:10.4319/lo.2000.45.3.0569, 2000.

Mohr, W., Grosskopf, T., Wallace, D., and LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rates, PLoS One, 5, e12 583, doi:10.1371/journal.pone.0012583, 2010.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular cyanobacterial distributions broaden the oceanic $N_2$ fixation domain, Science, 327, 1512 –1514, doi:10.1126/science.1185468, 2010.

Montoya, J. P., Voss, M., Kahler, P., and Capone, D. G.: A simple, high-precision, high-sensitivity tracer assay for $N_2$ fixation, Applied and Environmental Microbiology, 62, 986–993, 1996.

Moore, C. M., Mills, M. M., Langlois, R., Milne, A., Achterberg, E. P., La Roche, J., and Geider, R. J.: Relative influence of nitrogen and phosphorous availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean, Limnology and Oceanography, 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., Marañón, 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, Nature Geoscience, 6, 701–710, doi:10.1038/ngeo1765, 2013.

Nausch, M., Bach, L., Czerny, J., Goldstein, J., Grossart, H. P., Hellemann, D., Hornick, T., Achterberg, E., Schulz, K., and Riebesell, U.: Effect of $CO_2$ perturbation on phosphorus pool sizes and uptake in a mesocosm experiment during a low productive summer season in the northern Baltic Sea, Biogeosciences Discussions, 12, 17 543–17 593, doi:10.5194/bgd-12-17543-2015, 2015.

Ohlendieck, U., Stuhr, A., and Siegmund, H.: Nitrogen fixation by diazotrophic cyanobacteria in the Baltic Sea and transfer of the newly fixed nitrogen to picoplankton organisms, Journal of Marine Systems, 25, 213–219, doi:10.1016/S0924-7963(00)00016-6, 2000.

Ohlendieck, U., Gundersen, K., Meyerhoefer, M., Fritsche, P., Nachtigall, K., and Bergmann, B.: The significance of nitrogen fixation to new production during early summer in the Baltic Sea, Biogeosciences, 4, 63–73, doi:10.5194/bg-4-63-2007, 2007.

Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., and Niemkiewicz, E.: Biovolumes and size-classes of phytoplankton in the Baltic Sea, HELCOM Baltic Sea Environment Proceedings No. 106, p. 144, Helsinki Commision, Helsinki, Finland, 2006.

Patey, M. D., Rijkenberg, M. J. A., Statham, P. J., Stinchcombe, M. C., Achterberg, E. P., and Mowlem, M.: Determination of nitrate and phosphate in seawater at nanomolar concentrations, TrAC Trends in Analytical Chemistry, 27, 169–182, doi:10.1016/j.trac.2007.12.006, 2008.

Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community, Biogeosciences, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015.

Pliński, M. and Jóźwiak, T.: Temperature and N:P ratio as factors causing blooms of blue-green algae in the Gulf of Gdańsk, Oceanologia, 41, 73 – 80, 1999.

Ploug, H.: Cyanobacterial surface blooms formed by *Aphanizomenon* sp. and *Nodularia spumigen*a in the Baltic Sea: Small-scale fluxes, pH, and oxygen microenvironments, Limnology and Oceanography, 53, 914–921, doi:10.4319/lo.2008.53.3.0914, 2008.

Ploug, H., Musat, N., Adam, B., Moraru, C. L., Lavik, G., Vagner, T., Bergman, B., and Kuypers, M. M. M.: Carbon and nitrogen fluxes associated with the cyanobacterium *Aphanizomenon sp.* in the Baltic Sea, The ISME Journal, 4, 1215–1223, doi:10.1038/ismej.2010.53, 2010.

R Core Team: R: A language and environment for statistical computing, URL `http://www.r-project.org/`, 2014.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R., and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean change research, Biogeosciences, 10, 1835–1847, doi:10.5194/bg-10-1835-2013, 2013.

Sahlsten, E. and Sörensson, F.: Planktonic nitrogen transformations during a declining cyanobacteria bloom in the Baltic Sea, Journal of Plankton Research, 11, 1117 – 1128, doi:10.1093/plankt/11.6.1117, 1989.

Schluter, L., Garde, K., and Kaas, H.: Detection of the toxic cyanobacteria *Nodularia spumigena* by means of a 4-keto-myxoxanthophyll-like pigment in the Baltic Sea, Marine Ecology Progress Series, 275, 69–78, doi:10.3354/meps275069, 2004.

Shapiro, J.: The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes, Freshwater Biology, 37, 307–323, doi:10.1046/j.1365-2427.1997.00164.x, 1997.

Sharp, J.: Improved analysis for particulate organic carbon and nitrogen from seawater, Limnology and Oceanography, 19, 984–989, doi:10.4319/lo.1974.19.6.0984, 1974.

Shi, D., Kranz, S. A., Kim, J.-M., and Morel, F. M. M.: Ocean acidification slows nitrogen fixation and growth in the dominant diazotroph *Trichodesmium* under low-iron conditions, Proceedings of the National Academy of Sciences, 109, E3094–E3100, doi:10.1073/pnas.1216012109, 2012.

Stal, L. J., Albertano, P., Bergman, B., Bröckel, K. v., Gallon, J. R., Hayes, P. K., Sivonen, K., and Walsby, A. E.: BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea—responses to a changing environment, Continental Shelf Research, 23, 1695–1714, doi:10.1016/j.csr.2003.06.001, 2003.

Tamminen, T.: Nitrate and ammonium depletion rates and preferences during a Baltic spring bloom, Marine Ecology Progress Series, 120, 123–133, doi:10.3354/meps120123, 1995.

Tamminen, T. and Andersen, T.: Seasonal phytoplankton nutrient limitation patterns as revealed by bioassys over Baltic Sea gradients of salinity and eutrophication, Marine Ecology Progress Series, 340, 121 – 138, doi:10.3354/meps340121, 2007.

Tomas, C. R.: Identifying Marine Phytoplankton, Academic Press, San Diego, U.S.A/London, United Kingdom, 1997.

Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P., Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N., and Wulff, F.: Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea, AMBIO, 36, 186–194, doi:10.1579/0044-7447(2007)36[186:IEFENC]2.0.CO;2, 2007.

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of *Nodularia spumigena* to $pCO_2$ – Part I: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973–2988, doi:10.5194/bg-9-2973-2012, 2012.

Wannicke, N., Korth, F., Liskow, I., and Voss, M.: Incorporation of diazotrophic fixed $N_2$ by mesozooplankton — Case studies in the southern Baltic Sea, Journal of Marine Systems, 117–118, 1–13, doi:10.1016/j.jmarsys.2013.03.005, 2013.

Wasmund, N.: Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions, Internationale Revue Der Gesamten Hydrobiologie, 82, 169–184, doi:10.1002/iroh.19970820205, 1997.

Wasmund, N., Voss, M., and Lochte, K.: Evidence of nitrogen fixation by non-heterocystous cyanobacteria in the Baltic Sea and re-calculation of a budget of nitrogen fixation, Marine Ecology Progress Series, 214, 1–14, doi:10.3354/meps214001, 2001.

Yamamoto, Y.: Environmental factors that determine the occurrence and seasonal dynamics of *Aphanizomenon flos-aquae*, Journal of Limnology, 68, 122–132, doi:10.4081/jlimnol.2009.122, 2009.

Yamamoto, Y. and Nakahara, H.: The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length, Limnology, 6, 1–6, doi:10.1007/s10201-004-0138-1, 2005.

Zehr, J. P., Bench, S. R., Carter, B. J., Hewson, I., Niazi, F., Shi, T., Tripp, H. J., and Affourtit, J. P.: Globally distributed uncultivated oceanic $N_2$-fixing cyanobacteria lack oxygenic photosystem II, Science, 322, 1110 –1112, doi:10.1126/science.1165340, 2008.

Zhang, J.-Z. and Chi, J.: Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide, Environmental Science & Technology, 36, 1048–1053, doi:10.1021/es011094v, 2002.

# Tables and figures

**Table 3.1:** Summary of linear regression analyses of $fCO_2$ and nutrient stoichiometry, dissolved silicate drawdown, abundance of large (>20 µm) dominant diatom species present (*Chaetoceros* sp., *Skeletonema marinoi*), $N_2$-fixation rates and stable nitrogen isotope natural abundance. Numbers in bold indicate variable had a negative correlation with average $fCO_2$. Dashes indicate no regression was completed to avoid any bias in the conclusions because either no data or no complete data set is available. Degrees of freedom, n = 4.

| **Variable** | Phase | *p* | F-statistic | $R^2$ | **Variable** | Phase | *p* | F-statistic | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| **$N_2$-fixation rate** | I | 0.764 | 0.104 | 0.025 | ***Chaetoceros* sp. abundance** | I | 0.737 | 0.129 | 0.031 |
| | II | – | – | – | | II | – | – | – |
| | III | – | – | – | | III | 0.075 | 5.726 | 0.589 |
| **$\delta^{15}$N in suspended particulate matter** | I | 0.417 | 0.819 | 0.17 | ***Skeletonema marinoi* abundance** | I | 0.772 | 0.097 | 0.024 |
| | II | – | – | – | | II | – | – | – |
| | III | – | – | – | | III | – | – | – |
| **$\delta^{15}$N in sinking particulate matter** | I | 0.289 | 1.494 | 0.272 | **Excess phosphate (P*)** | I | 0.493 | 0.569 | 0.125 |
| | II | – | – | – | | II | 0.783 | 0.086 | 0.021 |
| | III | – | – | – | | III | **0.004** | **37.56** | **0.904** |
| **DSi drawdown** | I | 0.927 | 0.01 | 0.002 | **DIN:DIP (includes $NH_4^+$)** | I | 0.647 | 0.569 | 0.125 |
| | II | 0.52 | 0.496 | 0.11 | | II | 0.556 | 0.412 | 0.093 |
| | III | 0.966 | 0.001 | 0.002 | | III | 0.797 | 0.076 | 0.019 |

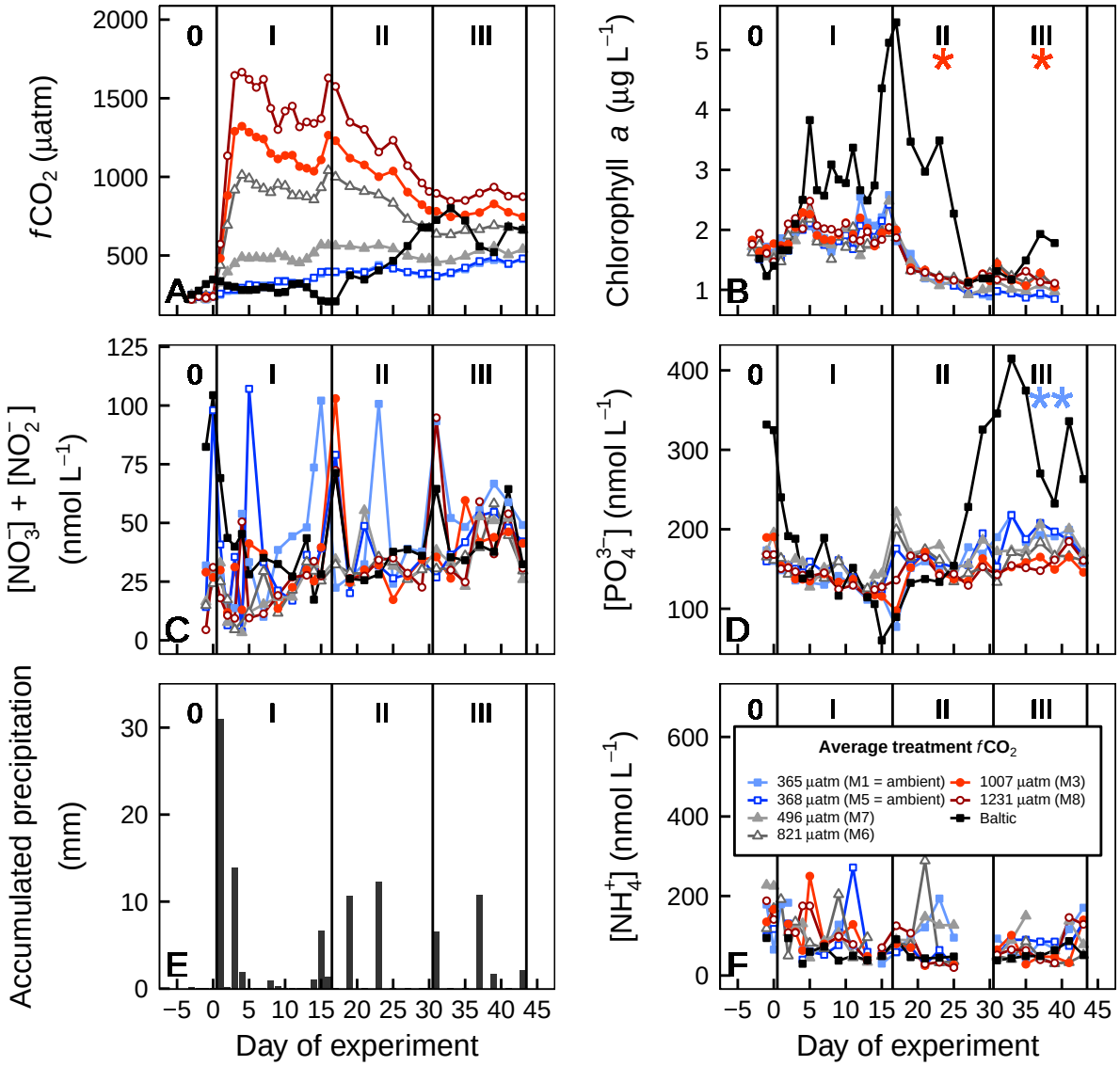


**Figure 3.1:** Temporal development in A) calculated $fCO_2$ using measured DIC and pHT, B) chlorophyll a concentrations, C) dissolved inorganic nitrate concentrations, D) dissolved inorganic phosphate concentrations over the study period, E) accumulated precipitation between sampling days recorded at the Hanko weather station (ID no.: GHCND:FIE00142025, latitude: 59.8439, longitude: 23.2517), and F) measured dissolved ammonium concentrations. Data for A – D, and F is from Paul et al. (2015) and for E from National Oceanographic Data Center, NOAA. * = $p < 0.05$, ** = $p < 0.01$ where red indicates positive and blue a negative detected effect of $fCO_2$. Legend indicates colours and symbols for each mesocosm. Average treatment $fCO_2$ was calculated for each mesocosm between $t1$ and $t43$.

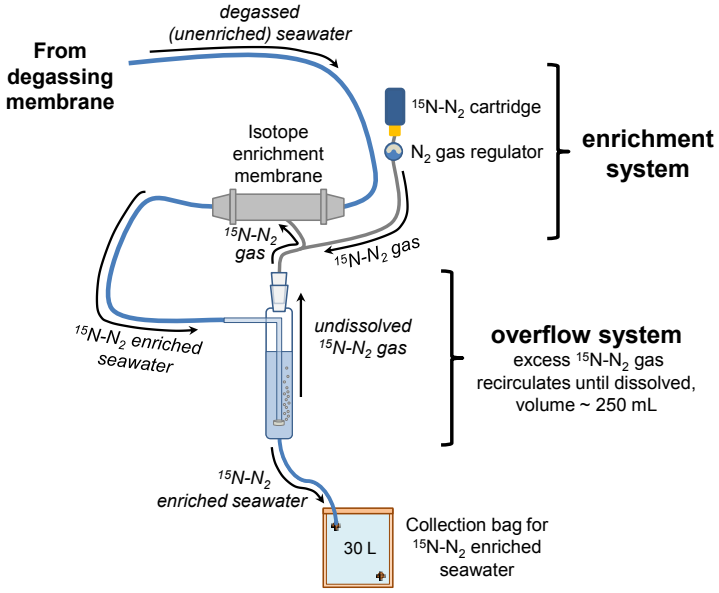


**Figure 3.2:** Diagram of set-up used for large-scale preparation of $^{15}$N-N$_2$-enriched seawater which was added to selected mesocosms.

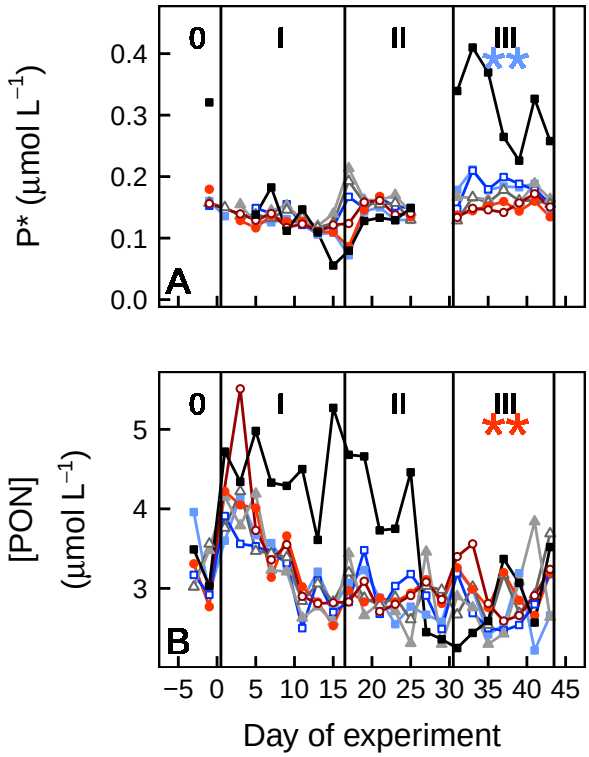


**Figure 3.3:** Temporal development in A) excess dissolved inorganic phosphate (P*) calculated according to Eqn. 3.1, and B) suspended particulate organic nitrogen (PON) concentration. Data and statistical significance is from Paul et al. (2015). Colours and symbols are the same as for Fig. 3.1. ** = $p < 0.01$ where red indicates positive and blue a negative detected effect of $fCO_2$.

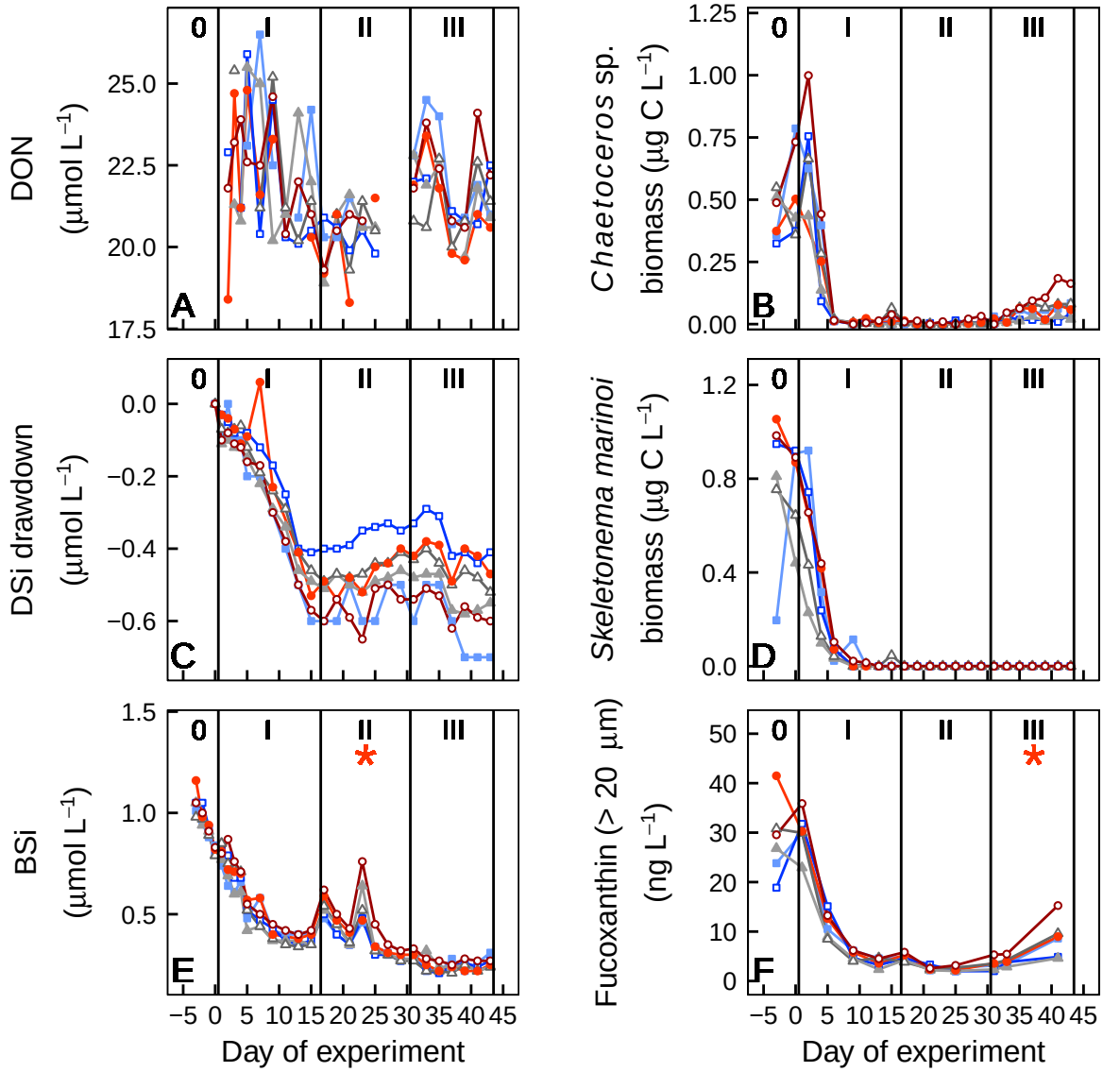


**Figure 3.4:** Temporal development in A) dissolve organic nitrogen concentrations (DON), C) dissolved silicate (DSi) drawdown and E) particulate biogenic silicate (BSi) concentrations (data from Paul et al. 2015), the abundances of the two dominant diatom species determined by microscopy (B, D) and F), Fucoxanthin marker pigment concentrations (>20 µm), a key pigment in diatoms. Colours and symbols are the same as for Fig. 3.1. Red asterisk denotes significant positive effect of $CO_2$ (* = $p < 0.05$).

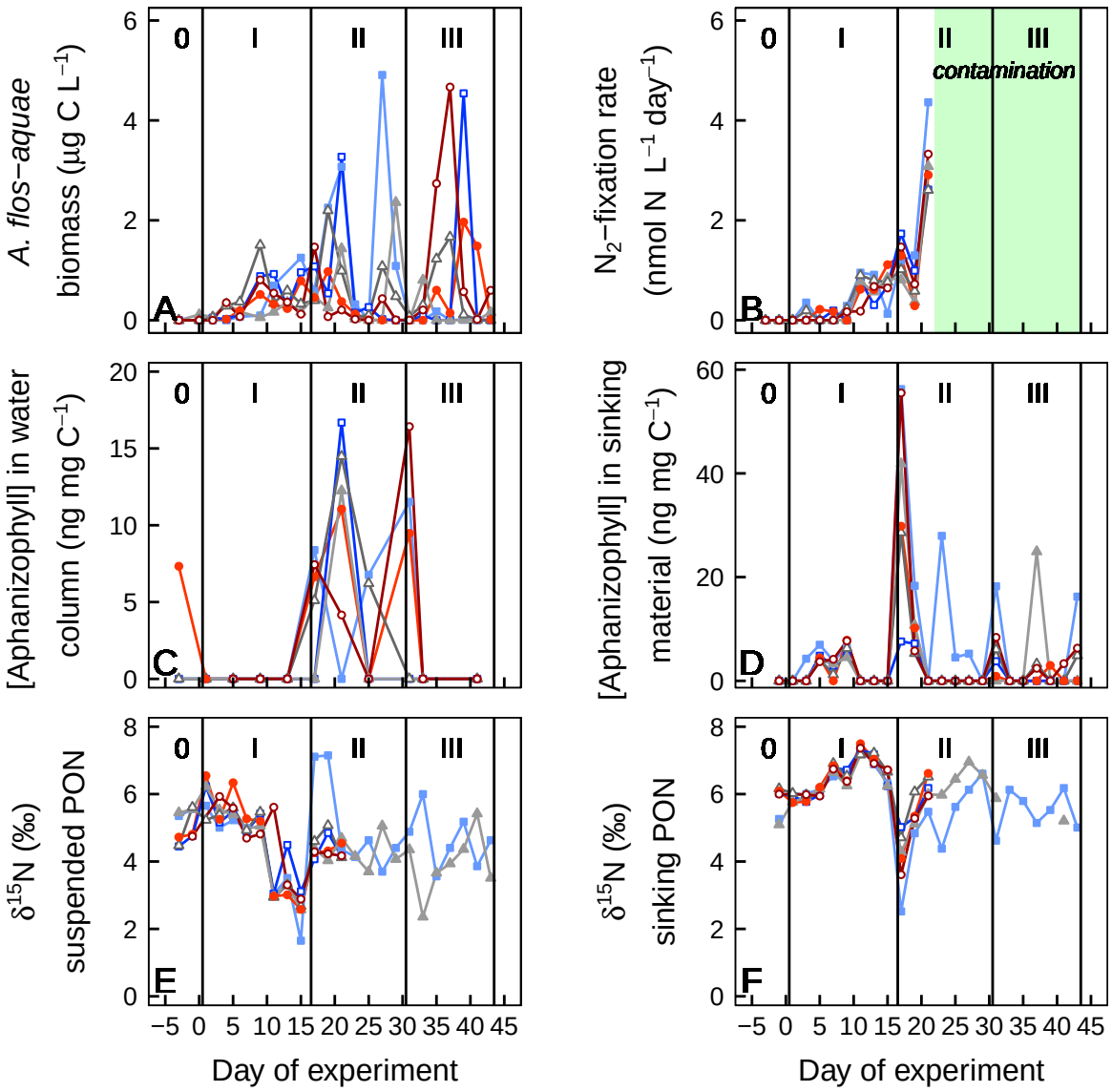


**Figure 3.5:** Variables indicating abundance and activity of filamentous diazotrophic cyanobacteria: A) biomass of *A. flos-aquae* calculated from microscopy abundance data, B) $N_2$-fixation rates determined by stable isotope incubations, C) carbon-normalised Aphanizophyll marker pigment concentration relative as a proxy for *A. flos-aquae* abundance in the water column and D) in the sediment trap material, E) natural abundance $\delta^{15}$N of particulate organic nitrogen (PON) in the water column and F) natural abundance $\delta^{15}$N in the sinking particle organic nitrogen collected in the sediment trap determined by analyses on an isotope ratio mass spectrometer. The green shaded area in B) between *t*23 and *t*43 indicates when contaminated $^{15}$N-$N_2$ gas was used in incubations (see Dabundo et al. 2014). Colours and symbols are as described in Fig. 3.1.

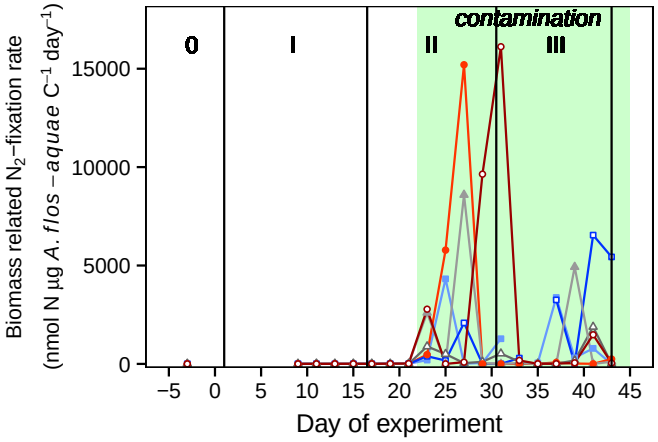


**Figure 3.6:** *A. flos-aquae* carbon-normalised $N_2$-fixation rates over the study period. Where data points are missing before $t9$, rates were either below detection limit (0.15 nmol N $L^{-1}$ $d^{-1}$) or did not coincide with sampling for phytoplankton abundance counts. Green shaded area between $t23$ and $t43$ indicates when contaminated $^{15}N$-$N_2$ gas was used in incubations (see Dabundo et al. 2014) and added to mesocosms. Colours and symbols are as described in Fig. 3.1.

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# Growth of key diazotrophic species negatively affected by ocean acidification and warming

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# Abstract

Nitrogen fixation is a key source of nitrogen in the Baltic Sea which counteracts nitrogen loss processes in the deep anoxic basins. Laboratory and field studies have indicated that single-strain $N_2$-fixing cyanobacteria from the Baltic Sea are sensitive to ocean acidification and warming, two drivers of marked future change in the marine environment. Here we enclosed a natural plankton community in twelve indoor mesocosms (volume ∼1400 L) and manipulated $pCO_2$ to yield six $CO_2$ treatments each two different temperature treatments (16.6°C and 22.4°C, $pCO_2$ range = 360 – 2030 µatm). We followed the filamentous diazotrophic cyanobacteria community (primarily *Nodularia spumigena*) over four weeks. Our results confirmed results from single-strain studies, and show that filamentous diazotrophic cyanobacteria may become less competitive in natural plankton communities under ocean acidification due to the negative effects of elevated $CO_2$ on their growth rate. This may be exacerbated by warming and have consequences for new nitrogen inputs and primary and secondary production in the Baltic Sea in future.

## 4.1 Introduction

The Baltic Sea is a semi-enclosed water body with under high anthropogenic stress due to the large and highly-populated catchment area (Sweitzer et al., 1996) and long water residence times of over 30 years due to low water exchange through the Danish Straits (Voipio, 1981). Eutrophication from increased nutrient loads from agricultural run-off has been of particular concern in the Baltic Sea over the past few decades. This has increased phytoplankton growth, thereby stimulating oxygen consumption and nitrogen (N) loss processes in the deep anoxic basins, and increasing the phosphate pool available for supporting bloom development of filamentous, diazotrophic ($N_2$-fixing), and potentially toxic cyanobacteria (Vahtera et al., 2007).

*Nodularia spumigena* is a filamentous diazotrophic cyanobacteria species found in the Baltic Sea which commonly blooms during the N-limited summer period. These extensive blooms are reported as a common occurrence since around 1960's (Finni et al., 2001) and form an important annual N source. *N. spumigena* and other diazotrophic cyanobacteria assimilate the residual phosphate present after the spring-bloom (Kononen et al., 1996) and fix atmospheric $N_2$, thereby supplying the wider plankton community with bioavailable N. This N input replenishes N pools which are lost through annamox and denitrification in the anoxic bottom waters (Vahtera et al., 2007) and supports between 20-45% of primary productivity in the region annually (Gustafsson et al., 2013).

The threat of climate change introduces new potential drivers of aquatic ecosystem change: increasing anthropogenic $CO_2$ emissions not only leads to warming of the atmosphere and the oceans, but also dissolves and reduces pH in the surface waters (Rhein et al., 2013). These changes are termed ocean warming and ocean acidification, respectively. Increases in summer surface temperature of 2-4$^{\circ}$C (Storch et al., 2015), concurrent with an average surface ocean pH decrease of up to 0.4 pH units (Schneider et al., 2015) are projected for the Baltic Sea by the year 2100. In some regions such as the Baltic Sea, short-term and seasonal fluctuations already markedly exceed projected changes (Thomsen et al., 2010). However these fluctuations may be exacerbated under ocean acidification (Omstedt et al., 2012). Diazotrophic organisms appear to be sensitive to ocean acidification (Barcelos e Ramos et al., 2007; Czerny et al., 2009; Eichner et al., 2014; Fu et al., 2008; Hutchins et al., 2007, 2013, 2015; Wannicke et al., 2012) and warming (Fu et al., 2014). To date there are only a couple of studies from the Pacific Ocean investigating the effects of elevated $CO_2$ on dizaotrophic organisms in a plankton community (Böttjer et al., 2014; Law et al., 2012) and the Baltic Sea remains even less studied.

In this mesocosm study, we wanted to stimulate a bloom of *N. spumigena* and observe the response of diazotroph growth and activity to future ocean acidification and warming scenarios in a natural plankton community to observe any potential changes in new N inputs. When using a natural community, important factors such as grazing and resource availability and competition will likely modulate bloom initiation and the response of *N. spumigena* to increasing $CO_2$ concentrations, in contrast to the physiological response observed in single-strain culture studies. This experimental set-up used shallow mesocosms ($\sim$1.5 m deep) with high light availability, similar to the conditions present in surface waters in the Baltic Sea during summer, where strong stratification, high light availability and high temperatures encourage development of extensive blooms of filamentous diazotrophic cyanobacteria. Based on laboratory experiments

using *N. spumigena* (Czerny et al., 2009; Eichner et al., 2014), we expected diazotroph activity and growth to be negatively affected by increased $CO_2$ concentrations under phosphate repletion but positively affected by increased temperature (Suikkanen et al., 2013), a controlling factor of diazotrophy in the Baltic Sea (Wasmund, 1997). Furthermore, we anticipated that $CO_2$- or temperature-related changes in diazotroph activity and growth will be visible in plankton community biomass due to potential relief of N-limitation in the plankton community.

## 4.2 Materials and methods

### 4.2.1 Experimental set-up and sampling

This mesocosm study took place using the indoor mesocosm facilities at GEOMAR Helmholtz Centre for Ocean Research Kiel between August $13^{th}$ and September $13^{th}$ 2013. In four temperature controlled rooms each with three mesocosms (volume ∼1400 L, surface area ∼ 1.54 $m^2$) we set-up a crossed experimental design with two temperature treatments (cold = 16.5°C, warm = 22.5°C) each containing a range of six target $pCO_2$ treatments spanning ambient (∼500 µatm) up to of 3000 µatm. The temperature in the warm and cold treatments was increased and decreased by 3°C, respectively, from ambient temperature to exclude the effect of any potential temperature shock.

The twelve mesocosms were filled simultaneously on August $13^{th}$ (*t*-3 = three days before first $CO_2$ enrichment) with unfiltered seawater from the Kiel Firth collected from approximately 2 m deep containing a natural summer plankton community from the western Baltic Sea including bacteria, phytoplankton and protozoa. In addition, 1.75 L of *Nodularia spumigena* culture (cell density = $4.131 \times 10^6$ cells $L^{-1}$) was added to each mesocosm on August $14^{th}$ (*t*-2) to encourage formation of a filamentous diazotrophic cyanobacteria bloom. Mesozooplankton (90% copepods, mixed species and stages) collected from the Kiel Bight were also added to the mesocosms on *t*-1 at a density of 20 individuals $L^{-1}$, simulating levels reported for this region in the summer season (Behrends, 1997) to replenish those lost during mesocosm filling. This addition was made as described in Garzke et al. (2015).

A light permeable, polyvinylchloride cover on each mesocosm maintained a headspace above the water surface to reduce outgassing of $CO_2$ during the experiment. In each mesocosm an electrical propeller gently mixed the water and ensured homogenous distribution of particulate matter and reduced sedimentation. The irradiance and period reflected natural conditions for this latitude and season as calculated by the astronomic model of Brock (1981). The five spotlights (100 W HIBay-LED lamps, lamp unit: HL3700 and Profilux II) were computer controlled (GHL Groß Hard- und Softwarelösungen, Kaiserslautern, Germany). The light:dark cycle of 14.05:9.95 h and included a sunrise/sundown simulation of approximately two hours long and an average maximum irradiance of 382.7 µmol photons $m^{-2} s^{-1}$ around noon. Irradiance was measured using a LICOR Li-250A light meter.

Regular sampling from all mesocosms took place every Monday, Wednesday and Friday between *t*-2 (Wednesday $14^{th}$ August 2013) and *t*28 (Friday $13^{th}$ September 2013) between 07:00 and 09:30 local time (LT). Samples for dissolved inorganic carbon (DIC) were taken directly from the centre of the mesocosms at approximately 0.5 m depth using flexible silicon tubing. Samples for total alkalinity (TA) were taken on the previous day to enable analyses in time for calculating required $CO_2$ enrichments on each sampling day. TA fluctuations over the 48-72 sampling periods were small and thus the difference in sampling timing had minimal influence on calculation of $CO_2$ system. Dissolved organic matter was also sampled directly from the mesocosms to minimise the risk of contamination. Water for all other variables (e.g. particulate matter, dissolved inorganic nutrients) was collected into plastic carboys for subsequent sub-sampling. Samples for analysis of nutrient concentrations in the water used for the $CO_2$-

enrichments were collected on the day of mesocosm filling as well as on the last day of $CO_2$ enrichment ($t26$).

For a more comprehensive description of the experimental set-up, mesocosm infrastructure and sampling procedures used, see Paul et al. (under revision).

### 4.2.2 Carbonate system manipulations

A range of $CO_2$ treatments were attained by the addition of $CO_2$-saturated seawater. Different amounts of $CO_2$ enriched seawater were added to each treatment to set up and maintain a regression of on average ∼420 – 1670 atm $pCO_2$ in the warm treatment and ∼365 – 1920 atm $pCO_2$ in the cold treatments throughout the study period. $CO_2$-enrichment started on $t$-2 and took place every sampling day after general sampling until $t10$ where $CO_2$-enriched seawater was added every day, apart from $t15$, $t16$ and $t23$, to maintain more stable $CO_2$ treatments. Seawater for the $CO_2$ enrichments for the whole experiment was collected at the beginning of the study when the mesocosms were being filled, sterile filtered (0.2 µm) and stored at 15°C. Water was bubbled with $CO_2$ gas for at least 6 hours on the day of addition.

### 4.2.3 Analytical procedures

#### Carbonate chemistry (TA, DIC)

Samples for total alkalinity (TA) and dissolved inorganic carbon concentrations (DIC) were taken directly from the centre of each mesocosm and gently pressure-filtered (Sarstedt Filtropur PES 0.2 µm) to exclude particulate material before analysis. DIC samples were collected as gas samples into 50 mL glass flasks (Schott Duran) with at least 100 mL of overflow. TA was analysed by potentiometric titration on an autosampler Metrohm 869 Sample Changer and a 907 Titrando Dosing unit according to the open cell method described in Dickson et al. (2007). DIC was analysed by infrared detection of $CO_2$ by a LICOR LI-7000 on an AIRICA system (MARIANDA, Kiel). Reported values were calculated as the mean of the three best out of four measurements with typical precision of 1.5 µmol $\mathrm{kg}^{-1}$. Certified reference material provided by Andrew Dickson (CRM 115, Scripps Institute for Oceanography of the University of California, San Diego) was used to correct for any drift during analyses for both TA and DIC between sampling days and within a run. Carbonate system parameters and $pCO_2$ were calculated using measured DIC and TA and the carbonic acid dissociation constants of Millero et al. (2006) in the CO2SYS program (Pierrot et al., 2011) taking into account measured ambient nutrient concentrations, temperature and salinity.

#### Dissolved inorganic and organic matter

Dissolved inorganic nitrate (DIN = $NO_3^-$ + $NO_2^-$), phosphate ($PO_4^{3-}$) and ammonium ($NH_4^+$) were filtered (cellulose acetate, 0.8 µm pore size, Sartorius Stedim) and frozen until analysis at -20°C. Concentrations were determined on an auto-analyser (Skalar, SANPLUS Breda/Netherlands) as described by Hansen and Koroleff (1999). The actual detection limit varied between sampling

days but was on average 0.57 µmol $L^{-1}$, 0.09 µmol $L^{-1}$ and 0.34 µmol $L^{-1}$ for DIN, $PO_4^{3-}$ and $NH_4^+$ respectively. Phosphate excess (P*, Deutsch et al. 2007) was calculated from dissolved inorganic phosphate, nitrate and ammonium concentrations according to:

$$P^* = [PO_4^{3-}] - \frac{[NO_3^-] + [NH_4^+]}{16} \tag{4.1}$$

For dissolved organic nitrogen and phosphorus (DON and DOP, respectively) analyses, 60mL of samples was filtered through pre-combusted GF/F filters (450°C, 6 hours) and collected in acid-rinsed, high density polyethylene (HDPE) bottles and stored at -20°C until analysis. Total dissolved nitrogen and phosphate were converted to inorganic nitrate and phosphate using an autoclave (20 mins) and an oxidising solution and concentrations were determined colorimetrically as described by Hansen and Koroleff (1999). DON concentrations were calculated from total dissolved nitrate by subtracting dissolved inorganic nitrate and ammonium concentrations. DOP concentrations were calculated from total dissolved phosphate by subtracting dissolved inorganic phosphate concentrations.

Both DOP concentrations and P* were analysed to detect the as indicators of diazotrophic activity (Deutsch et al., 2007; Deutsch and Weber, 2012). Filamentous diazotrophic cyanobacteria can also use DOP as a source of phosphorus (Unger et al., 2013). $N_2$-fixation also increases N while consuming P hence differences in DOP drawdown and P* between treatments may be attributed to differences in $N_2$-fixation.

**Particulate matter (C, N, P)**

Samples for particulate nitrogen analyses were collected on GF/F filters (Whatman, nominal pore size of 0.7 µm, diameter 25 mm) by filtration under reduced vacuum (< 200 mbar) between $t1$ and $t28$. Filtration volumes ranged between 100 and 250 mL to ensure sufficient biomass on the filters for analysis. Samples for analysis by mass spectrometry were stored at -20°C until analysis before drying overnight at 60°C and packing into tin capsules. Particulate nitrogen was converted to $N_2$ gas using the method of Sharp (1974), and the stable isotope ratio ($^{15}N/^{14}N$) was analysed on a Finnigan DeltaPlus isotope ratio mass spectrometer coupled by a Conflo II to an elemental analyser (EuroEA). In addition to the standard calibration at the beginning of each run, standard materials (caffeine, peptone, acetanilide) were also included within runs to identify any drift and ensure accuracy and full combustion of the samples during analysis. Isotope enrichment in particulate N ($\delta^{15}$N-PN) is reported in per mil (‰) compared to atmospheric $N_2$ (AIR). Due to analytical problems (S.D. > 0.2 ‰ in standard material $\delta^{15}$N) with the samples on $t1$ (biomass too low) and $t28$ (problems with calibration), these data points were excluded from analyses.

The isotopic composition of particulate N ($\delta^{15}$N-PN) can be used as an indicator of diazotroph activity (Carpenter et al., 1997). Atmospheric N fixed by diazotrophs is isotopically light (Delwiche and Steyn, 1970) thus can be distinguished from uptake of the more $^{15}$N-enriched nitrate (Montoya et al., 2002).

**Statistical analyses**

We tested the effects of temperature and measured $pCO_2$ on diazotroph abundance (Nostocales) and indicators of diazotroph activity ($\delta^{15}$N-PN, P*, DOP) by building non-linear mixed-effects models (NLME) using the R-package nlme (Pinheiro et al., 2015) and R software (R Core Team, 2014). The experiment was divided into a bloom and a post-bloom phase, as described in Paul et al. (under revision). An NLME was chosen for this particular analysis because the longitudinal data set included repeated measures, potentially non-constant correlation between observations and non-linear variable response (Lindstrom and Bates, 1990).

Diazotroph abundance was zero-skewed therefore data was log-transformed to satisfy the assumption of normality in the model residuals. We used $pCO_2$ and temperature as fixed effects to test Nostocales abundance in the first model ($pCO_2$ x temperature). We then tested the effect of diazotroph biomass, $CO_2$ and temperature on key biogeochemical indicators ($\delta^{15}$N-PN, P*, DOP) using the model Nostocales abundance x $pCO_2$ x temperature. In both models, mesocosm was included as a random effect. We simplified the models to exclude all non-significant terms ($p > 0.05$). Normality of residuals and heteroscedascity were inspected visually and satisfied model assumptions before performing an ANOVA to test significance of each variable. No collinearity was detected between $pCO_2$ and Nostocales abundance.

## 4.3 Results

### 4.3.1 Carbonate system and environmental variables

Temperature treatment levels of 22.4 ± 0.1°C (warm) and 16.6 ± 0.4°C (cold) were reached (mean ± S.D.) by $t2$ and remained within 0.4°C of these values until the end of the experiment on $t28$. A gradient of $CO_2$ treatments was present from $t3$ onwards with average $pCO_2$ ranging from 420 to 1760 µatm in the warm treatment, and from 360 to 2030 µatm in the cold treatment (Fig. 4.1). However there was a high level of variability within each treatment, particularly in the highest $pCO_2$ treatments primarily due to the high concentration difference to the atmospheric level and corresponding high level of outgassing in the shallow mesocosms. Daily additions of $CO_2$-enriched seawater appeared to improve stability within each treatment.

Salinity ranged between 15.2 and 15.3 (Paul et al., under revision), and TA remained relatively stable and ranged between 1950 and 1970 µmol $\mathrm{kg}^{-1}$. There was a small TA drawdown later in the study period during the post-bloom phase. This was slightly higher under lower $CO_2$ and higher temperature.

### 4.3.2 Filamentous diazotrophic cyanobacteria abundances and contribution to phytoplankton community biomass

*Nodularia spumigena* was the dominant filamentous diazotrophic cyanobacterium identified in all the mesocosm. Biomass increased with additions of *N. spumigena* culture to all mesocosms with an average biomass on $t0$ of 3.08 ± 0.50 and 3.07 ± 0.58 µg C $\mathrm{L}^{-1}$ (mean ± S.D.) in the warm and cold treatments respectively (Fig. 4.2). The next dominant species identified in the

Nostocales genus was *Anabaena* sp. which contributed on average to less than 15% of Nostocales biomass (*N. spumigena* = 1.4 ± 1.7 µg C $L^{-1}$, *Anabaena* sp. = 0.2 ± 0.5 µg C $L^{-1}$, mean ± S.D.), but up to 100% of biomass in some warm treatments during the post-bloom phase when *N. spumigena* was no longer present.

Total phytoplankton biomass declined during the experiment and remained low (89.7 ± 66.7 µg C $L^{-1}$, mean ± S.D.) and within the range of reported values for the Kiel Fjord (Wasmund and Siegel, 2008). There was a higher variability in starting phytoplankton community biomass on *t*-2 between mesocosms in the cold treatment (157.6 ± 49.5 µg C $L^{-1}$, mean ± S.D) than in the warmer treatment (168.3 ± 13.1 µg C $L^{-1}$, mean ± S.D). In general, Nostocales biomass declined over time, and was considerably lower than other studies with artificial blooms (∼460 µg C $L^{-1}$, Engström-Öst et al. 2002) contributing on average to less than 3% of total phytoplankton biomass throughout the study period. In some cold treatment mesocosms the contribution of Nostocales to phytoplankton community biomass increased after *t*20 to up to 23% (Fig. 4.2).

No significant direct effect of temperature was detected but there was a strong effect of $CO_2$ on Nostocales biomass in both the bloom and post-bloom periods and in both temperature treatments. A highly significant interactive effect between $CO_2$ and temperature was detected ($p < 0.0001$, Table 4.1). Hence biomass was generally highest under low $CO_2$ and low temperature and lowest under high $CO_2$ and high temperature (Fig. 4.2). Net growth of Nostocales after the *N. spumigena* culture addition on *t*0 was only discernible in the lowest $CO_2$ treatments.

### 4.3.3 Indicators of diazotrophic activity

While $N_2$-fixation rates were analysed as described by Mohr et al. (2010), contaminated $^{15}N$-$N_2$ gas was used (Dabundo et al., 2014) and thus the measured rates are considered unreliable for this study. Nevertheless, we analysed $\delta^{15}$N-PN, DOP and P* as indicators of diazotrophic activity as described in the following two sections.

#### Stable isotope abundances in particulate nitrogen

$\delta^{15}$N-PN decreased from 3.5 ± 1.2 ‰ on *t*3 to reach a minimum of -0.2 ± 1.2 ‰ on *t*10 before increasing again in the post-bloom period to reach 2.2 ± 1.7 ‰ on *t*26. There was a significant negative effect of temperature on $\delta^{15}$N-PN in both the bloom and post-bloom periods ($p = 0.0054$ and $p = 0.0008$, Table 4.2), but no significant effect of $CO_2$ concentration. In the post-bloom period, there was a significant interaction between $CO_2$ concentration and Nostocales abundance on $\delta^{15}$N-PN which suggested that there was a crossover effect, possibly with some carryover in the signal from the bloom period. This means that the negative relationship between $CO_2$ concentration and $\delta^{15}$N-PN, insignificant in the bloom phase, became significant in the post-bloom period and changed to a positive relationship as Nostocales abundances increased between $CO_2$ treatments ($p$ <0.0001, Table 4.2).

**Inorganic and organic nutrient concentrations**

Nutrient concentrations directly after mesocosms were filled on *t*-3 were 0.6, 1.83 and 0.66 µmol $L^{-1}$ for $NO_3^-$, $NH_4^+$, $PO_4^{3-}$ respectively. $NO_3^-$ and $NH_4^+$ concentrations in almost all samples were below the detection limits which were on average 0.6 and 0.34 respectively as is common for the summer season in the Kiel Fjord (Smetacek, 1985). There was an excess of inorganic phosphate in all mesocosms throughout the study period (Fig. 4.3, 0.33 ± 0.10 µmol $L^{-1}$, mean ± S.D.), compared to inorganic nitrogen (N) according to the Redfield ratio of 106C:16N:1P (Redfield, 1958). As $NO_3^-$ and $NH_4^+$ were below detection limits, they are reported as 0 µmol $L^{-1}$ and P* therefore was equivalent to $PO_4^{3-}$ concentration. During the bloom phase, Nostocales abundance, $pCO_2$ and temperature all had a significant negative effect on $PO_4^{3-}$ (Table 4.2), whereas in the post-bloom period only temperature still had a significant negative effect ($p < 0.0001$).

Through the regular additions of $CO_2$-enriched seawater to maintain more constant $pCO_2$ in the mesocosms, we also inadvertently added small amounts of inorganic nutrients ($NH_4^+$, $NO_3^-$ and $PO_4^{3-}$). However overall amounts added (0.017 µmol $L^{-1}$ $NO_3^-$, 0.024 µmol $L^{-1}$ $PO_4^{3-}$, 0.071 µmol $L^{-1}$ $NH_4^+$) were small and remained well below the ambient N pool size (e.g. DON ∼17 µmol $L^{-1}$, PON ∼ 2 µmol $L^{-1}$) in the mesocosms. We consider the minor nutrient input during $CO_2$ manipulations had no remarkable effect on Nostocales growth or relief of N-limitation of the phytoplankton present. Amounts of added $NH_4^+$ were not of a magnitude shown to affect *N. spumigena* growth or activity (Huber, 1986; Lehtimaki et al., 1997; Sanz-Alférez and Campo, 1994; Vintila and El-Shehawy, 2010).

DOP concentrations in the mesocosms were on average 0.32 ± 0.08 µmol $L^{-1}$ (mean ± S.D.) over the entire study period and there was no significant effect of time, Nostocales abundance, $pCO_2$ or temperature.

## 4.4 Discussion

### 4.4.1 Ocean acidification had a more significant effect than temperature on diazotroph growth

In this mesocosm study, rising $CO_2$ suppressed net growth of filamentous diazotrophic cyanobacteria of the order Nostocales, predominantly, *N. spumigena* and *Anabaena* sp., under a phosphate excess. Thus, this mesocosm study using a natural plankton community agrees with the results of two monoculture studies which indicated reduced growth rates in *N. spumigena* with rising $CO_2$ (Czerny et al., 2009; Eichner et al., 2014). However despite warm temperatures (>16°C), light and phosphate availability fitting a suggested requirements for diazotrophic growth (Wasmund, 1997), no large bloom of *N. spumigena* was observed. The small artificial *N. spumigena* bloom was sustained for longer in the lowest $CO_2$ treatments in both the warm and cold treatments indicating net growth (Fig. 4.2). In contrast, there was no clear period of net positive growth in all $CO_2$ treatments above 1000 µatm (average treatment $pCO_2$) in the warm treatment. The $CO_2$ treatment levels selected here (up to an average of 2000 µatm) spanned a much wider range than the widely-recognise projections by 2100 (up to ∼1000 µatm, Ciais et al. 2013) but are within the natural range of the Kiel Fjord which can reach up to 3000 µatm during late summer

(Thomsen et al., 2010). Nevertheless the clearest difference between treatments appeared early on, before $pCO_2$ was close to the target values, indicating a potential threshold for *N. spumigena* growth of under 1000 µatm. Similarly, the most significant changes in diazotroph activity and growth in a variety of taxa in culture were evident across a comparable $pCO_2$ range (Hutchins et al., 2013) suggesting that most critical changes in diazotroph physiology may occur within the range of realistic future average $pCO_2$ levels.

Rising temperature has been proposed as a key driver of the observed increased filamentous cyanobacteria presence in summer in the Baltic Sea (Suikkanen et al., 2013) with suggestions that future warming will continue this positive trend (HELCOM, 2013). Warmer temperatures have been shown to promote the development in slow-growing diazotrophs, enabling them to increase in abundance and effectively compete with other autotrophic organisms in plankton communities (Paerl, 2012), and stimulate blooms of *N. spumigena* in the field (Kononen et al., 1996; Pliński and Jóźwiak, 1999). However, we could not identify any direct relationship between temperature and Nostocales abundance, in either the bloom or post-bloom phases as the warm temperature treatment (22.4°C) was exactly in the reported optimum temperature range of 20 – 25°C for *N. spumigena* growth (Lehtimaki et al., 1994). We only observed a significant effect on the interaction between temperature and $CO_2$ concentrations. In the highest temperature treatment, the effect of increasing $CO_2$ was exacerbated during both the bloom and post-bloom phases. Higher temperature also induced a faster loss of Nostoscales biomass consistent with the idea that heterotrophic activity and biomass is more enhanced with warming than autotrophic activity or biomass as shown in food web studies (Biermann et al., 2014; O'Connor et al., 2009; Wohlers et al., 2009).

Furthermore, elevated $CO_2$ had a stronger effect than higher temperature on Nostocales biomass. This is contrary to the response of the wider plankton community in this experiment (Paul et al., under revision) as well as in numerous studies on the interactive effects of $CO_2$ and temperature in plankton communities (Coello-Camba et al., 2014; Hare et al., 2007; Kim et al., 2011; Maugendre et al., 2015; Sett, 2014; Sommer et al., 2015) and on growth parameters in single strain culture experiments in the laboratory (Fu et al., 2007) and structural equation modelling (Alsterberg et al., 2013). This may be due to the experimental design used in this study. Unlike many previous experiments, where $pCO_2$ has fluctuated with the plankton bloom dynamics (Engel et al., 2008; Schulz et al., 2013), daily additions of $CO_2$-enriched seawater were used to maintain $pCO_2$ levels as close to target values as possible. Thus, the maintenance of $CO_2$ treatments, and hence maintenance of treatment stress, may have contributed to the comparatively strong $CO_2$ response in diazotroph biomass in this study. However, we would be careful to extrapolate this lack of any individual temperature effect that we observed outside the framework of this particular experimental set-up, considering the substantial evidence and widespread agreement in the literature on the critical role of temperature on growth of filamentous diazotrophic cyanobacteria (Breitbarth et al., 2007; Paerl and Huisman, 2008; Staal et al., 2003) including in the Baltic Sea (Suikkanen et al., 2013; Wasmund, 1997). Selected temperature treatments (16.6°C and 22.4°C) may have also spanned the species and strain specific optimum temperature for Nostocales growth, as observed in other phytoplankton species (e.g. Zhang et al. 2014). Thus, the warm and cold treatments in this study may lie an equal distance above and below the ambient temperature for the species and strains present in this study.

### 4.4.2 Negative effect of ocean acidification on net diazotroph growth not reflected in indicators of diazotroph activity

$\delta^{15}$N-PN decreased during the period of highest Nostocales biomass ($t3 - t10$) in both temperature treatments, thereby indicating potential increased uptake of isotopically light diazotrophic N into plankton community biomass. Yet there is no direct evidence that $\delta^{15}$N-PN, a possible proxy for diazotroph activity, was at all affected by $CO_2$, despite the strong negative response to $CO_2$ observed in Nostocales biomass (Table 4.1, see discussion above).

In contrast to Nostocales abundances, it appears as though temperature had a stronger influence than $CO_2$ on $\delta^{15}$N-PN. Warmer temperature had a negative effect on $\delta^{15}$N-PN however this could not be attributed to higher diazotroph biomass (Table 4.2). Additional factors such as an high importance of regenerative production (Sigman et al., 2009) and differential nitrate isotope fractionation between phytoplankton species (Needoba et al., 2003) and N source concentration (Pennock et al., 1996; Waser et al., 1998) also complicate interpretation of this diazotrophic $\delta^{15}$N signature in $\delta^{15}$N-PN, particularly as exact inorganic N concentrations remain unknown (below detection limit). Indeed, higher zooplankton abundances (Paul et al., under revision), and presumably also zooplankton organic matter respiration in the warmer treatments fits well to this picture and could thereby account for the overriding negative temperature signal on $\delta^{15}$N-PN (Table 4.2). Hence increased regeneration of isotopically light inorganic nitrogen by zooplankton appears to better explain variation in $\delta^{15}$N-PN than diazotroph activity.

Other indicators of diazotroph activity, such as P*, are also contradictory. On the one hand, Nostocales abundance had a strongest negative effect on P*, fitting with the idea from Deutsch et al. (2007) that diazotrophs add N and use P thereby reducing P*. However P* was also strongly negatively correlated with both $CO_2$ and temperature (Table 4.2), which if true, would suggest higher $N_2$-fixation activity under higher $CO_2$ and higher temperature. This is in disagreement with the negative correlation between P* and both Nostocales biomass and $CO_2$ (Table 4.2). Considering the very low abundances, or even absence of $N_2$-fixing cyanobacteria in the highest $CO_2$ treatments, we believe that additional factors, not explicitly incorporated in the model used in the statistical analysis, were more important for determining P* than Nostocales abundance. Other phytoplankton present were in much higher abundance than Nostocales, thus would have had a much stronger influence on nutrient concentrations than the dominant diazotrophic organisms present. Further more, non-diaztrophic phytoplankton may have consumed relatively more P than that dictated by the Redfield ratio (Geider and La Roche, 2002). Hence, while diazotroph biomass was clearly affected by elevated $CO_2$, we have no clear indication of any temperature or $CO_2$ effect on diazotroph activity within the study time frame, based on our analysis of $\delta^{15}$N-PN and P*, as proxies of diazotrophic N supply.

### 4.4.3 Potential influence of diazotrophs on N supply to the Baltic Sea in future

The negative growth response of the dominant diazotrophic cyanobacterium *N. spumigena* to increasing $CO_2$ reported in physiological studies, verified here in a natural plankton community, strengthens projections of reduced filamentous diazotrophic cyanobacteria biomass under ocean

acidification. The direction of this negative physiological response was not modified through resource competition with other phytoplankton functional groups or by top-down grazing pressure. Nostocales did not dominate the plankton community in this study and generally contributed to less than 1% of total phytoplankton biomass (Fig. 4.2). This is considerably less than field studies reporting large surface aggregates contributing around 20 to 30% of plankton community biomass (Andersson et al., 2015; Stal et al., 1999), consequently their influence on biogeochemical pools was limited. However, if we assume that this negative $CO_2$ effect on biomass cascades through the food web over longer time periods, this may lead to a decrease in diazotroph N inputs, with consequences for productivity in the wider plankton community. Results from field and laboratory experiments suggest that zooplankton growth and development can be supported by the presence of filamentous diazotrophic cyanobacteria (Brutemark and Engström-Öst, 2013; Hogfors et al., 2014), particularly during bloom decay, despite their poor food quality and hepatoxicity (Karlson et al. 2015 and references therein). This study also supports the idea of this potential link indicating a negative correlation between both grazing pressure (Paul et al., under revision) and diazotroph biomass (this study) with increasing $CO_2$. Hence the potentially negative effect of ocean acidification on diazotroph biomass in future may also reduce secondary production and therefore also transfer of energy to higher trophic levels such as fish (Hansson et al., 2007). Nonetheless, the Baltic Sea sedimentary record indicates that ocean warming increases anoxia (Kabel et al., 2012) which in turns sustains cyanobacterial blooms through hypoxia-driven phosphate recycling (Funkey et al., 2014) although this may not result in increased $N_2$-fixation (Neumann et al., 2012). How this negative response of diazotrophs in the Baltic Sea to warming and acidification may be modulated by hypoxia and eutrophication remains an open question.

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# References


Alsterberg, C., Eklöf, J. S., Gamfeldt, L., Havenhand, J. N., and Sundbäck, K.: Consumers mediate the effects of experimental ocean acidification and warming on primary producers, Proceedings of the National Academy of Sciences, 110, 8603–8608, doi:10.1073/pnas.1303797110, 2013.

Andersson, A., Höglander, H., Karlsson, C., and Huseby, S.: Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea, Estuarine, Coastal and Shelf Science, 164, 161 – 171, doi:10.1016/j.ecss.2015.07.013, 2015.

Barcelos e Ramos, J., Biswas, H., Schulz, K., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochemical Cycles, 21, GB2028, doi:10.1029/2006GB002898, 2007.

Behrends, G.: Long-term investigation of seasonal mesozooplankton dynamics in Kiel Bight, Germany, Proceedings of the 13th Baltic Marine Biologists Symposium, pp. 93 –98, 1997.

Biermann, A., Engel, A., and Riebesell, U.: Changes in organic matter cycling in a plankton community exposed to warming under different light intensities, Journal of Plankton Research, p. fbu005, doi:10.1093/plankt/fbu005, URL `http://plankt.oxfordjournals.org/content/early/2014/02/09/plankt.fbu005`, 2014.

Breitbarth, E., Oschlies, A., and LaRoche, J.: Physiological constraints on the global distribution of *Trichodesmium* - Effect of temperature on diazotrophy, Biogeosciences, 4, 53–61, doi:10.5194/bg-4-53-2007, 2007.

Brock, T. D.: Calculating solar radiation for ecological studies, Ecological Modelling, 14, 1 – 19, doi:http://dx.doi.org/10.1016/0304-3800(81)90011-9, 1981.

Brutemark, A. and Engström-Öst, J.: Does the presence of zooplankton influence growth and toxin production of *Nodularia spumigena*?, International Review of Hydrobiology, 98, 225–234, doi:10.1002/iroh.201301633, 2013.

Böttjer, D., Karl, D. M., Letelier, R. M., Viviani, D. A., and Church, M. J.: Experimental assessment of diazotroph responses to elevated seawater $pCO_2$ in the North Pacific Subtropical Gyre, Global Biogeochemical Cycles, 28, 601–616, doi:10.1002/2013GB004690, 2014.

Carpenter, E. J., Harvey, H. R., Fry, B., and Capone, D. G.: Biogeochemical tracers of the marine cyanobacterium *Trichodesmium*, Deep Sea Research Part I: Oceanographic Research Papers, 44, 27 – 38, doi:10.1016/S0967-0637(96)00091-X, 1997.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., LeQuee C., Myneni, R., Piao, S., and Thornton, P.: Carbon and Other Biogeochemical Cycles, p. 465–570, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, doi:10.1017/CBO9781107415324.015, 2013.

Coello-Camba, A., Agustí, S., Holding, J., Arrieta, J. M., and Duarte, C. M.: Interactive effect of temperature and $CO_2$ increase in Arctic phytoplankton, Global Change and the Future Ocean, 1, 49, doi: 10.3389/fmars.2014.00049, 2014.

Czerny, J., Ramos, J., and Riebesell, U.: Influence of elevated $CO_2$ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, Biogeosciences, 6, 1865–1875, doi:10.5194/bg-6-1865-2009, 2009.

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., and Granger, J.: The contamination of commercial $^{15}N_2$ gas stocks with $^{15}N$-labeled nitrate and ammonium and consequences for nitrogen fixation measurements, PLoS ONE, 9, e110 335, doi:10.1371/journal.pone.0110335, 2014.

Delwiche, C. and Steyn, P.: Nitrogen isotope fractionation in soils and microbial reactions, Environmental Science & Technology, 4, 929–935, doi:10.1021/es60046a004, 1970.

Deutsch, C. and Weber, T.: Nutrient ratios as a tracer and driver of ocean biogeochemistry, Annual Review of Marine Science, 4, 113–141, doi:10.1146/annurev-marine-120709-142821, 2012.

Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., and Dunne, J. P.: Spatial coupling of nitrogen inputs and losses in the ocean, Nature, 445, 163–167, doi:10.1038/nature05392, 2007.

Dickson, A. G., Sabine, C. L., and Christian, J. R., eds.: Guide to best practices for ocean $CO_2$ measurements, PICES Special Publication 3, 2007.

Eichner, M., Rost, B., and Kranz, S. A.: Diversity of ocean acidification effects on marine $N_2$ fixers, Journal of Experimental Marine Biology and Ecology, 457, 199–207, doi:10.1016/j.jembe.2014.04.015, 2014.

Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R., Delille, B., and Schartau, M.: Effects of $CO_2$ on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), Biogeosciences, 5, 509–521, doi:10.5194/bg-5-509-2008, 2008.

Engström-Öst, J., Viitasalo, M., Jónasdóttir, S., Repka, S., Sivonen, K., Koski, M., and Schmidt, K.: Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*, Limnology and Oceanography, 47, 878–885, doi:10.4319/lo.2002.47.3.0878, 2002.

Finni, T., Kononen, K., Olsonen, R., and Wallström, K.: The History of Cyanobacterial Blooms in the Baltic Sea, AMBIO, 30, 172–178, doi:10.1579/0044-7447-30.4.172, 2001.

Fu, F.-X., Warner, M., Zhang, Y., Feng, Y., and Hutchins, D.: Effects of increased temperature and $CO_2$ on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria), Journal of Phycology, 43, 485–496, doi:10.1111/j.1529-8817.2007.00355.x, 2007.

Fu, F.-X., Mulholland, M., Garcia, N., Beck, A., Bernhardt, P., Warner, M., Sanudo-Wilhelmy, S., and Hutchins, D.: Interactions between changing $pCO_2$, $N_2$ fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*, Limnology and Oceanography, 53, 2472–2484, doi: 10.4319/lo.2008.53.6.2472, 2008.

Fu, F.-X., Yu, E., Garcia, N. S., Gale, J., Luo, Y., Webb, E. A., and Hutchins, D. A.: Differing responses of marine $N_2$-fixers to warming and consequences for future diazotroph community structure, Aquatic Microbial Ecology, 72, 33–46, doi:10.3354/ame01683, 2014.

Funkey, C. P., Conley, D. J., Reuss, N. S., Humborg, C., Jilbert, T., and Slomp, C. P.: Hypoxia sustains cyanobacteria blooms in the Baltic Sea, Environmental Science & Technology, 48, 2598–2602, doi: 10.1021/es404395a, 2014.

Garzke, J., Ismar, S. M. H., and Sommer, U.: Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance, Oecologia, 177, 849–860, doi:10.1007/ s00442-014-3130-4, 2015.

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

Gustafsson, ., Gelting, J., Andersson, P., Larsson, U., and Roos, P.: An assessment of upper ocean carbon and nitrogen export fluxes on the boreal continental shelf: A 3-year study in the open Baltic Sea comparing sediment traps, $^{234}$Th proxy, nutrient, and oxygen budgets, Limnology and Oceanography: Methods, 11, 495–510, doi:10.4319/lom.2013.11.495, 2013.

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

Hansson, S., Hjerne, O., Harvey, C., Kitchell, J. F., Cox, S. P., and Essington, T. E.: Managing Baltic Sea fisheries under contrasting production and predation regimes: Ecosystem model analyses, AMBIO: A Journal of the Human Environment, 36, 265–271, doi:10.1579/0044-7447(2007)36[265:MBSFUC]2. 0.CO;2, 2007.

Hare, C. E., Leblanc, K., DiTullio, G., Kudela, R. M., Zhang, Y., Lee, P. A., Riseman, S., and Hutchins, D. A.: Consequences of increased temperature and $CO_2$ for phytoplankton community structure in the Bering Sea, Marine Ecology Progress Series, 352, 9–16, doi:doi:10.3354/meps07182, 2007.

HELCOM: Climate change in the Baltic Sea Area: HELCOM thematic assessment in 2013, Tech. rep., Helsinki Commission, Helsinki, 2013.

Hogfors, H., Motwani, N. H., Hajdu, S., El-Shehawy, R., Holmborn, T., Vehmaa, A., Engström-Öst, J., Brutemark, A., and Gorokhova, E.: Bloom-forming cyanobacteria support copepod reproduction and development in the Baltic Sea, Plos One, 9, e112 692, doi:10.1371/journal.pone.0112692, 2014.

Huber, A.: Nitrogen-fixation by *Nodularia-Spumigena* Mertens (cyanobacteriaceae). 2. Laboratory Studies, Hydrobiologia, 133, 193–202, doi:10.1007/BF00005590, 1986.

Hutchins, D., Fu, F., Zhang, Y., Warner, M., Feng, Y., Portune, K., Bernhardt, P., and Mulholland, M.: $CO_2$ control of *Trichodesmium* $N_2$ fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry, Limnology and Oceanography, 52, 1293–1304, doi:10.4319/lo.2007.52.4.1293, 2007.

Hutchins, D. A., Fu, F.-X., Webb, E. A., Walworth, N., and Tagliabue, A.: Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations, Nature Geoscience, 16, 790–795, doi:10.1038/ngeo1858, 2013.

Hutchins, D. A., Walworth, N. G., Webb, E. A., Saito, M. A., Moran, D., McIlvin, M. R., Gale, J., and Fu, F.-X.: Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide, Nature Communications, 6, 8155, doi:10.1038/ncomms9155, 2015.

Kabel, K., Moros, M., Porsche, C., Neumann, T., Adolphi, F., Andersen, T. J., Siegel, H., Gerth, M., Leipe, T., Jansen, E., and Damste, J. S. S.: Impact of climate change on the Baltic Sea ecosystem over the past 1,000 years, Nature Climate Change, 2, 871–874, doi:10.1038/NCLIMATE1595, 2012.

Karlson, A. M. L., Duberg, J., Motwani, N. H., Hogfors, H., Klawonn, I., Ploug, H., Svedén, J. B., Garbaras, A., Sundelin, B., Hajdu, S., Larsson, U., Elmgren, R., and Gorokhova, E.: Nitrogen fixation by cyanobacteria stimulates production in Baltic food webs, AMBIO, 44, 413–426, doi: 10.1007/s13280-015-0660-x, 2015.

Kim, J.-M., Lee, K., Shin, K., Yang, E. J., Engel, A., Karl, D. M., and Kim, H.-C.: Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions, Geophysical Research Letters, 38, L08 612, doi:10.1029/2011GL047346, 2011.

Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J., Pavelson, J., and Nõmmann, S.: Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea, Limnology and Oceanography, 41, 98–112, doi:10.4319/lo.1996.41.1.0098, 1996.

Law, C. S., Breitbarth, E., Hoffmann, L. J., McGraw, C. M., Langlois, R. J., LaRoche, J., Marriner, A., and Safi, K. A.: No stimulation of nitrogen fixation by non-filamentous diazotrophs under elevated $CO_2$ in the South Pacific, Global Change Biology, 18, 3004–3014, doi:10.1111/j.1365-2486.2012.02777.x, 2012.

Lehtimaki, J., Sivonen, K., Luukkainen, R., and Niemela, S.: The effects of incubation-time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains, Archiv Fur Hydrobiologie, 130, 269–282, 1994.

Lehtimaki, J., Moisander, P., Sivonen, K., and Kononen, K.: Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria, Applied and Environmental Microbiology, 63, 1647–1656, 1997.

Lindstrom, M. L. and Bates, D. M.: Nonlinear mixed effects models for repeated measures data, Biometrics, 46, 673–687, doi:10.2307/2532087, 1990.

Maugendre, L., Gattuso, J.-P., Louis, J., Kluijver, A. d., Marro, S., Soetaert, K., and Gazeau, F.: Effect of ocean warming and acidification on a plankton community in the NW Mediterranean Sea, ICES Journal of Marine Science: Journal du Conseil, 72, 1744–1755, doi:10.1093/icesjms/fsu161, 2015.

Millero, F. J., Graham, T. B., Huang, F., Bustos-Serrano, H., and Pierrot, D.: Dissociation constants of carbonic acid in seawater as a function of salinity and temperature, Marine Chemistry, 100, 80–94, doi:10.1016/j.marchem.2005.12.001, 2006.

Mohr, W., Grosskopf, T., Wallace, D., and LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rates, PLoS One, 5, e12 583, doi:10.1371/journal.pone.0012583, 2010.

Montoya, J. P., Carpenter, E. J., and Capone, D. G.: Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic, Limnology and Oceanography, 47, 1617–1628, 2002.

Needoba, J. A., Waser, N. A., Harrison, P. J., and Calvert, S. E.: Nitrogen isotope fractionation in 12 species of marine phytoplankton during growth on nitrate, Marine Ecology Progress Series, 255, 81–91, doi:10.3354/meps255081, 2003.

Neumann, T., Eilola, K., Gustafsson, B., Muller-Karulis, B., Kuznetsov, I., Meier, H. E. M., and Savchuk, O. P.: Extremes of temperature, oxygen and blooms in the Baltic Sea in a changing climate, Ambio, 41, 574–585, doi:10.1007/s13280-012-0321-2, 2012.

O'Connor, M. I., Piehler, M. F., Leech, D. M., Anton, A., and Bruno, J. F.: Warming and resource availability shift food web structure and metabolism, PLoS Biol, 7, e1000 178, doi:10.1371/journal.pbio.1000178, URL `http://dx.doi.org/10.1371/journal.pbio.1000178`, 2009.

Omstedt, A., Edman, M., Claremar, B., Frodin, P., Gustafsson, E., Humborg, C., Hägg, H., Mörth, M., Rutgersson, A., Schurgers, G., Smith, B., Wällstedt, T., and Yurova, A.: Future changes in the Baltic Sea acid–base (pH) and oxygen balances, Tellus B, 64, 19 586, doi:10.3402/tellusb.v64i0.19586, 2012.

Paerl, H. W.: Marine Plankton, in: Ecology of Cyanobacteria II: Their Diversity in Space and Time, edited by Whitton, B. A., Springer Science & Business Media, Dordrecht, Heidelberg, New York, London, 2012.

Paerl, H. W. and Huisman, J.: Blooms like it hot, Science, 320, 57–58, doi:10.1126/science.1155398, 2008.

Paul, C., Sommer, U., Garzke, J., Moustaka-Gouni, M., Paul, A. J., and Matthiessen, B.: Effects of increased $CO_2$ concentrations on nutrient limited coastal summer plankton depend on temperature, under revision.

Pennock, J. R., Velinsky, D. J., Ludlam, J. M., Sharp, J. H., and Fogel, M. L.: Isotopic fractionation of ammonium and nitrate during uptake by *Skeletonema costatum*: Implications for delta $N^{15}$ dynamics under bloom conditions, Limnology and Oceanography, 41, 451–459, doi:10.4319/lo.1996.41.3.0451, 1996.

Pierrot, D., Wallace, D., and Lewis, E.: MS Excel Program Developed for $CO_2$ System Calculations, Tech. rep., URL `http://cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_XLS_v2.1/`, 2011.

Pinheiro, J., Bates, D., DebRoy, S., Sakar, D., and Team, R. C.: nlme: Linear and Nonlinear Mixed Effects Models, URL `http://CRAN.R-project.org/package=nlme`, 2015.

Pliński, M. and Jóźwiak, T.: Temperature and N:P ratio as factors causing blooms of blue-green algae in the Gulf of Gdańsk, Oceanologia, 41, 73 – 80, 1999.

R Core Team: R: A language and environment for statistical computing, URL `http://www.r-project.org/`, 2014.

Redfield, A. C.: The biological control of chemical factors in the environment, American Scientist, 46, 205–221, 1958.

Rhein, M., Rintoul, S. R., Aoki, S., Campos, E., Chambers, D., Feely, R. A., Gulev, S., Johnson, G. C., Josey, S. A., Kostianoy, A., Mauritzen, C., Roemmich, D., Talley, L. D., and Wang, F.: Observations: Ocean, in: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by Stocker, T. F., Qin, D., Plattner, G.-K., M. Tignor, Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., and Midgley, P. M., pp. 255 – 316, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013.

Sanz-Alférez, S. and Campo, F. F. d.: Relationship between nitrogen fixation and nitrate metabolism in the *Nodularia* strains M1 and M2, Planta, 194, 339–345, doi:10.1007/BF00197533, 1994.

Schneider, B., Eilola, K., Lukkari, K., Muller-Karulis, B., and Neumann, T.: Environmental Impacts—Marine Biogeochemistry, in: Second Assessment of Climate Change for the Baltic Sea Basin, edited by The BACC II Author Team, Regional Climate Studies, pp. 337–361, Springer International Publishing, Heidelberg, New York, Dordrecht, London, 2015.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences, 10, 161–180, doi: 10.5194/bg-10-161-2013, 2013.

Sett, S.: Interactive effects of ocean acidification and warming on marine phytoplankton - from physiology to biogeochemical cycling, PhD, Christian-Albrechts-Universität zu Kiel, Kiel, Germany, 97 p., 2014.

Sharp, J.: Improved analysis for particulate organic carbon and nitrogen from seawater, Limnology and Oceanography, 19, 984–989, doi:10.4319/lo.1974.19.6.0984, 1974.

Sigman, D. M., Karsh, K. L., and Casciotti, K. L.: Ocean process tracers: Nitrogen isotopes in the Ocean, in: Encyclopedia of Ocean Sciences, pp. 4138 – 4153, Academic Press, New York, 2009.

Sommer, U., Paul, C., and Moustaka-Gouni, M.: Warming and ocean acidification effects on phytoplankton—from species shifts to size shifts within species in a mesocosm experiment, PLoS ONE, 10, e0125 239, doi:10.1371/journal.pone.0125239, 2015.

Staal, M., Meysman, F. J. R., and Stal, L. J.: Temperature excludes $N_2$-fixing heterocystous cyanobacteria in the tropical oceans, Nature, 425, 504–507, doi:10.1038/nature01999, 2003.

Stal, L. J., Staal, M., and Villbrandt, M.: Nutrient control of cyanobacterial blooms in the Baltic Sea, Aquatic Microbial Ecology, 18, 165–173, doi:doi:10.3354/ame018165, 1999.

Storch, H. v., Omstedt, A., Pawlak, J., and Reckermann, M.: Introduction and Summary, in: Second Assessment of Climate Change for the Baltic Sea Basin, edited by The BACC II Author Team, Regional Climate Studies, pp. 1–22, Springer International Publishing, Heidelberg, New York, Dordrecht, London, 2015.

Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., and Brutemark, A.: Climate change and eutrophication induced shifts in northern summer plankton communities, PLoS ONE, 8, e66 475, doi:10.1371/journal.pone.0066475, 2013.

Sweitzer, J., Langaas, S., and Folke, C.: Land cover and population density in the Baltic Sea drainage basin: A GIS database, AMBIO, 25, 191–198, 1996.

Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M., and Melzner, F.: Calcifying invertebrates succeed in a naturally $CO_2$-rich coastal habitat but are threatened by high levels of future acidification, Biogeosciences, 7, 3879–3891, doi:10.5194/bg-7-3879-2010, 2010.

Unger, J., Endres, S., Wannicke, N., Engel, A., Voss, M., Nausch, G., and Nausch, M.: Response of Nodularia spumigena to pCO(2) - Part 3: Turnover of phosphorus compounds, Biogeosciences, 10, 1483–1499, doi:10.5194/bg-10-1483-2013, wOS:000317010600018, 2013.

Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P., Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N., and Wulff, F.: Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea, AMBIO, 36, 186–194, doi:10.1579/0044-7447(2007)36[186:IEFENC]2.0.CO;2, 2007.

Vintila, S. and El-Shehawy, R.: Variability in the response of the cyanobacterium *Nodularia spumigena* to nitrogen supplementation, Journal of Environmental Monitoring, 12, 1885–1890, doi:10.1039/c002863k, 2010.

Voipio, A.: The Baltic Sea, Elsevier Scientific Publishing Company, Amsterdam, The Netherlands, 1981.

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of *Nodularia spumigena* to $pCO_2$ – Part I: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973–2988, doi:10.5194/bg-9-2973-2012, 2012.

Waser, N. A., Yin, K. D., Yu, Z. M., Tada, K., Harrison, P. J., Turpin, D. H., and Calvert, S. E.: Nitrogen isotope fractionation during nitrate, ammonium and urea uptake by marine diatoms and coccolithophores under various conditions of N availability, Marine Ecology Progress Series, 169, 29–41, doi:10.3354/meps169029, 1998.

Wasmund, N.: Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions, Internationale Revue Der Gesamten Hydrobiologie, 82, 169–184, doi:10.1002/iroh.19970820205, 1997.

Wasmund, N. and Siegel, H.: Phytoplankton, in: State and Evolution of the Baltic Sea, 1952–2005 : a detailed 50-year survey of meteorology and climate, physics, chemistry, biology and marine environment, edited by Feistel, R., Nausch, G., and Wasmund, N., pp. 441–481, John Wiley & Sons, Inc., Hoboken, New Jersey, U.S.A., 2008.

Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jürgens, K., Hoppe, H.-G., Sommer, U., and Riebesell, U.: Changes in biogenic carbon flow in response to sea surface warming, Proceedings of the National Academy of Sciences, 106, 7067–7072, doi:10.1073/pnas.0812743106, URL `http://www.pnas.org/content/106/17/7067`, 2009.

Zhang, Y., Klapper, R., Lohbeck, K. T., Bach, L. T., Schulz, K. G., Reusch, T. B. H., and Riebesell, U.: Between- and within-population variations in thermal reaction norms of the coccolithophore *Emiliania huxleyi*, Limnology and Oceanography, 59, 1570–1580, doi:10.4319/lo.2014.59.5.1570, 2014.

# Tables and figures

**Table 4.1:** Summary of detected significant fixed effects in mixed effects model analyses for diazotroph abundances (Nostocales sp.). Initial model tested ($pCO_2$ x temperature) was simplified to remove all insignificant fixed effects. Bloom and post-bloom indicate $t$-2 to $t$10 and $t$12 to $t$28 respectively with degrees of freedom (df) indicated in brackets. Nostocales sp. was log-transformed to satisfy assumption of normally-distributed residuals in the simplified model. Dashes (–) indicate fixed effect had no significant effect on variable.

| | **log(Nostocales sp. abundance)** | |
|---|---|---|
| ***Significant fixed effect*** | bloom (df = 46) | post-bloom (df = 82) |
| $pCO_2$ | 39.243 *** ↓ | 9.556 ** ↓ |
| temperature | – | – |
| $pCO_2$ x temperature | 23.517 *** ↓ | 57.044 *** ↓ |

**Table 4.2:** Summary of detected significant fixed effects in mixed effects model analyses of and indirect indicators of diazotroph activity ($\delta^{15}$N in particulate nitrogen ($\delta^{15}$N-PN) and dissolved inorganic phosphate concentration ($PO_4^{3-}$). Initial model tested (Nostocales sp. abundance x $pCO_2$ x temperature) was simplified to remove all insignificant fixed effects. Bloom and post-bloom indicate $t$-2 to $t$10 and $t$12 to $t$28 respectively with degrees of freedom (df) indicated in brackets. Dashes (–) indicate fixed effect had no significant effect on variable.

| | **$\delta^{15}$N-PN** | | **$PO_4^{3-}$** | |
|---|---|---|---|---|
| ***Significant fixed effect*** | bloom (df = 35) | post-bloom (df = 69) | bloom (df = 55) | post-bloom (df = 81) |
| Nostocales sp. abundance | – | – | 23.700 *** ↓ | – |
| $pCO_2$ | – | – | 20.890 *** ↓ | – |
| temperature | 8.806 ** ↓ | 12.241*** ↓ | 8.793 ** ↓ | 19.609 *** ↓ |
| Nostocales sp. abundance x temperature | – | 16.499 *** ↓ | – | 7.296 ** ↓ |
| $pCO_2$ x temperature | – | – | – | – |
| Nostocales sp. abundance x $pCO_2$ x temperature | – | – | – | – |

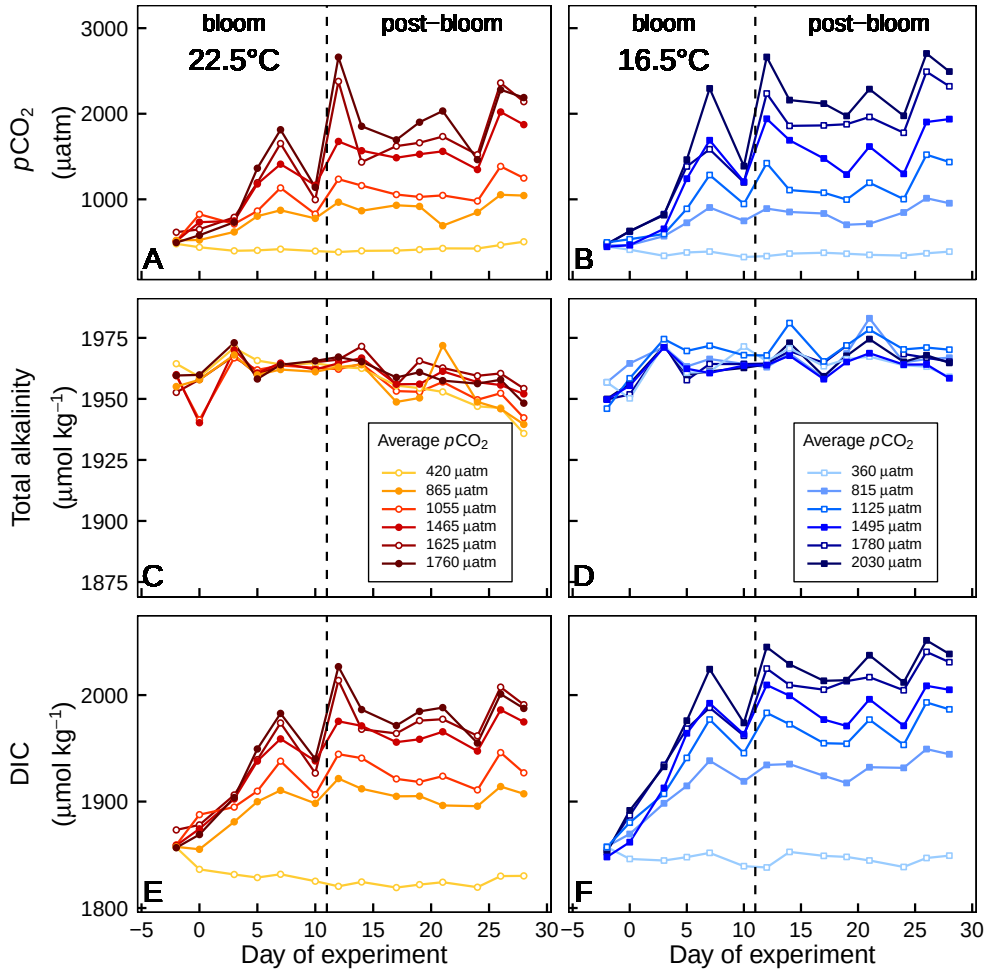


**Figure 4.1:** Carbonate chemistry variables during the study period: A) and B) calculated $pCO_2$ (µatm), C) and D) measured total alkalinity (µmol $kg^{-1}$), and E) and F) measured dissolved inorganic carbon (DIC, in µmol $kg^{-1}$), with the left column for the 16.5°C and the right column for the 22.5°C treatments respectively. Vertical line indicates division between bloom and post-bloom phases.

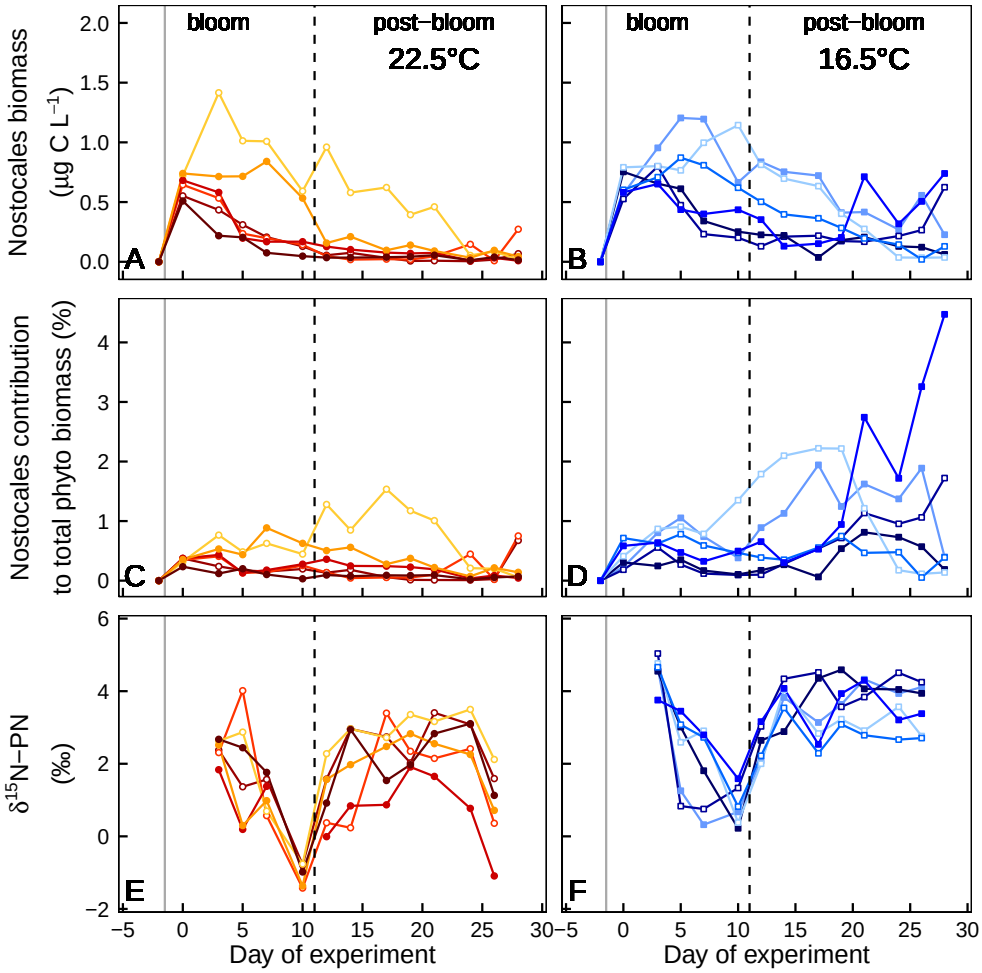


**Figure 4.2:** Development of A) and B) filamentous diazotrophic cyanobacteria biomass (order: Nostocales), calculated from microscopy counts of *Nodularia spumigena* and *Anabaena* sp. using reported biovolumes (Olenina et al., 2006) and cellular carbon content (Menden-Deuer and Lessard, 2000); C) and D) % contribution to phytoplankton community biomass; and E) and F) $\delta^{15}$N-PN (‰) is reported as potential indicator of diazotroph activity during the study period, with the left column for the 16.5°C and the right column for the 22.5°C treatments respectively. The grey vertical line indicates when the *N. spumigena* culture was added to the mesocosms. Colours and symbols are as described in Fig. 4.1.

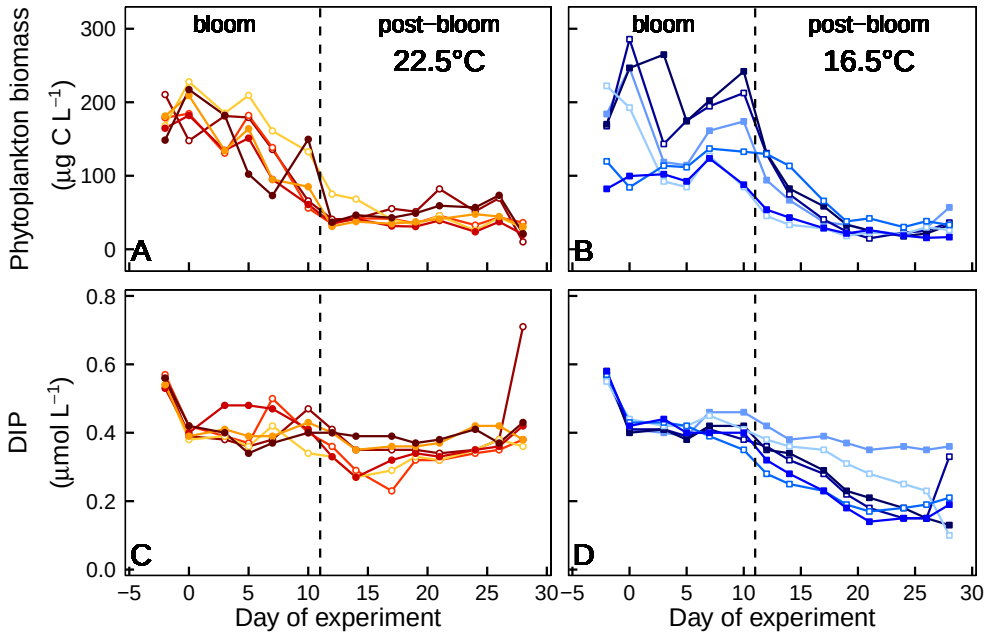


**Figure 4.3:** A) and B) total phytoplankton community biomass (µg C $L^{-1}$), calculated from microscopy phytoplankton abundances using reported biovolumes (Olenina et al., 2006) and cellular carbon content (Menden-Deuer and Lessard, 2000), and C) and D) dissolved inorganic phosphate (DIP in µmol $L^{-1}$) dynamics during the study period, with the left column for the 16.5°C and the right column for the 22.5°C treatments respectively. Colours and symbols are as described in Fig. 4.1.

# 5 | Synthesis

In this chapter, ideas and common themes arising from the combined knowledge gained in studies presented in Chapters 2 - 4 are discussed and amalgamated to contribute to a better understanding of the impacts of ocean acidification and warming on filamentous diazotrophic cyanobacteria and biogeochemical cycling in N-limited growing seasons and regions such as the Baltic Sea.

Based on this, I then draw attention to and suggest areas where future research efforts should be made. The considerable methodological issues which limit the scope of conclusions from this study are discussed including suggestions for future application of an ecosystem scale isotope tracer approach.

## 5.1 Potential consequences of ocean acidification and warming on filamentous diazotrophic cyanobacteria in the Baltic Sea

In Chapters 3 and 4, results were presented from two plankton community $CO_2$-manipulation studies with different filamentous diazotrophic cyanobacteria, *A. flos-aquae* and *N. spumigena* respectively. Experimental set-up in both mesocosm studies were designed to facilitate cyanobacteria bloom development by providing conditions fitting the niche for this group with excess phosphate, low inorganic N, high light intensities and warm temperatures. However in these two mesocosm studies, abundances of filamentous diazotrophic cyanobacteria remained well below levels characteristic of annual Baltic Sea blooms. While in Chapter 3, ambient seawater temperatures remained below the threshold for cyanobacteria bloom development (16°C, Wasmund, 1997) and likely contributed to the absence of a bloom, unidentified factors seem to have been more important to bloom formation than the phosphate excess and warm temperatures in Chapter 4. It is unlikely that either system was limited by iron availability as concentrations are not thought to be limiting in the Baltic Sea (Breitbarth et al., 2009) or in mesocosm studies where strict trace-metal clean procedures are not followed (L. Hoffmann, pers. comm.).

Despite low abundances, the results of two independent physiological studies on *N. spumigena* and ocean acidification (Eichner et al., 2014; Czerny et al., 2009) were confirmed in the indoor mesocosm experiment in Chapter 4. Hence, grazing pressure and resource competition did not override the direction of the negative physiological growth response to $CO_2$ observed in culture studies. On this basis, there is increased confidence that the presence of *N. spumigena* in the summer in the Baltic Sea may decrease under ocean acidification, with warming potentially enhancing this effect. There was no significant effect of temperature alone on *N. spumigena* abundances, contrary to current suggestions from the literature (Breitbarth et al., 2007; Hobson and Fallowfield, 2003; Karlberg and Wulff, 2013; Paerl and Huisman, 2008; Suikkanen et al., 2013; Yamamoto, 2009). Hence, selected $CO_2$ treatments had a stronger influence on diazotrophic cyanobacteria abundances than the selected temperature treatments in this study. The upper end of average treatment $pCO_2$ during the study period of 2030 µatm was much higher than typical $pCO_2$ during *N. spumigena* blooms during summer, but was within the range of locally observed $pCO_2$ in the Kiel Fjord in late summer and autumn (Thomsen et al., 2010) . Thus, the strong negative effect observed even below 1000 µatm in both this mesocosm study (Chapter 3) and culture studies (Czerny et al., 2009; Eichner et al., 2014) implies that the changes in *N.*

*spumigena* abundance in the Baltic Sea may occur within the relevant $CO_2$ range for projected ocean acidification of up to around 1000 µatm by 2100 (Collins et al., 2013).

Unfortunately, there is no conclusive data on $CO_2$- or temperature related differences in diazotroph activity as the more sensitive $N_2$-fixation rate measurements were unreliable due to contamination of the supplied gas (Dabundo et al., 2014, see also Chapters 3 and 4). It is therefore challenging to draw and justify strong conclusions on potential consequences on the Baltic Sea N cycle based on the available data sets. Nevertheless, it is reasonable to assume that lower abundances of *N. spumigena* the dominant filamentous diazotrophic cyanobacterium species in the open Baltic Sea, observed at higher $pCO_2$ in Chapter 4 would translate into to a decline in total diazotrophic N inputs in the Baltic Sea in the future, as it is unlikely that such dramatic loss in abundance could be compensated by any potential large increase in cell-normalised $N_2$-fixation rates. Considering the importance of $N_2$-fixation to the N cycle in the Baltic Sea, any decrease in $N_2$-fixation may have marked impacts on benthic and pelagic food webs (Karlson et al., 2015) and the oxygen inventory below the halocline (Vahtera et al., 2007). However, a decrease in abundance should not be viewed as an entirely negative consequence of ocean acidification and warming. *N. spumigena* is a toxic species of filamentous cyanobacteria, hence fewer toxic blooms may indeed be of economic benefit to regional tourism and the fishing industry.

## 5.2 Potential modification of seasonal diazotrophic niche under ocean acidification and warming

The response of an organism to an environmental variable generally has an optimum curve shape, indicating a particular range where growth (or any physiological process) is possible. This has been nicely shown for phytoplankton growth in response to temperature (Eppley, 1972) and more recently for calcification in coccolithophores in response to $CO_2$ (Bach et al., 2011; Sett et al., 2014). The benefit of increased substrate (carbon) availability with increased $pCO_2$, is balanced with the costs due to increased presence of an inhibitor (protons) through decreased pH. Under low pH, extra energy is required to maintain net calcification. The relative success of *Emiliania huxleyi* is then dependent on the ratio of cost to benefit: *E. huxleyi* is only competitive in this natural environment when this ratio lies above the threshold value ('Cost-Benefit Threshold', Bach et al. 2015). Hence, in a monoclonal culture study, the range in $CO_2$ where a species can survive, defines the fundamental niche for $CO_2$, whereas, outside monoclonal culture environments additional factors such as grazing pressure and resource availability may increase this cost-benefit threshold thereby reducing the range in $CO_2$ where the organism remains competitive. This is viewed as the realised niche, where an organism may be found in the environment (Hutchinson, 1957). As such, the formation of extensive blooms of filamentous diazotrophic cyanobacteria in the Baltic Sea is due to summer conditions satisfying their niche conditions with warm temperatures, high light and excess phosphate (see Section 1.2.2).

Using the observations from the indoor mesocosm experiment which manipulated both $CO_2$ concentrations and temperature (Chapter 4), combined with reported threshold on bloom initiating conditions and the physiological response to $pCO_2$ and temperature from the literature (Wasmund, 1997; Eichner et al., 2014; Czerny et al., 2009; Eppley, 1972), this same concept developed by Bach et al. (2015) was applied to growth in *N. spumigena* in response to $CO_2$ and

temperature (Fig. 5.1). In principle, this idea can be applied to any species or strain as this would simply change the thresholds and boundary conditions which need to be satisfied for the fundamental and realised niche.

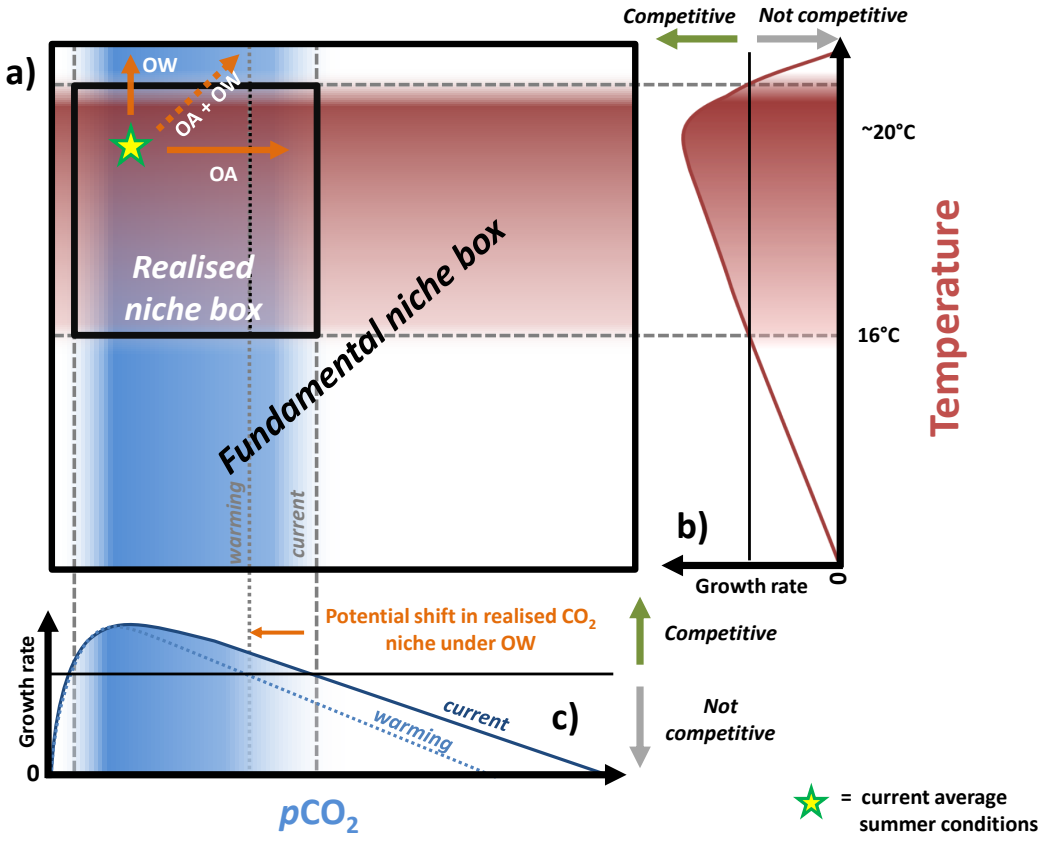


**Figure 5.1:** Conceptual figure showing how the current realised niche box for *N. spumigena* may be influenced by changes in $pCO_2$ (OA = ocean acidification) and temperature (OW = ocean warming) as well as how OA and OW may create conditions outside the realised niche (orange arrows). The fundamental niche box (a), is based on the physiological capabilities of *N. spumigena* to (b) temperature and (c) $pCO_2$ under two temperature scenarios. Results from studies in this doctoral dissertation and commonly cited observations from the literature (Wasmund, 1997) were used to define the response curves and the black horizontal lines defining the thresholds for competitiveness indicated here in (b) and (c). The smaller realised niche box indicates the conditions where *N. spumigena* may be found.

The large box (a) defines the fundamental niche for $CO_2$/temperature. That is, in the absence of grazing pressure and resource competition, such as in a monoclonal culture experiment, growth in *N. spumigena* can be expected (Fig. 5.1 (b) and (c)). Within the bounds of the 'realised niche box', presence and potential bloom formation can be expected in *N. spumigena* as it lies within the realised niche of $CO_2$ and temperature range of *N. spumigena*. Nevertheless it is important to recognise that the interaction of additional environmental variables with the $CO_2$ and temperature response curves would in turn modify the area of the realised niche. For example, in Chapter 4 the negative growth response in *N. spumigena* to $CO_2$ was exacerbated in the highest temperature treatment. Hence, shifts in environmental conditions ($CO_2$/pH and temperature) may not only push *N. spumigena* outside the range of the realised niche, but also reduce the width of the niche $CO_2$ conditions (Fig. 5.1 (c)), thereby reducing the overall area of

the realised niche under ocean acidification and warming.

This can be also viewed on a seasonal basis (Fig. 5.2). A projected temperature increase of up to 4°C (HELCOM, 2013) would mean that the threshold temperature for bloom development would be reached earlier in summer. Hence the period where bloom formation is possible is longer. In addition there is also a minimum in $pCO_2$ after strong drawdown during the spring bloom (Thomas and Schneider, 1999). However under projections of increased $CO_2$ of up to 1000 µatm (Ciais et al., 2013), this drawdown may not reach current levels of between 100 - 250 µatm in May/June (Thomas and Schneider, 1999). This would have the opposing effect to increased temperature and reduce the time window where cyanobacteria, with their efficient carbon concentrating mechanism (Price et al., 2008) are highly competitive and have potentially positive net growth (Chapter 4, Czerny et al. 2009, Eichner et al.2014) when sufficient excess $PO_4^{3-}$ is present. How the combination of these two factors will play out in determining the seasonal realised niche for diazotrophic filamentous cyanobacteria such as *N. spumigena* is yet to be investigated.

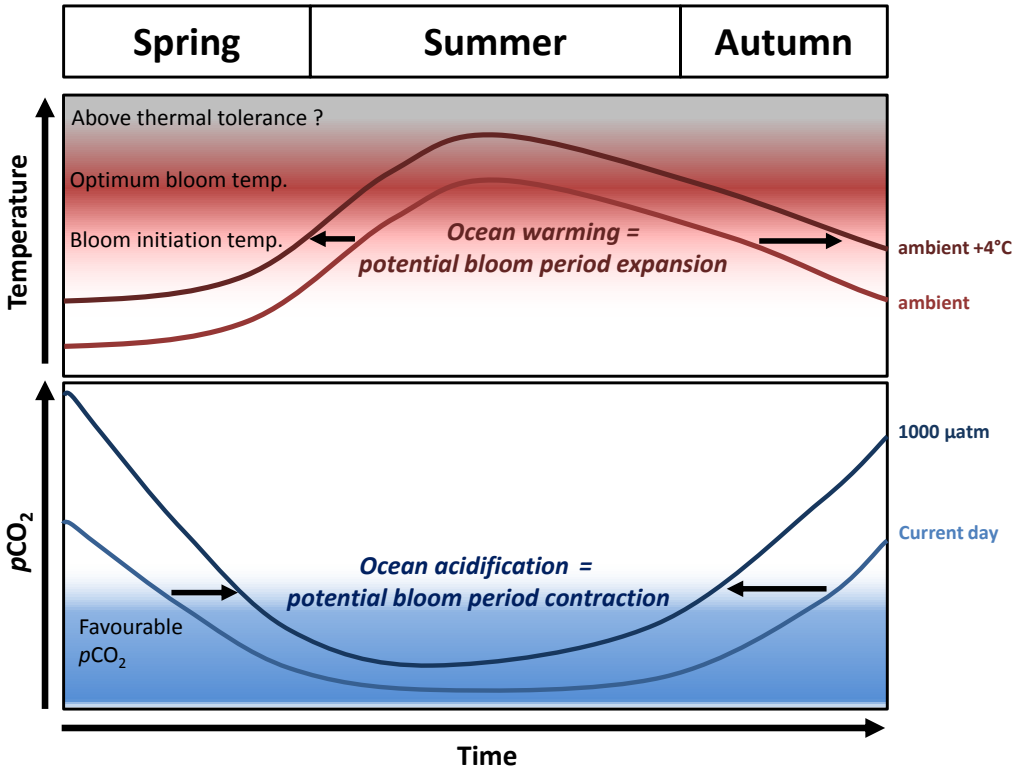


**Figure 5.2:** Schematic indicating variation in temperature (top panel) and $pCO_2$ (bottom panel) between spring and autumn in the Baltic Sea and how this may be expected to change in future. Key temperature and $pCO_2$ to fit the niche for *N. spumigena* are indicated in text in the figure. The arrows show how the temperature window for bloom formation in *N. spumigena* may expand in future (increase in presence of seasonal niche), whereas ocean acidification may have the opposite effect.

Neither migration and evolutionary adaptation are explicitly incorporated in either model above and thus are assumed to be constant. However these may also modify the realised niche area. Warming of the open ocean is suggested to lead to latitudinal shift in species distribution (Boyd et al., 2013; Thomas et al., 2012) as organisms may migrate to remain within their thermal

tolerance windows (Perry et al., 2005). If migration is not an option, organisms must then either adapt to the new and changing environmental conditions or face becoming uncompetitive within the plankton assemblage. However any potential retreat of Baltic Sea species northwards is limited geographically by the northern enclosed end of the Baltic Sea. In addition these organisms must also be able to cope or adapt to the decrease in salinity from south to north (Leppäranta and Myrberg, 2009) and the projected further decrease under climate change (HELCOM, 2013). An experimental evolution study with *Trichodesmium* indicates that there is potential for long-term changes in regulation of $N_2$-fixation due to increased $CO_2$ (Hutchins et al., 2015). Nevertheless, there is still uncertainty as to how the upregulation of this energetically demanding process may influence the ability of *Trichodesmium* to occupy it's current realised niche in future.

## 5.3 Variable response of diazotrophic cyanobacteria growth to ocean acidification

Results from the two mesocosm studies in this dissertation indicated variability in the response to the $CO_2$ gradient between these two Baltic Sea species of filamentous, heterocystous diazotrophic cyanobacteria. This was not expected based on physiological experiments, where both *N. spumigena* and *A. flos-aquae* responded negatively in terms of growth and/or activity in single-strain culture studies to increased $pCO_2$ and/or decreased pH under replete $PO_4^{3-}$ availability (Yamamoto and Nakahara, 2005; Czerny et al., 2009; Eichner et al., 2014).

The response to $CO_2$ was less clear for *A. flos-aquae* despite evidence of the sensitivity of bloom formation in this species to pH (Yamamoto and Nakahara, 2005). However, low abundances and natural patchiness in the distribution of filamentous cyanobacteria may have introduced noise into the data set during sub-sampling thereby masking any potential $CO_2$ effect. Furthermore, there were a number of differences in the experimental designs in the two studies such as artificial vs. natural light conditions, plankton community composition and resource availability, and temperature. One noteable difference between experimental set-ups is that $PO_4^{3-}$ concentrations were twice as high in the indoor mesocosm study (Chapter 4), compared to the study in the Finnish Archipelago Sea (Chapter 3). $PO_4^{3-}$ concentration has been hypothesised to modulate the magnitude or even direction of the $CO_2$-response of a filamentous diazotrophic cyanobacteria due to shifts in the allocation of energy between cellular processes (Eichner et al., 2014), as has been suggested for carbon assimilation and $N_2$-fixation in *Trichodesmium* under varied irradiance (Kranz et al., 2010). Hence a difference in allocation of energy between $PO_4^{3-}$ uptake and $N_2$-fixation in these diazotrophic species may have lead to the different growth response observed. Lower $PO_4^{3-}$ concentrations may have diminished the response of *A. flos-aquae* species in the Baltic Sea (Chapter 3), compared to *N. spumigena* (Chapter 4), thereby supporting Eichner et al. (2014).

A diverse response to $CO_2$ between taxa has been previously reported between filamentous and unicellular morphologies of tropical diazotrophic species (*Trichodesmium*, and *Crocosphaera* respectively, Hutchins et al. 2013) and between diazotrophs of a variety of morphologies and from environments of salinities/temperature (*Calothrix*, *Cyanothece*, *Nodularia*, Eichner et al. 2014). In the experiments in Chapter 4, a clear negative response in *N. spumigena* growth to increased $CO_2$ was observed which contradicts the expected response of diazotrophic

organisms derived from species such as *Trichodesmium* sp., where ocean acidification promotes cellular carbon and $N_2$-fixation (Barcelos e Ramos et al., 2007; Hutchins et al., 2007; Levitan et al., 2007; Kranz et al., 2010; Garcia et al., 2011; Hutchins et al., 2013, 2015).

While we have an increasing appreciation of the diverse response within autotrophic diazotrophic taxa, the question remains is whether $CO_2$ or pH (or both) is the driving factor(s) in the cyanobacterial response to ocean acidification. Indeed, the mechanism of potential pH/$CO_2$-sensitivity remains unknown. Cyanobacteria have an efficient carbon concentrating mechanism which saturates the RuBISCO (carboxylating enzyme for carbon fixation) with $CO_2$ even when ambient concentrations are low (Price et al., 2008). Hence, in general, cyanobacteria are thought to be less competitive in plankton communities under ocean acidification and high $CO_2$. Nonetheless this does not explain the range in direction of autotrophic diazotrophs to increased $CO_2$. Czerny et al. (2009) suggested that lower pH interferes with transport of charged amino acids between heterocysts and neighbouring vegetative cells, cellular structures which *Trichodesmium* and unicellular cyanobacteria do not have. The exact process of fixed N transfer is not known, however, this intercellular transfer probably occurs within the periplasm, a continuous space between the inner cytoplasmic membrane and the outer membrane of the bacterial cell wall (Flores et al., 2006; Montesinos et al., 1995), which conceivably may buffer changes in seawater pH to some degree.

## 5.4 Nutrient-poor regions and seasons as drivers of change

Biomass in both plankton communities were dominated by small phytoplankton (<2 µm, Chapter 2, C. Paul, pers. comm.), as is typical in regions and seasons where ambient inorganic nutrient concentrations are low. There were no clear bloom periods in either mesocosm study. Instead suspended particulate matter and Chl *a* concentrations declined, in part due to grazing. The estimated contribution of filamentous diazotrophic cyanobacteria in Chapters 3 and 4 to the overall N cycle and other biogeochemical element pools was minimal, primarily due to the low observed abundances. Yet there were significant detected $CO_2$-related differences in phytoplankton biomass, despite very low inorganic N availability which was presumed to restrict biomass development (Chapter 2). If it is assumed that $CO_2$ is a rate-limiting resource (Low-Décarie et al., 2014) and nutrient availability limited biomass yield (sensu Liebig), this would suggest that there was either undetectable but relevant differences in N input from diazotrophs, or that $CO_2$ was a co-limiting or even the proximate limiting nutrient for picoplankton. This supported sustained higher biomass of these organisms under higher $CO_2$ and low inorganic N concentrations.

However, there is no strong evidence from Chapter 2 to support the idea of $CO_2$ as a rate-limiting resource as $CO_2$-related difference in organic matter pools could be attributed to differences in respiration and bacterial activity, rather than primary production. Thus, it is difficult to disentangle any effects of $CO_2$ on net biomass production as nutrient supply via continuous consumption of organic matter and regeneration of inorganic N by zooplankton and bacteria in the water column capped biomass accumulation. Processes such as amino acid and sugar hydrolysis are enzymatically-degraded by bacteria therefore may directly affected by pH (Endres et al., 2014; Piontek et al., 2010) but not directly by $CO_2$. Evidence from other field studies for stim-

ulated production under ocean acidification is also inconclusive (Hein and Sand-Jensen, 1997; Egge et al., 2009; Engel et al., 2013; Kim et al., 2011; Lomas et al., 2012; Sala et al., 2015), thus giving weight to the idea that organic matter loss processes (respiration, organic matter remineralisation, zooplankton grazing e.g. Lewandowska et al. 2014) are more affected by changes in pH and temperature than production is by $CO_2$ concentrations under low N availability (Sala et al., 2015).

Additionally, in nutrient-poor regions, production is generally tightly coupled and organic nutrient regeneration supports supply along with $N_2$-fixation. Hence, looking at the bigger picture in terms of biogeochemical cycling can mean that key internal processes and turnover rates within the N cycle may have be overlooked such as in Chapters 3 and 4. It may take some time for small, $CO_2$-driven differences in organic matter turnover (e.g. Endres et al. 2014) to be above analytical detection limits in bulk biogeochemical pools. In Chapter 2, $CO_2$-related differences in particulate and dissolved matter pools were revealed three weeks after initial $CO_2$-manipulation, during a low productive phase when picoplankton were the dominant size class of Chl *a*-containing organisms. This appeared to be a positive and sustained effect detected over more than a two week period before the study ended with around 25% difference in particulate carbon and phosphorus pools sizes between the lowest and highest $CO_2$ treatments (Chapter 2). A similar positive response to increased $CO_2$ in Chl *a* was reported by Sala et al. (2015) under low nutrient concentrations with no response observed under high nutrient concentrations during the 9 day study period. Picoplankton biomass (Chl *a* as a biomass proxy), responded positively to increased $CO_2$ regardless of nutrient status.

Many studies on the biological effects of ocean acidification on a plankton community have ranged from 1.5 days to three weeks (see Appendix, Table 5.1). In others, phytoplankton blooms were induced via naturally and artificially available inorganic nutrients. Thus, a sustained, and initially underlying, response in tightly coupled plankton assemblages such as observed in Chapter 2 may have been missed in previous short-term incubations. Small and sustained changes in these tightly coupled ecosystems have the potential to shift foodweb structure and lead to long-term change in organic matter fluxes, as alluded to in Chapter 2. Evidence for permanent changes in regulation of physiological processes from long-term, high $CO_2$ exposure studies with select phytoplankton is mixed (Hutchins et al., 2015; Schaum and Collins, 2014). Hence, the question remains if higher picoplankton biomass and particulate and dissolved matter pools under elevated $CO_2$ is a sustained or transient response over even longer periods covering multiple generations and growing seasons.

## 5.5 Future research perspectives

> *The amount of nitrogen fixed biologically is difficult to estimate because of the heterogeneity of nitrogen-fixing organisms, the heterogeneity of their distribution, the heterogeneity of the environment in which they function, and our extremely limited but improving database.*
>
> Hardy and Havelka (1975)

This quote by Hardy and Havelka was made in reference to terrestrial $N_2$-fixation 40 years ago, nonetheless it is also an apt description of the difficulties and current stand of current aquatic $N_2$-fixation research: there is not only a paucity of data on the distribution and activity of diazotrophic organisms (visualised in Luo et al. 2012 and 2014), but also unresolved methodological issues, detection of diaztrophy in previously unconsidered environments and organism groups (Blais et al., 2012; Farnelid et al., 2013; Fernandez et al., 2011; Loescher et al., 2014) and an incoherent response of diazotrophs across well-recognised diazotrophic taxa (Eichner et al., 2014; Hutchins et al., 2013).

Current methods to directly estimate $N_2$-fixation rates are continuously improving but still do not incorporate many key pathways of newly-fixed N. This may mean an even greater underestimation of the importance of diazotrophic N in the global N budget than previously suggested (Großkopf et al., 2012). The picture is even less clear how cell morphology (also interactions with grazers and symbionts), $N_2$-fixing strategy (autotrophic, heterotrophic) may play in modulating diazotrophic N inputs may interact with environmental drivers such $CO_2$ and temperature. Detailed here are some experimental approaches, on a microscopic to plankton assemblage-scale which could be used to gain a better appreciation of the contribution of diazotrophic organisms within marine foodwebs and how this could be incorporated into studies of environmental change.

### Diazotroph-specific isotope tracer applicable from molecular level processes to ecosystem scale studies

Low N ecosystems, such as the Baltic Sea in the summer are typically reliant primarily on remineralised and/or diazotrophically-fixed N to support autotrophic growth: N fluxes are likely well-balanced and N pools remain stable. In these nutrient-poor plankton communities, elemental budgeting is particularly challenging as the absolute changes in biogeochemical pools can be so small during study periods (usually days to weeks) that it is not detectable above analytical detection limits. This was a considerable limitation in the studies in Chapters I and II where most rate measurements were hindered due to methodological problems. A solid estimation of $N_2$-fixation rates may have enabled the N elemental budget to be closed in the two low N plankton communities studied. Hence, it is difficult to determine if there was any underlying effect of increased $CO_2$ and/or temperature on diazotrophic N inputs.

Annual diazotrophic N inputs are regarded as very important to the Baltic Sea region and can be traced throughout the food web (Karlson et al., 2015) via natural abundance isotope

signals (Loick-Wilde et al., 2012; Rolff, 2000), and genetic material (Engström-Öst et al., 2011). However, the impact of diazotrophs on particulate and dissolved matter pools was minimal, if not undetectable in both plankton community studies presented in this dissertation (Chapter 2 and 4), possibly due to the low abundances (generally <1% of phytoplankton biomass, Chapter 4). In Chapter 4, $CO_2$- or temperature-related differences in filamentous cyanobacteria abundances did not appear to cascade through the food web or into particulate or dissolved N or P pools. A considerable proportion of the N fixed by filamentous diazotrophs is thought to be retained in the cell until cell death and degradation during bloom decay, a stage not included in the study period. Any immediate release and uptake of newly fixed N from *N. spumigena* (Wannicke et al., 2013) likely remained below analytical detection limits in dissolved and particulate N pools. Thus, diazotrophic N may be important on longer time-scales that were not covered in this study timeframe, is not incorporated in proportions or rates which could be distinguished by bulk biogeochemical pools, or plankton biomass assessments on this scale of experimentation when the abundances are low.

In light of the reported contaminated gas stocks, it is critical that the tracer is firstly checked for presence of isotopically labelled contaminants. If present, these contaminants will interfere with estimations of $N_2$-fixation, particularly when ambient nutrient concentrations are very low as described in Chapter 3. However, even if the problem of contamination of the $^{15}N$-$N_2$ gas is excluded, currently accepted methods may not accurately estimate gross diazotrophic N inputs. Despite a number of studies reporting direct release of $NH_4^+$ or DON by a variety of diazotrophic species, these $^{15}N$-$N_2$ assays only assess uptake into particulate N. Thus how much diazotrophic N is directly released as dissolved N remains an unknown but important component which may be largely missed in current estimates of diazotrophic nitrogen inputs. Is this the critical missing input in the global marine nitrogen budget? A better appreciation of the baseline in $N_2$-fixation on both a regional and global level is important for tracking future global change. This in itself is not a new idea, but is particularly important in light of potentially shifting baselines in biogeochemical elemental cycling in the aquatic realm.

Considering the high energetic investment that $N_2$-fixation requires, active DON liberation by diazotrophic organisms (Ploug et al., 2010; Wannicke et al., 2013; Mulholland et al., 2004; Glibert and Bronk, 1994) seems counterproductive for the $N_2$-fixers. However there is currently no mechanistic understanding of how this exudation occurs and any critical controls on this process. Direct release of bioavailable N, in particular $NH_4^+$ and DON, may have a direct impact on primary productivity or bacterial production and shape the plankton community (Berg et al., 2003), although this is still poorly understood (McDonald et al., 2010). Filamentous diazotrophic cyanobacteria reportedly liberate DON making the use of the $^{15}N$-$N_2$ tracer ideal for probing the molecular characteristics of freshly produced DON and ensuing microbial reworking of labile DON over time. It is conceivable that this may be a $CO_2$/pH- and temperature-sensitive process as suggested for DOC exudation by phytoplankton (Engel et al., 2013; Riebesell et al., 2007; Taucher et al., 2012). It would be interesting to take closer look at the isotope tracers used in relation to the reported rates of DON or $NH_4^+$ released is perhaps required to exclude any possible interference of $^{15}N$-labelled dissolved contaminants before any concrete conclusions are drawn on the magnitude of this process.

Furthermore, diverse heterotrophic bacteria with the ability to fix $N_2$ have been recently dis-

covered in the Baltic Sea (Farnelid et al., 2013), after these two studies took place. Although not explicitly studied in this thesis, estimated rates until *t*21 reported in Chapter 3 include $N_2$ fixed by all organisms larger than 0.7 µm, capturing some bacteria present. Indications from ecosystems where inorganic N availability is high (Fernandez et al., 2011) and oxygen minimum zones (OMZs) indicate $N_2$-fixation may be overlooked below the euphotic zone which may internally balance N-loss at the anoxic-oxic interface (Loescher et al., 2014). Temperature, pH and particulate and dissolved organic matter availability influence heterotrophic processes with increased organic matter degradation through changes in bacterial growth and enzyme efficiency with ocean acidification and warming (Endres et al., 2014; Piontek et al., 2009, 2010). Here, higher DOC concentrations were observed under elevated $CO_2$ during the steady state period in a N-starved plankton assemblage indicating potential for stimulated heterotrophic $N_2$-fixation under ocean acidification. In contrast to autotrophic $N_2$-fixation, heterotrophic $N_2$-fixation would act as a positive feedback on $CO_2$ concentrations via concurrent production of inorganic carbon with production of bioavailable N.

Assessment of heterotrophic $N_2$-fixation using common analyses in bulk seawater particulate matter contains numerous hurdles as it is challenging to accurately estimate only the heterotrophic contribution $N_2$-fixation rates, and not $N_2$ fixed by autotrophic diazotrophs who fix $N_2$ in the dark, such as *Crocosphaera watsonii* and *Cyanothece* (Berman-Frank et al., 2007; Mohr et al., 2010). No known cultures of these organisms exist and their physiological characterisation is poor. Hence how important they are for the N budget, or environmental controls on $N_2$-fixation in these organisms is still unknown. Widely used incubation methods must be applied carefully and in combination with genetic marker identification to accurately estimate the contribution of heterotrophic organisms to $N_2$-fixation in regions like OMZs. These organisms may not be trapped on the GF/F filters used to collect particulate matter for isotope abundance analysis. By analysing the isotope uptake into total N (dissolved and particulate components) would capture both the contribution of heterotrophic $N_2$-fixing organisms as well as release of DON and $NH_4^+$ in the bioassays. Complimentary to this would be the determination of cellular $N_2$-fixation rates using a dual-isotope labelling ($^{13}$C-glucose and $^{15}$N-$N_2$) and analysed by nano secondary ion mass spectrometry (nanoSIMS). Cells which contain both the $^{13}$C and $^{15}$N label indicate uptake organic C as well as fix $N_2$, thereby providing evidence of heterotrophic organisms fixing N.

The isotope tracer, $^{15}$N-$N_2$ gas, used frequently in bottle assays for $N_2$-fixation rate estimations can also be used to determine the fate of newly-fixed N in the food web in situ and at a much higher sensitivity than assessment of bulk biogeochemical standing stocks. The value of using this particular tracer lies in the high specificity for diazotrophic N and has potential to be a powerful tool when combined with ecosystem scale experimental infrastructure such as large-scale mesocosms. It enables quantification of not only net $N_2$-fixation rates, as in bottle assays (uptake into particulate N), but also comprehensive sampling of all relevant N pools (DON, DIN, particulate N, zooplankton, N sinking flux) in a closed system over extended periods of time. This facilitates estimation of gross $N_2$-fixation rates within an ecosystem and a clearer idea of how this newly-fixed N cascades through the food web and internal cycling within trophic levels.

Variable sensitivity within the diazotrophic community to increased temperature and $CO_2$ (Eichner et al., 2014; Fu et al., 2014) will likely change the competitiveness and hence dominance of particular diazotrophic species under ocean acidification and temperature. In addition,

there may also be differing responses between metabolic processes within an organism. For example, ocean acidification stimulated $N_2$-fixation in the symbiotic *Calothrix* but did not affect growth rates or organic matter production (Eichner et al., 2014). Furthermore, a recent study indicated that different diazotroph morphologies have different $N_2$-fixation and N-utilisation strategies with consequences on sinking particulate flux (Berthelot et al., 2015). Higher proportions of production were collected in the 15 m deep sediment trap when fuelled by UCYN-C $N_2$-fixation, than by diatom-diazotroph associations, even though there was no significant difference in the proportion of primary production supported by diazotrophic N (Berthelot et al., 2015). Thus, it is not only the amount of N which is fixed, but also the shape of the diazotroph assemblage which may affect the efficiency of the biological carbon pump. What proportion of diazotrophic N remains within the microbial food web compared to consumers such as zooplankton? Does diazotrophic N remain in the upper water column? How fast is it transferred into sinking fluxes? These are highly under-explored research avenues, including any potential changes in these processes under projected ocean acidification and warming which could be tackled using this diazotroph-specific tracer.

## Probing the underlying physiological mechanisms

The apparent interaction between $PO_4^{3-}$ availability, $PO_4^{3-}$ requirements and the response of diazotrophic organisms to ocean acidification has been proposed both in this doctoral dissertation and in a previous study (Eichner et al., 2014). This deserves further attention as it is particularly relevant for the Baltic Sea and other coastal areas, where nutrient run-off and upwelling of $PO_4^{3-}$-rich waters occurs. One possible parallel study site to probe the sensitivity of diverse diazotrophic organisms could be the North Atlantic and South Atlantic Oceans. These sub-tropical gyres have contrasting contributions of dissolved inorganic and organic species to the available phosphorus pool (Mather et al., 2008).

Although there is a growing body of literature which shows a range of sensitivity in $N_2$-fixing organisms to ocean acidification, the mechanism of pH/$CO_2$-sensitivity still remains unknown. Decoupled carbonate system studies with a variety of $N_2$-fixing species such as that on *E. huxleyi* (Bach et al., 2011), could disentangle the influence of increased $CO_2$ and decreased pH on C-fixation and $N_2$-fixation, thus improving our understanding of the physiological mechanisms.

## Importance of long-term monitoring data

Chapter 2 revealed the relatively long time it took for the impacts of elevated $CO_2$ to become apparent in bulk biogeochemical pools in a plankton assemblage under low inorganic N availability. While manipulation studies are useful to highlight potential for changes in biogeochemical cycling and plankton community structure, these need to be set against these natural observations. The complexity of entire ecosystem cannot be incorporated into any feasible experimental set-up. There are often limitations to how long even such large-scale infrastructure (> 1000 L) can be used. Sufficient replication for strong statistical analyses becomes increasingly difficult with increasing size of experiment enclosure for practical reasons.

As seen in a number of $CO_2$-manipulation studies (Sala et al., 2015; Brussaard et al., 2013;

Newbold et al., 2012), picoplankton were the key plankton size class driving the changes in community functioning, despite depleted inorganic N availability in some cases. Currently, phytoplankton counts under a microscope are commonly used to determine abundances in long-term monitoring programs in the Baltic Sea (Suikkanen et al., 2007, 2013). Hence these monitoring data sets may have overlooked crucial underlying shifts in community structure which may impact biogeochemical cycling and ecosystem function. One way to resolve this would be to include flow cytometry and phytoplankton pigment analyses in time-series sampling regimes to observe changes in lower community structure over time as has been done for the Hawaii Ocean Time-series (HOT) and Bermuda Atlantic Time-Series (BATS). Flow cytometry, in particular, is a simple and relatively quick and cheap technique to use.

By utilising satellite data on surface blooms of filamentous diazotrophic cyanobacteria in combination with large data bases on environmental variables (e.g. HELCOM) could be used to better evaluate changes in niche over time and provide further evidence to better constrain the niche model developed in Section 5.2. Introducing $N_2$-fixation assays into long-term monitoring programmes would be helpful to reduce variability in the data sets, particularly as these filamentous cyanobacteria are often patchy in their distribution.

# References


Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnology and Oceanography, 56, 2040–2050, doi:10.4319/lo.2011.56.6.2040, 2011.

Bach, L. T., Riebesell, U., Gutowska, M. A., Federwisch, L., and Schulz, K. G.: A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework, Progress in Oceanography, 135, 125 – 138, doi:http://dx.doi.org/10.1016/j.pocean.2015.04.012, 2015.

Barcelos e Ramos, J., Biswas, H., Schulz, K., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochemical Cycles, 21, GB2028, doi:10.1029/2006GB002898, 2007.

Berg, G. M., Balode, M., Purina, I., Bekere, S., Béchemin, C., and Maestrini, S. Y.: Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen, Aquatic Microbial Ecology, 30, 263–274, doi:doi:10.3354/ame030263, 2003.

Berman-Frank, I., Quigg, A., Finkel, Z. V., Irwin, A. J., and Haramaty, L.: Nitrogen-fixation strategies and Fe requirements in cyanobacteria, Limnology and Oceanography, 52, 2260–2269, doi:10.4319/lo.2007.52.5.2260, 2007.

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

Blais, M., Tremblay, J.-r., Jungblut, A. D., Gagnon, J., Martin, J., Thaler, M., and Lovejoy, C.: Nitrogen fixation and identification of potential diazotrophs in the Canadian Arctic, Global Biogeochemical Cycles, 26, GB3022, doi:10.1029/2011GB004096, 2012.

Boyd, P. W., Rynearson, T. A., Armstrong, E. A., Fu, F., Hayashi, K., Hu, Z., Hutchins, D. A., Kudela, R. M., Litchman, E., Mulholland, M. R., Passow, U., Strzepek, R. F., Whittaker, K. A., Yu, E., and Thomas, M. K.: Marine phytoplankton temperature versus growth responses from polar to tropical waters – Outcome of a scientific community-wide study, PLoS ONE, 8, e63 091, doi:10.1371/journal.pone.0063091, 2013.

Breitbarth, E., Oschlies, A., and LaRoche, J.: Physiological constraints on the global distribution of *Trichodesmium* - Effect of temperature on diazotrophy, Biogeosciences, 4, 53–61, doi:10.5194/bg-4-53-2007, 2007.

Breitbarth, E., Gelting, J., Walve, J., Hoffmann, L. J., Turner, D. R., Hassellöv, M., and Ingri, J.: Dissolved iron (II) in the Baltic Sea surface water and implications for cyanobacterial bloom development, Biogeosciences, 6, 2397–2420, doi:10.5194/bg-6-2397-2009, 2009.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated $CO_2$ levels, Biogeosciences, 10, 719–731, doi:10.5194/bg-10-719-2013, 2013.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., LeQuee C., Myneni, R., Piao, S., and Thornton, P.: Carbon and Other Biogeochemical Cycles, p. 465–570, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, doi:10.1017/CBO9781107415324.015, 2013.

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A., and Wehner, M.: Long-term Climate Change: Projections, Commitments and Irreversibility, p. 1029–1136, Cambridge University Press, Cambridge, United Kingdom, Cambridge, United Kingdom and New York, NY, USA, doi:10.1017/CBO9781107415324.024, 2013.

Czerny, J., Ramos, J., and Riebesell, U.: Influence of elevated $CO_2$ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, Biogeosciences, 6, 1865–1875, doi:10.5194/bg-6-1865-2009, 2009.

Dabundo, R., Lehmann, M. F., Treibergs, L., Tobias, C. R., Altabet, M. A., Moisander, P. H., and Granger, J.: The contamination of commercial $^{15}N_2$ gas stocks with $^{15}N$-labeled nitrate and ammonium and consequences for nitrogen fixation measurements, PLoS ONE, 9, e110 335, doi:10.1371/journal.pone.0110335, 2014.

Egge, J. K., Thingstad, T. F., Larsen, A., Engel, A., Wohlers, J., Bellerby, R. G. J., and Riebesell, U.: Primary production during nutrient-induced blooms at elevated $CO_2$ concentrations, Biogeosciences, 6, 877–885, doi:10.5194/bg-6-877-2009, 2009.

Eichner, M., Rost, B., and Kranz, S. A.: Diversity of ocean acidification effects on marine $N_2$ fixers, Journal of Experimental Marine Biology and Ecology, 457, 199–207, doi:10.1016/j.jembe.2014.04.015, 2014.

Endres, S., Galgani, L., Riebesell, U., Schulz, K.-G., and Engel, A.: Stimulated Bacterial Growth under Elevated $pCO_2$: Results from an Off-Shore Mesocosm Study, PLoS ONE, 9, e99 228, doi:10.1371/journal.pone.0099228, 2014.

Engel, A., Borchard, C., Piontek, J., Schulz, K. G., Riebesell, U., and Bellerby, R.: $CO_2$ increases $^{14}C$ primary production in an Arctic plankton community, Biogeosciences, 10, 1291–1308, doi:10.5194/bg-10-1291-2013, 2013.

Engström-Öst, J., Hogfors, H., El-Shehawy, R., De Stasio, B., Vehmaa, A., and Gorokhova, E.: Toxin-producing cyanobacterium *Nodularia spumigena*, potential competitors and grazers: testing mechanisms of reciprocal interactions, Aquatic Microbial Ecology, 62, 39–48, doi:10.3354/ame01456, 2011.

Eppley, R.: Temperature and phytoplankton growth in the sea, Fishery Bulletin, 70, 1063 – 1085, 1972.

Farnelid, H., Bentzon-Tilia, M., Andersson, A. F., Bertilsson, S., Jost, G., Labrenz, M., Jürgens, K., and Riemann, L.: Active nitrogen-fixing heterotrophic bacteria at and below the chemocline of the central Baltic Sea, The ISME Journal, 7, 1413–1423, doi:10.1038/ismej.2013.26, 2013.

Fernandez, C., Farias, L., and Ulloa, O.: Nitrogen fixation in denitrified marine waters, PLoS ONE, 6, e20 539, doi:10.1371/journal.pone.0020539, 2011.

Flores, E., Herrero, A., Wolk, C. P., and Maldener, I.: Is the periplasm continuous in filamentous multicellular cyanobacteria?, Trends in Microbiology, 14, 439 – 443, doi:http://dx.doi.org/10.1016/j.tim.2006.08.007, 2006.

Fu, F.-X., Yu, E., Garcia, N. S., Gale, J., Luo, Y., Webb, E. A., and Hutchins, D. A.: Differing responses of marine $N_2$-fixers to warming and consequences for future diazotroph community structure, Aquatic Microbial Ecology, 72, 33–46, doi:10.3354/ame01683, 2014.

Garcia, N. S., Fu, F.-X., Breene, C. L., Bernhardt, P. W., Mulholland, M. R., Sohm, J. A., and Hutchins, D. A.: Interactive effects of irradiance and $CO_2$ on $CO_2$ fixation and $N_2$ fixation in the diazotroph *Trichodesmium erythraeum* (cyanobacteria), Journal of Phycology, 47, 1292–1303, doi:10.1111/j.1529-8817.2011.01078.x, 2011.

Glibert, P. and Bronk, D.: Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium* spp., Applied and Environmental Microbiology, 60, 3996–4000, 1994.

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.

Hein, M. and Sand-Jensen, K.: $CO_2$ increases oceanic primary production, Nature, 388, 526–527, doi:10.1038/41457, 1997.

HELCOM: Climate change in the Baltic Sea Area: HELCOM thematic assessment in 2013, Tech. rep., Helsinki Commission, Helsinki, 2013.

Hobson, P. and Fallowfield, H. J.: Effect of irradiance, temperature and salinity on growth and toxin production by *Nodularia spumigena*, Hydrobiologia, 493, 7–15, doi:10.1023/A:1025447318487, 2003.

Hutchins, D., Fu, F., Zhang, Y., Warner, M., Feng, Y., Portune, K., Bernhardt, P., and Mulholland, M.: $CO_2$ control of *Trichodesmium* $N_2$ fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry, Limnology and Oceanography, 52, 1293–1304, doi:10.4319/lo.2007.52.4.1293, 2007.

Hutchins, D. A., Fu, F.-X., Webb, E. A., Walworth, N., and Tagliabue, A.: Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations, Nature Geoscience, 16, 790–795, doi:10.1038/ngeo1858, 2013.

Hutchins, D. A., Walworth, N. G., Webb, E. A., Saito, M. A., Moran, D., McIlvin, M. R., Gale, J., and Fu, F.-X.: Irreversibly increased nitrogen fixation in *Trichodesmium* experimentally adapted to elevated carbon dioxide, Nature Communications, 6, 8155, doi:10.1038/ncomms9155, 2015.

Hutchinson, G. E.: Concluding Remarks, Cold Spring Harbor Symposia on Quantitative Biology, 22, 415–427, doi:10.1101/SQB.1957.022.01.039, 1957.

Karlberg, M. and Wulff, A.: Impact of temperature and species interaction on filamentous cyanobacteria may be more important than salinity and increased $pCO_2$ levels, Marine Biology, 160, 2063–2072, doi:10.1007/s00227-012-2078-3, 2013.

Karlson, A. M. L., Duberg, J., Motwani, N. H., Hogfors, H., Klawonn, I., Ploug, H., Svedén, J. B., Garbaras, A., Sundelin, B., Hajdu, S., Larsson, U., Elmgren, R., and Gorokhova, E.: Nitrogen fixation by cyanobacteria stimulates production in Baltic food webs, AMBIO, 44, 413–426, doi: 10.1007/s13280-015-0660-x, 2015.

Kim, J.-M., Lee, K., Shin, K., Yang, E. J., Engel, A., Karl, D. M., and Kim, H.-C.: Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions, Geophysical Research Letters, 38, L08 612, doi:10.1029/2011GL047346, 2011.

Kranz, S. A., Levitan, O., Richter, K.-U., Prasil, O., Berman-Frank, I., and Rost, B.: Combined effects of $CO_2$ and light on the $N_2$-fixing cyanobacterium *Trichodesmium* IMS101: Physiological responses, Plant Physiology, 154, 334–345, doi:10.1104/pp.110.159145, 2010.

Leppäranta, M. and Myrberg, K.: The Baltic Sea: History and Geography, vol. Physical Oceanography of the Baltic Sea, pp. 1–54, Praxis Publishing Ltd., Chichiester, United Kingdom, 2009.

Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., Prasil, O., and Berman-Frank, I.: Elevated $CO_2$ enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*, Global Change Biology, 13, 531–538, doi:10.1111/j.1365-2486.2006.01314.x, 2007.

Lewandowska, A. M., Boyce, D. G., Hofmann, M., Matthiessen, B., Sommer, U., and Worm, B.: Effects of sea surface warming on marine plankton, Ecology Letters, 17, 614–623, doi:10.1111/ele.12265, 2014.

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, The ISME Journal, 8, 2180–2192, doi: 10.1038/ismej.2014.71, 2014.

Loick-Wilde, N., Dutz, J., Miltner, A., Gehre, M., Montoya, J. P., and Voss, M.: Incorporation of nitrogen from $N_2$ fixation into amino acids of zooplankton, Limnology and Oceanography, 57, 199–210, doi: 10.4319/lo.2012.57.1.0199, 2012.

Lomas, M. W., Hopkinson, B. M., Ryan, J. L. L. D. E., Shi, D. L., Xu, Y., and Morel, F. M. M.: Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic, Aquatic Microbial Ecology, 66, 211–222, doi:10.3354/ame01576, 2012.

Low-Décarie, E., Fussmann, G. F., and Bell, G.: Aquatic primary production in a high-$CO_2$ world, Trends in Ecology & Evolution, 29, 223–232, doi:10.1016/j.tree.2014.02.006, 2014.

Mather, R. L., Reynolds, S. E., Wolff, G. A., Williams, R. G., Torres-Valdes, S., Woodward, E. M. S., Landolfi, A., Pan, X., Sanders, R., and Achterberg, E. P.: Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres, Nature Geoscience, 1, 439–443, doi:10.1038/ngeo232, 2008.

McDonald, S. M., Plant, J. N., and Worden, A. Z.: The mixed lineage nature of nitrogen transport and assimilation in marine eukaryotic phytoplankton: A case study of *Micromonas*, Molecular Biology and Evolution, 27, 2268–2283, doi:10.1093/molbev/msq113, 2010.

Mohr, W., Intermaggio, M. P., and LaRoche, J.: Diel rhythm of nitrogen and carbon metabolism in the unicellular, diazotrophic cyanobacterium *Crocosphaera watsonii* WH8501, Environmental Microbiology, 12, 412–421, doi:10.1111/j.1462-2920.2009.02078.x, 2010.

Montesinos, M. L., Herrero, A., and Flores, E.: Amino acid transport systems required for diazotrophic growth in the cyanobacterium *Anabaena* sp. strain PCC 7120., Journal of Bacteriology, 177, 3150–3157, 1995.

Mulholland, M. R., Bronk, D. A., and Capone, D. G.: Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101, Aquatic Microbial Ecology, 37, 85–94, doi:10.3354/ame037085, 2004.

Newbold, L. K., Oliver, A. E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M., Andersen, G., van der Gast, C. J., and Whiteley, A. S.: The response of marine picoplankton to ocean acidification, Environmental Microbiology, 14, 2293–2307, doi:10.1111/j.1462-2920.2012.02762.x, 2012.

Paerl, H. W. and Huisman, J.: Blooms like it hot, Science, 320, 57–58, doi:10.1126/science.1155398, 2008.

Perry, A. L., Low, P. J., Ellis, J. R., and Reynolds, J. D.: Climate change and distribution shifts in marine fishes, Science, 308, 1912–1915, doi:10.1126/science.1111322, 2005.

Piontek, J., Hel, N., Langer, G., Wohlers, J., Riebesell, U., and Engel, A.: Effects of rising temperature on the formation and microbial degradation of marine diatom aggregates, Aquatic Microbial Ecology, 54, 305–318, doi:10.3354/ame01273, 2009.

Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., and Engel, A.: Acidification increases microbial polysaccharide degradation in the ocean, Biogeosciences, 7, 1615–1624, doi:10.5194/bg-7-1615-2010, 2010.

Ploug, H., Musat, N., Adam, B., Moraru, C. L., Lavik, G., Vagner, T., Bergman, B., and Kuypers, M. M. M.: Carbon and nitrogen fluxes associated with the cyanobacterium *Aphanizomenon sp.* in the Baltic Sea, The ISME Journal, 4, 1215–1223, doi:10.1038/ismej.2010.53, 2010.

Price, G. D., Badger, M. R., Woodger, F. J., and Long, B. M.: Advances in understanding the cyanobacterial $CO_2$-concentrating-mechanism (CCM): functional components, $C_i$ transporters, diversity, genetic regulation and prospects for engineering into plants, Journal of Experimental Botany, 59, 1441–1461, doi:10.1093/jxb/erm112, 2008.

Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J., and Zöllner, E.: Enhanced biological carbon consumption in a high $CO_2$ ocean, Nature, 450, 545–548, doi:10.1038/nature06267, 2007.

Rolff, C.: Seasonal variation in $^{13}C$ and $^{15}N$ of size-fractionated plankton at a coastal station in the northern Baltic proper, Marine Ecology Progress Series, 203, 47–65, doi:10.3354/meps203047, 2000.

Sala, M. M., Aparicio, F. L., Balagu., Boras, J. A., Borrull, E., Cardels, C., Cros, L., Gomes, A., L-Sanz, A., Malits, A., Martz, R. A., Mestre, M., Movilla, J., Sarmento, H., Vuez-Domuez, E., Vaqu., Pinhassi, J., Calbet, A., Calvo, E., Gasol, J. M., Pelejero, C., and Marras.: Contrasting effects of ocean acidification on the microbial food web under different trophic conditions, ICES Journal of Marine Science: Journal du Conseil, doi:10.1093/icesjms/fsv130, 2015.

Schaum, C. E. and Collins, S.: Plasticity predicts evolution in a marine alga, Proceedings of the Royal Society of London B: Biological Sciences, 281, 20141 486, doi:10.1098/rspb.2014.1486, 2014.

Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater $pCO_2$, PLoS ONE, 9, e88 308, doi:10.1371/journal.pone.0088308, 2014.

Suikkanen, S., Laamanen, M., and Huttunen, M.: Long-term changes in summer phytoplankton communities of the open northern Baltic Sea, Estuarine Coastal and Shelf Science, 71, 580–592, doi:10.1016/j.ecss.2006.09.004, 2007.

Suikkanen, S., Pulina, S., Engström-Öst, J., Lehtiniemi, M., Lehtinen, S., and Brutemark, A.: Climate change and eutrophication induced shifts in northern summer plankton communities, PLoS ONE, 8, e66 475, doi:10.1371/journal.pone.0066475, 2013.

Taucher, J., Schulz, K. G., Dittmar, T., Sommer, U., Oschlies, A., and Riebesell, U.: Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment, Biogeosciences, 9, 3531–3545, doi:10.5194/bg-9-3531-2012, 2012.

Thomas, H. and Schneider, B.: The seasonal cycle of carbon dioxide in Baltic Sea surface waters, Journal of Marine Systems, 22, 53 – 67, doi:http://dx.doi.org/10.1016/S0924-7963(99)00030-5, 1999.

Thomas, M. K., Kremer, C. T., Klausmeier, C. A., and Litchman, E.: A global pattern of thermal adaptation in marine phytoplankton, Science, 338, 1085–1088, doi:10.1126/science.1224836, 2012.

Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., Eisenhauer, A., Körtzinger, A., Wahl, M., and Melzner, F.: Calcifying invertebrates succeed in a naturally $CO_2$-rich coastal habitat but are threatened by high levels of future acidification, Biogeosciences, 7, 3879–3891, doi:10.5194/bg-7-3879-2010, 2010.

Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkänen, H., Savchuk, O. P., Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N., and Wulff, F.: Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea, AMBIO, 36, 186–194, doi:10.1579/0044-7447(2007)36[186:IEFENC]2.0.CO;2, 2007.

Wannicke, N., Korth, F., Liskow, I., and Voss, M.: Incorporation of diazotrophic fixed $N_2$ by mesozooplankton — Case studies in the southern Baltic Sea, Journal of Marine Systems, 117–118, 1–13, doi:10.1016/j.jmarsys.2013.03.005, 2013.

Wasmund, N.: Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions, Internationale Revue Der Gesamten Hydrobiologie, 82, 169–184, doi:10.1002/iroh.19970820205, 1997.

Yamamoto, Y.: Environmental factors that determine the occurrence and seasonal dynamics of *Aphanizomenon flos-aquae*, Journal of Limnology, 68, 122–132, doi:10.4081/jlimnol.2009.122, 2009.

Yamamoto, Y. and Nakahara, H.: The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length, Limnology, 6, 1–6, doi:10.1007/s10201-004-0138-1, 2005.

# Appendix

**Table 5.1:** Summary of known published studies to date testing the effect of ocean acidification on phytoplankton communities, current as of 11th October, 2015. Short-term studies over a few days were completed in bottle incubations, whereas longer studies over a few weeks commonly used seawater volumes of >100 L. Low indicates inorganic nitrogen concentrations (< 2 uM), and replete indicates relatively high nutrient concentrations which were either naturally present or artificially supplemented. Deplete followed by addition indicates that the nutrient addition did not occur at the beginning of the study period and did not coincide with $CO_2$-enrichment.

| Location | Nutrient status | Length of study | Citation(s) (Author, Year, Journal) |
|---|---|---|---|
| Baltic Sea, coastal | low | 44 days | Paul et al., 2015, Biogeosciences |
| Sea of Okhotsk | low | 14 days | Yoshimura et al., 2010, Journal of Experimental Marine Biology and Ecology |
| Arctic, coastal | low followed by addition | 36 days | Schulz et al., 2013, Biogeosciences |
| Bering Sea, shelf and oceanic | low followed by addition | 9 - 10 days | Hare et al., 2007, Marine Ecology Progress Series |
| Korea Straits, coastal | low followed by addition | 14 days | Kim et al., 2006, Limnology and Oceanography |
| Mediterranean, coastal | low followed by addition | 7 days | Mercado et al., 2014, Aquatic Biology; Neale et al,. 2014, Aquatic Biology; Sobrino et al., 2014, Aquatic Biology |
| North Atlantic, coastal | low followed by addition | 20 days | Calbet et al., 2014, PLoS ONE |
| North Atlantic, coastal | low followed by addition | 22 days | Hopkins et al., 2010, Proceedings of the National Academy of Sciences |
| North Atlantic, shelf sea | low followed by addition | 4 days | Richier et al., 2014, Biogeosciences; MacGilchrist et al., 2014 Biogeosciences |
| North Atlantic, tropical, oceanic | low followed by addition | 1.5 - 3.2 days | Lomas et al., 2012, Aquatic Microbial Ecology |
| North Pacific, sub-tropical | low followed by addition | 3 - 4 days | Losh et al., 2012, Marine Ecology Progress Series |
| Australia, subtropical estuary | replete | 14 days | Nielsen et al. 2011, Aquatic Microbial Ecology |
| Baltic Sea, coastal | replete | 21 days | Engel et al., 2014, Journal of Plankton Research; Schulz and Riebesell 2012, Marine Biology |
| Baltic Sea, coastal | replete | 28 days | Rossoll, et al., 2013, Marine Ecology Progress Series |
| Bay of Bengal, coastal | replete | 5 days | Biswas et al., 2012, Biodiversity and Conservation |
| Equatorial Pacific, coastal Peru | replete | 12 days | Tortell et al., 2002, Marine Ecology Progress Series |
| Korea, coastal | replete | 20 days | Kim et al., 2011, Geophysical Research Letters |
| North Atlantic, coastal | replete | 26 days | Engel et al., 2005, Limnology and Oceanography |
| North Atlantic, coastal | replete | 19 days | Engel et al., 2008, Biogeosciences |
| North Atlantic, coastal | replete | 22 days | Schulz et al., 2008, Biogeosciences |
| North Pacific, coastal | replete | 14 days | Hama et al., 2012, Journal of Oceanography |
| Oresund Strait/Baltic Sea, coastal | replete | 14 days | Nielsen et al., 2010, Marine Biology Research |
| Ross Sea, HNLC | replete | 18 days | Feng et al., 2010, Deep-Sea Research I |
| Weddell Sea | replete | 18 - 30 days | Hoppe et al., 2013, PLoS ONE |
| Bering Sea, HNLC | replete, Fe replete | 14 days | Yoshimura et al., 2013, Journal of Oceanography, Yoshimura et al., 2014, Deep Sea Research I |
| Gulf of Alaska, HNLC | replete, Fe replete | 3.5 - 5.5 days | Hopkinson et al. 2010, L&O |
| Subarctic Pacific, HNLC | replete, Fe replete | 14 days | Yoshimura et al., 2013, Journal of Oceanography, Yoshimura et al., 2014, Deep Sea Research I |

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# Eidesstattliche Erklärung

Hiermit bestätige ich, dass die vorliegende Arbeit mit dem Titel:

**Ocean acidification and warming in the Baltic Sea:**
**Effects on diazotrophy and pelagic biogeochemistry**

von mir selbstständig verfasst worden ist und keine weiteren Quellen und Hilfsmittel als die angegebenen verwendet wurden. Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden und wurde weder im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt noch veröffentlicht. Veröffentlichte oder zur Veröffentlichung eingereichte Manuskripte wurden kenntlich gemacht.

Ich erkläre mich einverstanden, dass diese Arbeit an die Bibliothek des GEOMAR Helmholtz Zentrum für Ozeanforschung Kiel und die Universitätsbibliothek der Christian-Albrechts-Universität zu Kiel weitergeleitet wird.

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**Paul, A. J.**, Achterberg, E. P., Bach, L. T., Boxhammer, T., Czerny, J., Haunost, M., Lischka, S., Meyerhöfer, M., Schulz, K.-G., Stuhr, A., Trense, Y., and Riebesell, U.: Insight into the fate of newly-fixed nitrogen in a high-$CO_2$ ocean, IMBER Open Science Conference, Bergen (Norway), June 2014

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**Paul, A. J.**, Bach, L. T., Achterberg, E. P., Boxhammer, T., Schulz, K.-G., Stuhr, A., and Riebesell, U.: New nitrogen supply in a future acidified ocean, Sustainable Ocean Development Symposium, New York (U.S.A.), September 2015

## List of publications

*Published manuscripts*

**Paul, A. J.**, Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M. and Riebesell, U.: Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community, *Biogeosciences*, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015

Miller, B. J., Du, L., Steel, T. J., **Paul, A. J.**, Södergren, A. H., Lane, J. R., Henry, B. R. and Kjaergaard, H. G.: Absolute intensities of NH-stretching transitions in dimethylamine and pyrrole, *Journal of Physical Chemistry A*, 116(1), 290–296, 2012

*Manuscripts under review*

**Paul, A. J.**, Achterberg, E. P., Bach, L. T., Boxhammer, T., Czerny, J., Haunost, M., Schulz, K.-G., Stuhr, A. and Riebesell, U.: No observed effect of ocean acidification on nitrogen biogeochemistry in a Baltic Sea plankton community, *Biogeosciences Discussions*, 12, 17507-17541, doi:10.5194/bgd-12-17507-2015, 2015

Almén, A.K., Vehmaa, A., Brutemark, A., Bach, L. T., Lischka, S., Stuhr, A., Furuhagen, S., **Paul, A. J.**, Bermúdez, J. R., Riebesell, U., and Engström-Öst, J.: Food quantity and quality affect reproduction of a common copepod more than ocean acidification, *Biogeosciences Discussions*, 12, 17093-17123, doi:10.5194/bgd-12-17093-2015, 2015

Paul, C., Sommer, U., Garzke, J., Moustaka-Gouni, M., **Paul, A. J.**, and Matthiessen, B.: Effects of increased $CO_2$ concentration on nutrient limited coastal summer plankton depend on temperature, *Under revision*

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