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Nitrogen assimilation and phytoplankton communities in changing environments

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Content

Abbreviations

ARP – Amazon River plume

C - carbon

Chl *a* - Chlorophyll *a*

DDA – Diatom-Diazotroph association

DOC - dissolved organic carbon

DON - dissolved organic nitrogen

HPLC - high precision liquid chromatography

IRMS - isotope ratio mass spectrometry

LOD - limit of detection

MLD – mixed layer depth

N - nitrogen

N_2 - dinitrogen

NBC - North Brazil Current

NH_4^+ - ammonium

NO_3^- - nitrate

P - phosphorus

PO_4^{3-} - phosphate

SSS – sea surface salinity

SST – sea surface temperature

WTNA – Western Tropical North Atlantic

Summary

The future of dinitrogen (N_2) fixation in a changing ocean was studied to identify potential consequences for phytoplankton community interactions, as well as associated alterations of primary production and carbon sequestration in response to climate change. Therefore, a laboratory experiment was conducted at the Mediterranean Institute of Oceanography in Marseille, where two climate change scenarios were simulated in minicosms and the response of the globally significant diazotrophs *Crocosphaera watsonii* and *Trichodesmium* sp. was studied. In the scenarios, ocean acidification and ocean warming were mimicked simultaneously, which has rarely been done before, as most previous studies analysed the response of diazotrophs to a single changing variable only. The growth of the two species was monitored and N_2 fixation rates were measured on the community and the single cell level to observe the response of the two diazotrophs to the climate change scenarios. In addition, single cell uptake rates of nitrate (NO_3^-) and ammonium (NH_4^+) were measured via nanoscale secondary ion mass spectrometry (nanoSIMS) to get an information on the potential competition between diazotrophs and non-diazotrophs for dissolved inorganic nitrogen (DIN). To investigate the role of N_2 fixation for primary production on a local scale, field work in the Amazon River plume (ARP) was conducted during cruise M174 on RV Meteor in April/May 2021. The importance of different nitrogen sources for the phytoplankton community structure and primary production along a gradient of environmental variables was studied. The ARP posts ideal conditions to gain insights on nitrogen cycling and phytoplankton community interactions in a future ocean, as the system shows a strong stratification and nutrient availability changes rapidly along the plume affecting the community structure. These features correspond to a future ocean scenario (Gruber, 2011) where a stronger and shallower stratification is forecasted, as well as more acidic waters. If the stratification becomes stronger, nutrient input to the euphotic zone is restricted and this then limits the nutrient supply to the surface. During the Amazon cruise, for the first time in this region, uptake rates of amino acids, NO_3^- and NH_4^+ were measured using stable isotopes as tracers. Additionally, N_2 fixation and primary production rates were determined and the phytoplankton community was analysed via high precision liquid chromatography (HPLC) to link dominant phytoplankton groups to their nitrogen sources.

The results of the climate change experiment showed that *Crocosphaera watsonii* could potentially be in competition with non-diazotrophic phytoplankton for DIN. Inter community variability in the uptake of different nitrogen sources was high, implying that

cells have the potential to quickly react to changes in the nitrogen supply. Furthermore, it seemed that *C. watsonii* could overall cope with the increase in temperature and decrease of pH within the ranges tested, but the combination of stressors had a rather suppressing effect, if temperature exceeded the species' optimum. Small diazotrophs like *Crocosphaera watsonii* will likely be favoured by postulated future conditions over larger species and diazotrophs in general are not restricted to fully nitrogen limited regions. Furthermore, the response of N_2 fixers to climate change seems to be species specific. These findings should be considered in ecosystem models to improve predictions of global N_2 fixation. At the current state of our knowledge, it is hard to reliably forecast the behaviour of diazotrophs in a future ocean and the resulting consequences for global primary production. More multi-stressor experiments with different species like the one performed here are necessary to shed light on the complex response of N_2 fixers and also other phytoplankton taxa to climate change.

In a future ocean with highly stratified, nutrient limited conditions, N_2 fixation could become more important for primary production, alongside the recycling of nitrogen within the surface layer. From the field work in the ARP it could be concluded that recycling of nitrogen within the surface layer sustains primary production along the full gradient of stratification and nitrogen availability. Nitrogen in the ARP quickly becomes limiting, as the N:P ratio of river water is low, unlike that of most other rivers globally. DIN uptake is most important on the shelf, where diatoms and cryptophytes are dominant and nutrients are available at low concentrations. NH_4^+ is a major nitrogen source for phytoplankton in the plume, supporting to up to 90 % of primary production. Further north off the shelf, where cyanobacteria dominate and conditions are oligotrophic, N_2 fixation becomes important, but supports maximal 33 % of primary production, while NH_4^+ uptake rates are still quite high and sustain the largest fraction of primary production. The work presented here gives a baseline for nitrogen uptake processes in the ARP that is essential to monitor the effect of changes the Amazon catchment area is faced with. My observations can be used as an example for the potential phytoplankton community structure, nitrogen utilization and primary production in the future coastal ocean. The results also stress the role of N_2 fixation in fuelling productivity both directly and indirectly via the release of previously fixed nitrogen and remineralisation of diazotroph biomass. Altogether, the results from this thesis can help to improve models on global N_2 fixation and primary production in the future and therefore contribute to the estimate of the CO_2 storage capacity of the ocean

1 General introduction

1.1 Climate change and primary production

“Ocean warming dominates the increase in energy stored in the climate system” (IPCC, 2014) and warming of the upper 75 m of the surface ocean of 0.11 °C on average has been reported for the period of 1971 to 2010 (IPCC, 2014). In 2023, the highest temperature anomalies since 1850 were reported for global ocean surface water reaching +1.03°C in August (NOAA National Centers for Environmental information). As primary cause of global warming and thus ocean warming an increased carbon dioxide (CO_2) concentration in the atmosphere through anthropogenic activities such as burning of fossil fuels (Falkowski et al., 2000) and deforestation (Doney et al., 2009) has been identified. Stratification has increased by 5.3 % between 1960 and 2018 and resulted from temperature changes to more than 90 % (Li et al., 2020). Weakening of the Atlantic overturning circulation (Dima & Lohmann, 2010; Liu et al., 2017, 2020; Manabe & Stouffer, 2007) and shallow stratification may have considerable impacts on the productivity and oxygen concentrations in the near future. As higher temperatures are associated with higher growth rates and metabolic activity of phytoplankton (Brown et al., 2004; Eppley, 1972; Gillooly et al., 2001), ocean warming is generally thought to enhance primary production in the first place. However, an increase of stratification and the associated reduction of the vertical nutrient supply to the surface ocean could counteract this positive effect of an increase in temperature, as suggested for example by Marãnón et al. (2014). Furthermore, projected changes in rainfall (IPCC, 2019) can alter river runoff and modify nutrient supply to the ocean, which could intensify the problem of nutrient limitation for productivity.

The ocean and the overlaying atmosphere are in equilibrium in terms of CO_2. Consequently, CO_2 is exchanged across the air-sea interface when deviations from this equilibrium occur (Gruber & Sarmiento, 2002). CO_2 reacts with sea water to CO_2^{3-} and HCO_3^- ions, which buffer the pH of the ocean. Nowadays, the rate at which CO_2 is absorbed by the ocean is too fast to guarantee sufficient buffering, resulting in alterations of the concentrations of CO_2, CO_2^{3-} and HCO_3^-. These changes lead to a decrease in ocean pH, termed ocean acidification, but will likely also reduce the oceans ability to absorb carbon (Findlay & Turley, 2021; Sabine et al., 2004). A decrease in pH by 0.4 units by the year 2100 is predicted by global ocean models (Bopp et al., 2013; IPCC, 2019). Phytoplankton plays a crucial role in the carbonate cycle of the ocean: dissolved CO_2 is taken up by phytoplankton in the ocean during primary production and is thereby exported to the deep ocean as dead biomass sinks out of

the surface layer (Sarmiento & Gruber, 2002). Through this mechanism, referred to as the biological pump, the ocean has taken up between 23 % of anthropogenic carbon over the last decade (Friedlingstein et al., 2019) and 31 % between 1994 and 2007 (Gruber et al., 2019). Changes of the ocean's carbonate chemistry following ocean acidification (Doney et al., 2009), can therefore impact biological processes like for example primary production.

As primary production in the ocean accounts for one half of global primary production (Field et al., 1998) understanding its forcing and associated pathways is crucial to make predictions for the future (Falkowski et al., 2000).

1.2 Diazotrophy and the nitrogen cycle

Indisputably, nitrogen has an important role in controlling key aspects of the carbon cycle. As nitrogen is an important component of living organisms and is in short supply in terrestrial and marine ecosystems, it is an essential controlling factor of primary production (Gruber & Galloway, 2008; Moore et al., 2013; Tyrrell, 1999). Consequently, nitrogen availability can alter the strength of the biological pump and the subsequent impact of biological processes on atmospheric CO_2 (Gruber, 2004). Nitrogen can either be recycled with the surface layer of the ocean (regenerated nitrogen), or be introduced from external sources like rivers, atmospheric deposition, upwelling or via dinitrogen fixation (new nitrogen). In terms of climate change, the central question according to Gruber and Galloway (2008) is: "How will the availability of nitrogen affect the capacity of Earth's biosphere to continue absorbing CO_2 from the atmosphere?".

Dinitrogen fixation, the reduction of atmospheric dinitrogen (N_2) to biologically available ammonium (NH_4^+) by microorganisms called diazotrophs, plays an important role in this context. N_2 fixed from the atmosphere is the main source of new nitrogen in the ocean (Gruber, 2004; Gruber & Sarmiento, 1997; Jickells et al., 2017; Luo et al., 2012, 2014) and enables the phytoplankton community to sustain productivity over broad spatial and temporal scales (Mahaffey et al., 2005). In oligotrophic areas, N_2 fixation can account for up to 50 % of the new N supplied to an ecosystem (Karl et al., 1997). In other words, N_2 fixation contributes significantly to nutrient availability in the euphotic zone, and to the export of organic material to the deep ocean, hence to carbon storage (Karl et al., 2002).

N_2 fixation is catalysed by the enzyme nitrogenase, which consists of two subunits – the nitrogenase and the nitrogenase-reductase. The latter is encoded by the *nifH* gene. This gene

is highly conserved (Ruvkun & Ausubel, 1980) and phylogenetically diverse (Zehr et al., 1998), as it can be found amongst different strains of cyanobacteria, bacteria and archaea (Young, 1992). Therefore, the *nifH* gene can be used to analyse phylogenetic relationships (Zehr & Capone, 1996). Thanks to such molecular methods, the scientific community has made huge progress in identifying new diazotrophic organisms. The filamentous cyanobacterium *Trichodesmium* spp. was and still is the diazotroph studied the most (Capone et al., 1997; Karl et al., 2002), but in the last two decades, other organisms gained attention (Zehr et al., 1998, 2001). We now know that diazotrophs are taxonomically, physiologically and ecologically diverse (Karl et al., 2002) and surprisingly, non-cyanobacterial diazotrophs were found to dominate the *nifH* gene pool in some regions and are expected to be important for global N_2 fixation (Farnelid et al., 2011; Moisander et al., 2017). Diazotrophs have been detected in most regions of the world oceans, from surface waters in tropical, subtropical and temperate regions (Luo et al., 2012; Zehr, 2011; Zehr & Capone, 2020), to higher latitudes (Moisander et al., 2010), aphotic environments (Benavides et al., 2018) and deep sea sediments (Kapili et al., 2020).

N_2 fixers play a crucial role in global primary production via the release of previously fixed nitrogen to ambient water in the form of dissolved organic nitrogen (DON) or NH_4^+ (Capone et al., 1994; Glibert & Bronk, 1994; Mulholland, Bronk, et al., 2004). These nitrogen forms can be taken up by non-diazotrophic phytoplankton and sustain their primary production. As non-diazotrophic phytoplankton lack the ability to fix N_2 from the atmosphere, they rely on nitrogen in ambient waters resulting in close interactions between diazotrophs and non-diazotrophic phytoplankton that can be seen in the form of succession during phytoplankton blooms (Caffin et al., 2018). For example, Mulholland et al. (2004) and Chen et al. (2011) reported a high abundance of non-diazotrophic phytoplankton and diatoms, respectively, after a bloom of *Trichodesmium* sp.. In addition to their role as a nitrogen source, diazotrophs can likely also be in competition with other phytoplankton species for dissolved inorganic N (DIN), as several studies have shown that diazotrophs are able to assimilate DIN (Holl & Montoya, 2005; Knapp, 2012; Masuda et al., 2013; Masuda et al., 2022). Due to the link between N_2 fixation and the rest of the phytoplankton community, effects of climate change on diazotrophy and the C pump cannot be fully assessed without understanding the consequences of such effects for N assimilation by non-diazotrophic phytoplankton. Especially in a future ocean with stronger stratification and resulting nutrient limitation, the behaviour of N_2 fixers as a source or sink for nitrogen will be crucial to assess consequences for global primary production.

While it is obvious nowadays that the potential for N_2 fixation is widely distributed, environmental factors controlling this process, as well as the physiology of newly discovered diazotrophs are still not well understood and robust predictions of their behaviour in the future cannot be made at this stage of our knowledge (Benavides & Voss, 2015; Landolfi et al., 2018; Moisander et al., 2017; Tang et al., 2019). Diazotrophy is known to be regulated by physical (light, temperature, salinity, stability of the water column, turbulence, density fronts, mesoscale variability) and chemical factors (N:P ratio, iron availability, other trace metals) (Luo et al., 2014). These factors are postulated to change in the future due to climate change (Bopp et al., 2013) and as a consequence, N_2 fixation could change as well. Possible effects of ocean warming, ocean acidification, enhanced stratification and increasing nutrient limitation on diazotrophs and their N_2 and C fixation will be presented in the introduction of the first part of this thesis. In addition to improving our knowledge on the effects of climate change on N_2 fixation itself, it is also important to further explore potential consequences for the rest of the phytoplankton community, as they are closely linked to diazotrophy. If we understand the interaction and importance of different phytoplankton groups on a local scale, predictions for the future will become much more reliable. Even though N_2 fixation is an important process in this context, we cannot neglect other N assimilation pathways.

1.3 Focus of this thesis

This thesis was conducted as part of the project NOTION ("Nitrogen fixers structuring phytoplankton communities in the ocean under climate change"). As explained above, diazotrophs are important to sustain productivity in the oceans as they supply new nitrogen, which is essential for biomass production, to the surface layer. To date, models predicting the future of primary production under climate change conditions show contradictory results and N_2 fixation has been identified as a key driver for the response of global primary production to conditions in the future ocean (Bopp et al., 2022; Wrightson & Tagliabue, 2020). Consequently, the failure to adequately account for the response of N_2 fixation to conditions in the future ocean is a main reason for the high uncertainty in models. One major issue is that studies give an equivocal picture of the behaviour of N_2 fixers under climate change, which will be introduced in detail in the next section. In brief, some studies have shown that N_2 fixation rates increase under elevated pCO_2 in the water concentrations (Barcelos e Ramos et al., 2007; Fu et al., 2008; Garcia et al., 2013; Hutchins et al., 2007, 2013, 2015; Kranz et al., 2009; Levitan et al., 2007), while others show no effect (Gradoville,

White, Böttjer, et al., 2014; Hong et al., 2017; D. Shi et al., 2012) or even a reduction of rates (Czerny et al., 2009). A stimulation of N_2 fixation might be the result of reduced energy requirements to fulfil the organisms' carbon demand for photosynthesis and energy generation, if CO_2 is available at higher concentrations in the water (Barcelos e Ramos et al., 2007; Fu et al., 2008; Hutchins et al., 2007; Kranz et al., 2009; Levitan et al., 2007). On the other hand, a decrease of pH might have a suppressing effect on diazotrophs, as the process of N_2 fixation is sensitive to pH (Hong et al., 2017; Luo et al., 2019). The aim of the NOTION project was, to improve the knowledge and understanding of controlling factors of marine N_2 fixation and possible changes of the same due to climate change. As explained previously, to fully capture the impact of changing N_2 fixation on climate change and, more specifically, on the biological pump, the effect of such potential changes on phytoplankton community composition and productivity should be assessed, meaning that the importance of N_2 fixation for phytoplankton communities, both in general and in comparison to other N assimilation processes, needs to be understood at a local level. Results from our experiments will then be used to improve an ecosystem model on N_2 fixation by Domitille Louchard (ETH Zürich) and make more robust predictions for this process and associated productivity of phytoplankton communities in a future ocean. Applying a model including two different diazotrophic species, Louchard et al. (2023) could already show that the Amazon River plume enhances marine N_2 fixation by 74 %. The model will be developed further with results from our experiments that will help to investigate how climate change could affect N_2 fixation and primary production in the Western Tropical North Atlantic. The Amazon River plume (ARP) with its gradient of nutrient availability, salinity and turbidity that shapes phytoplankton communities along the plume represents an ideal study location for the importance of N_2 fixation for primary production and phytoplankton community interactions under different environmental conditions that are partly similar to predicted future conditions in the ocean. Therefore, the ARP is ideal to assess the environmental variables that control N_2 fixation, nitrogen assimilation, primary production and phytoplankton community structure on a local scale under current and potential future conditions.

This thesis consists of two parts that cover both issues mentioned. Firstly, a climate change experiment conducted in the laboratory at the Mediterranean Institute of Oceanography (MIO) in Marseille, France from July to September 2021 and secondly, the study of nitrogen uptake processes and phytoplankton community composition at the local level, here in the Amazon River plume (ARP) during cruise M174 on RV Meteor in April/May 2021. The two parts will be presented and discussed separately in the following chapters. In the end of this

thesis, implications from both parts will be combined and discussed. The general aim of this work was to improve our knowledge on nitrogen utilization by different phytoplankton groups under climate change conditions and in a highly stratified system, with an emphasis on the role of diazotrophs. Insights from the field and laboratory studies can be used to improve global models on the future of phytoplankton diversity, nitrogen utilization and in conclusion also primary production and the associated CO_2 storage capacity of the oceans.

Part I

Diazotrophs in times of climate change

– Laboratory experiment –

I-1 Introduction

Compared to other N assimilation processes, N_2 fixation is a highly cost intensive process (Großkopf & LaRoche, 2012). The required energy is obtained from photosynthesis, where CO_2 is transformed into glucose, which is then used as an energy source. Many diazotrophs possess carbon concentrating mechanisms (CCMs) (Badger & Price, 1992; Kaplan & Reinhold, 1999) to accumulate CO_2, enabling the organisms to meet their high energy demand. Elevated CO_2 concentrations in the future oceans may facilitate the uptake of CO_2, resulting in an increase in energy available for other processes - like N_2 fixation (Barcelos e Ramos et al., 2007; Fu et al., 2008; Hutchins et al., 2007; Kranz et al., 2009; Levitan et al., 2007). While this is one of the main reasons why N_2 fixation is thought to be enhanced by climate change, some studies did not confirm these findings but present alternative reactions of N_2 fixers to elevated levels of pCO_2, as will be discussed below.

Trichodesmium spp. and *Crocosphaera watsonii* (UCYN-B) are dominant cyanobacterial diazotrophs in tropical and subtropical regions of the ocean and are therefore globally significant sources of new nitrogen (Zehr & Capone, 2020). N_2 fixation of *Trichodesmium* spp. and *C. watsonii* increases with increasing CO_2 concentrations (Barcelos e Ramos et al., 2007; Fu et al., 2008; Garcia et al., 2013; Hutchins et al., 2007, 2013, 2015; Kranz et al., 2009; Levitan et al., 2007). Other studies have found no effect of an elevated pCO_2 on *Trichodesmium* spp. (Gradoville, White, Böttjer, et al., 2014; Hong et al., 2017; D. Shi et al., 2012) or a natural diazotroph community dominated mostly by *C. watsonii* (Böttjer et al., 2014). However, an increase in pCO_2 is associated with a decrease of the pH of sea water. Hong et al. (2017), Shi et al. (2012) and Luo et al. (2019) have shown that the nitrogenase, seems to be sensitive to a decrease of pH by 0.2 – 0.3 units and therefore the stimulating effect of elevated pCO_2 levels is offset. Due to a lack of studies, it is not clear to which extent these results apply to other diazotrophs and the few existing studies complicate the picture even more. For instance, increases in pCO_2 have been found to either enhance (Wannicke et al., 2012) or reduce (Czerny et al., 2009) growth rates and N_2 fixation in *Nodularia spumigena*, a heterocysteous cyanobacterium forming extensive blooms in the Baltic Sea. Hence, the response of diazotrophs to ocean acidification remains a complex issue. Luo et al. (2019) predict that growth rates of *Trichodesmium* sp. will decrease in the coming century due to ocean acidification as a result of reduced efficiency of the nitrogenase under lower pH. As a consequence, the authors state that N_2 fixation by this diazotroph could decline by 27 %, especially affecting iron-limited areas, as the nitrogenase requires iron and therefore

diazotrophs depend on sufficient iron supply. Nonetheless, effects of changes of other environmental variables like ocean warming are not taken into account in the model by Luo et al. (2019). The future of diazotrophy under climate change can hardly be examined without taking ocean warming into consideration. An increase in temperature mostly had a stimulating effect on growth of diazotrophs and N_2 fixation, as long as temperature did not exceed the upper threshold of the species investigated (Fu et al., 2014). Generally, it seems that warming favours the growth of important diazotrophs, as long as temperature does not exceed ~32-33°C (Gao et al., 2019). Boatman et al. (2017) investigated the effect of a combined increase in temperature and pCO_2 on the growth of *Trichodesmium* sp. and found that growth rates increased significantly under elevated pCO_2 between the minimum and optimum growth temperature of the diazotroph, but the effect above the optimum temperature was negligible. Same seems to hold true for growth rates of both *Trichodesmium* spp. and *C. watsonii* (Fu et al., 2014; Hutchins et al., 2007, 2015). It seems that diazotrophs have a great potential to cope with prospected conditions in the future oceans, but more studies investigating the effects of a combined alteration of pCO_2 and temperature are needed to create a more realistic scenario and obtain robust results on the response of N_2 fixers to climate change

Another aspect that needs more careful consideration in the cellular model of Luo et al. (2019) is the assumption that N_2 fixation is the only nitrogen source for *Trichodesmium* sp. and diazotrophy is inhibited by DIN, which is unlikely. Several studies have shown that nanomolar concentrations of dissolved inorganic nitrogen (DIN) do not inhibit N_2 fixation, while concentrations above 1 µM can reduce or completely inhibit N_2 fixation (Dekaezemacker & Bonnet, 2011; Holl & Montoya, 2005; Masuda et al., 2013). Walworth et al. (2018) have even suggested that climate change could lead to a changing N metabolism of *Trichodesmium* spp., reducing new N inputs by this species. Their study showed that *Trichodesmium* sp. switched from N_2 fixation to uptake of dissolved organic nitrogen (DON) in response to long-term adaptation to high CO_2 conditions, if either iron or phosphorus was limiting. If diazotrophs switch their N metabolism under stressful conditions, this could potentially result in competition of diazotrophs and non-diazotrophs for DIN in a future ocean, where ocean acidification an enhanced nutrient limitation due to stronger stratification are possible threats to N_2 fixers. Masuda et al. (2013, 2022) have shown that *C. watsonii* is capable of DIN uptake and N_2 fixation in parallel, which suggests that diazotrophs could potentially be in competition with non-diazotrophic phytoplankton for DIN. While it seems that future conditions could favour N_2 fixers, the consequences for non-

diazotrophic phytoplankton could be very different. Diazotrophs could potentially outcompete non-diazotrophs in areas where DIN is available in pulses. As another consequence, N release by diazotrophs could be reduced and less DIN would be available for other phytoplankton groups. How phytoplankton community structure and the associated primary production and carbon sequestration could be affected by such changes remains to be investigated. For example, a side effect of ocean warming is an intensification of the stratification of the water column, which will likely reduce the nutrient supply from deeper waters (Laufkötter et al., 2015; Sarmiento et al., 2004; Steinacher et al., 2010). Changes in N_2 fixation could alter N limitation that will likely arise from this projected intensified stratification (Wrightson & Tagliabue, 2020), possibly benefiting phytoplankton productivity. While diazotrophy has the potential to balance N limitation, the process of N_2 fixation is limited by nutrients itself. Iron (Fe) an/or phosphorus (P) have been shown to often limit diazotrophs (Luo et al., 2014; Mills et al., 2004; Sañudo-Wilhelmy et al., 2001). The supply of these nutrients could also change in the future. The flux of P into the surface layer from deeper, nutrient rich waters will likely be reduced as a result of weaker mixing. Additionally, less Fe could be available to phytoplankton due to changes in Fe availability under more acidic conditions (D. Shi et al., 2010).

Previous studies have investigated the interplay between Fe- and/or P-limitation and elevated pCO_2 on diazotrophs, simulating prospected conditions in the ocean. An increased pCO_2 leads to enhanced N_2 fixation only under Fe-replete conditions (Boatman et al., 2018; Fu et al., 2008; Law et al., 2012; D. Shi et al., 2012; Walworth et al., 2018). When they investigated co-limitation of Fe and P on cultured *Trichodesmium* sp., Walworth et al. (2018) found increases of N_2 fixation only under Fe-replete conditions, independent of P conditions (replete or limited). In general, the effect of P-limitation seem on N_2 fixation seems to be independent of a changing pCO_2 (Garcia et al., 2013; Hutchins et al., 2007; Walworth et al., 2016). The interplay of nutrient limitation and ocean acidification or warming is not yet fully understood and further impedes future predictions of N_2 fixation. It is generally agreed that Fe-limitation overrides the positive effects of increasing pCO_2 in cyanobacterial diazotrophs (Fu et al., 2008; Gao et al., 2019; Hong et al., 2017; D. Shi et al., 2012; Walworth et al., 2016). However, Jiang et al. (2018) observed a decrease of cellular Fe demand of *Trichodesmium* sp. under increased temperatures due to a higher Fe use efficiency. Same seems to be applicable for P use according to this study, implying an alleviation of Fe- and possibly P-limitation through global warming. This finding is supported by Deng et al. (2022), who found that the degree to which P limitation negatively affects growth, N_2 and C

fixation rates of *C. watsonii* seems to be temperature dependent and alleviated by warming as well. Additionally, the study of Yang et al. (2021) confirms that Fe-use efficiency of *C. watsonii* is enhanced by ocean warming within certain temperature limits. Such an alleviation could expand the niche for diazotrophs into areas where Fe is limiting at present conditions, resulting in a global increase of marine N_2 fixation by ~22% over the next century (Jiang et al., 2018).

Climate change induced nutrient limitation might favour some species more than others. Church et al. (2005) state that the low surface-area-to-volume ratio of unicellular cyanobacteria probably facilitates their ability to compete for nutrients in the oligotrophic ocean. Other studies show that the contribution of picophytoplankton to total phytoplankton biomass increases with increasing temperature (Agawin et al., 2000; Morán et al., 2010). For instance, *C. watsonii* has been shown to have a higher temperature optimum than larger species like Trichodesmium spp. (Fu et al., 2014) and to be more abundant in warm and P-depleted areas and therefore likely to thrive in a future ocean (Deng et al., 2022). Accordingly, smaller phytoplankton might be favoured by ocean warming. In addition, fast-growing r-selected species like unicellular cyanobacteria might be favoured by an elevated pCO_2 compared to slower growing K-selected species (Hutchins et al., 2013). These findings of previous studies suggest that a small species like *C. watsonii* might outcompete larger diazotrophs like *Trichodesmium* spp. in a future ocean. Nevertheless, Jacq et al. (2014) report a decrease of N_2 fixation and growth rate of *C. watsonii* under low Fe conditions.

Overall, there are not only many factors to take into account when studying N_2 fixation, but also conflicting opinions about the effects of changing environmental factors on N_2 fixation. In addition to the contradictory picture of the response of primary production and N_2 fixation to climate change, most studies worked with cultured diazotrophs only and did not simulate in-situ conditions with multiple stressors. To date there are only few studies investigating combined stressors in more realistic scenarios (Boyd et al., 2018), but such studies are needed to reliably predict the future of N_2 fixation and primary production in times of climate change. Studies on the behaviour of N_2 fixing organisms in climate change scenarios in natural communities under in-situ conditions are lacking, as such experiments are difficult to conduct in the field. Additionally, many diazotrophs thought to contribute significantly to global N_2 fixation have not yet been cultivated (Zehr & Capone, 2020) and thus effects of climate change on these species remain unstudied and unpredictable. We need to learn more about players, their ecological interactions and the factors that control N_2 fixation, as well as

consider a combination of environmental factors and emerging ecological interactions (Gao et al., 2019; Landolfi et al., 2018). Furthermore, models have to be improved by accounting for variable response of different functional groups of diazotrophs, i.e. different species, to climate change conditions (Bopp et al., 2022). Clearly, a species-specific response of N_2 fixers to climate change would influence the phytoplankton community structure and diversity of diazotrophs in the future ocean (Gradoville, White, Böttjer, et al., 2014; Hutchins et al., 2013).

Questions arising from the text above are:

1.) Does a decreasing pH offset the stimulating effect of elevated pCO_2 and temperature on N_2 fixation?
2.) Could diazotrophs be in competition for dissolved inorganic nitrogen with non-diazotrophic phytoplankton?
3.) Are unicellular cyanobacteria likely to outcompete larger species in a future ocean?

To answer these questions, we performed an experiment with cultures of *Trichodesmium* sp. and *Crocosphaera watsonii*, strain WH8501, also known as unicellular cyanobacteria group B (UCYN-B) grown at the Mediterranean Institute of Oceanography (MIO) in Marseille, France. In June to October 2021, the experiment was conducted at the MIO to investigate the effect of combined ocean acidification and warming on the two diazotrophs. In the experiment presented in the following, we investigated the response of two globally significant diazotrophs to climate change scenarios, with a focus on the competition between a unicellular and a larger filamentous species and potential competition for DIN between diazotrophs and non-diazotrophs.

I-2 Material & Methods

We conducted a joint minicosm experiment at the Mediterranean Institute of Oceanography (MIO) in Marseille, France in September 2021 with cultures of the unicellular diazotroph *Crocosphaera watsonii* strain WH8501 and the filamentous N_2 fixer *Trichodesmium erythraeum*.

Sea water used for the culturing of the species and the experiment was collected from the Mediterranean Sea in a 1 m³ polyethylene tank. While the water was pumped into the tank it was filtered by 0.2 µm. Water from the tank was transferred to containers by siphoning when needed and was filtered again by 0.2 µm before further use.

I-2.1 Growth and acclimation of cultures

Batch cultures of *C. watsonii* WH8501 and *T. erythraeum* were grown in nitrogen-free YBC-II medium based on 0.2 µm filtered sea water (Chen et al., 1996) at 26°C under a light:dark cycle of 12h:12h with a daytime photon flux of ~250 µmol photons m^{-2} s^{-1}. Cultures were gradually adapted to lower phosphate concentrations than normally used in the YBC-II medium for 8 weeks to approximate natural phosphate concentrations. The final phosphate concentrations in experimental cultures was 25 µM. Four weeks prior to the experiment, the acclimation of cultures to the experimental conditions, i.e. target levels of pH and temperature, was started. The three scenarios tested in our experiment were the control (26°C, pH = 8.1), an intermediate treatment (28°C, pH = 8.0) and an extreme treatment (30°C, pH = 7.9). Temperature in the cultures was elevated from 26°C to 28°C and 30°C, respectively at a rate of 0.5°C increase per day. After eight days, the thermal acclimation was completed. From these temperature-acclimated cultures, we prepared new parent cultures by bubbling water with CO_2 until the targeted pH levels of 8.1, 8.0 and 7.9 were reached prior to the addition of diazotroph cultures. These new cultures were grown for another seven days, corresponding to about four generations and were then used for the experiment.

I-2.2 Experimental Setup

We conducted a minicosm experiment in 8 cylindric 40 L tanks made from methacrylate (Figure I-1). Every tank was equipped with a manual stirrer, a thermostat, a CO_2 diffusor and a sensor to measure pH and temperature that were connected to an automated system (IKS aquastar, IKS Aquastar Computer System GmbH, Karlsbad, Germany) maintaining temperature and pH in the tanks at the target levels. The IKS system measured temperature and pH constantly and maintained both parameters by controlling the thermostat and CO_2 diffusor in the tanks. The CO_2 diffusor was connected to a CO_2 bottle ($CO_2 \geq 99{,}9$ %, Alphagaz, Air Liquide, Metz, France) and the IKS system opened or closed the valve of this bottle automatically to adjust the pH if necessary. In addition, every tank was continuously bubbled with O_2 to avoid stratification inside the tanks.

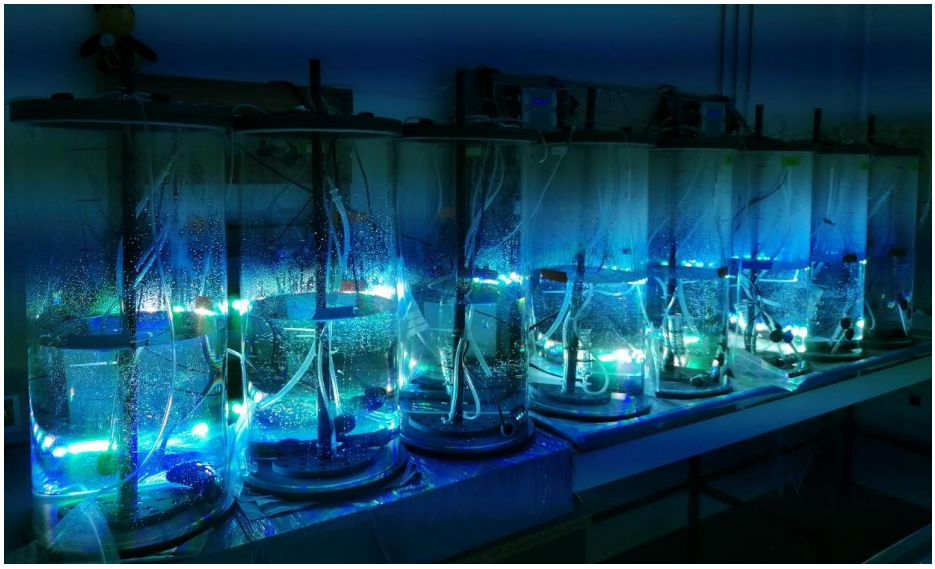

Figure I-1: *Experimental setup showing the minicosms under daylight conditions.*

Two tanks were used as control, with a temperature of 26°C and a pH of 8.1 (tanks 1A and 1B). Three tanks were used to simulate two different climate change scenarios corresponding to the RCP scenarios 4.5 (temperature of 28°C, pH 8.0; tanks 2A, 2B, 2C, hereafter referred to as intermediate treatment) or 6 (temperature of 30°C, pH 7.9; tanks 3A, 3B, 3C, hereafter referred to as extreme treatment), respectively (IPCC, 2014).

The tanks were kept in a temperature-controlled room with an ambient temperature of 26°C. Tanks were placed in front of lights simulating a natural daily light cycle with sunrise and sunset in the surface ocean (Alpheus LED lamps, Montgeron, France), such that every tank

received ~250 µmol photons m^{-2} s^{-1} during the daylight period. The light:dark cycle was 12h:12h.

The actual experiment was run for one week (day -2 to day 5). One day before the start of the experiment (day -3), sea water, which had been filtered twice by 0.2 µm by then, was transferred into the minicosms from the big tank. The tanks were filled with 25 L of sea water each and nutrients and trace metals corresponding to the concentrations in the YBC-II medium were added to the tanks. Phosphate was added to a final concentration of 25 µM only, corresponding to the concentration during the culturing process.

Diazotroph cultures were added to the minicosms on day -2 in a final concentration of ~1.5 x 10^4 cells ml^{-1} in each minicosm. On day 2, sodiumhydroxide solution (NaOH, 1 N) was added to the tanks to adjust the pH. An addition of 1 mL NaOH raised the pH by 0.06. Accordingly, 1 mL NaOH was added to tanks 2A and 2B, 1.5 mL to minicosm 2C and 0.5 mL to tanks 3A, 3B and 3C. The pH in the control treatments did not require an adjustment. From day -2 to day 5, temperature and salinity were measured daily with a conductivity-salinity-temperature meter (YSI pro30, Xylem Inc. New York, USA). In addition, daily samples were taken to determine cell abundance of the diazotrophs via microscopy counts and pH via spectrophotometry. Samples for pH measurements were collected into 10 cm cylindrical optical glass cells (120-OS.100 Hellma, Paris, France) and samples were kept at 25°C before and during spectrophotometry. The analysis was performed manually after the addition of purified Cresol Purple following the protocol described by Clayton and Byrne (1993). Absorbance was measured at three wavelengths with a Carry 60 instrument (Santa Clara, USA). The accuracy of the method was verified by the analysis of TRIS solutions (provided by Scripps Institution of Oceanography) and our measurements of the pH did not differ from the certified values by more than 0.005 pH units. In combination with measurements of total alkalinity (TA) at the beginning and end of the experiment (days -2 and 5), these pH measurements were used to monitor the carbonate chemistry. CO_2 concentrations and other parameters of the carbonate system were calculated using the *seacarb* R package. TA was determined from triplicate samples with a volume of 50 mL each by potentiometric titration on a 888 Titrando titrator coupled to a glass electrode plues (Metrohm, France) and a pt1000 thermometer. TA was calculated as described by Dickson et al. (2007).

Every other day, starting on day -2, samples for the determination of the natural abundance of $\delta^{15}N$ and $\delta^{13}C$ of particulate organic material (POM), concentrations of NO_2^-, NO_3^-,

PO_4^{3-}, NH_4^+, dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), as well as for counts of heterotrophic bacteria via flow cytometry were taken. Samples for nutrient concentrations were filtered by 0.2 µm and were stored frozen (-20°C) until analysis. Inorganic nutrient concentrations were measured with a segmented flow analyser (AAIII HR, Seal Analytical) according to Aminot and Kérouel (2009), except from NH_4^+ concentrations, which were determined via fluorometry directly after sampling. Concentrations of total dissolved nitrogen and phosphate were determined by high-temperature (120°C) persulfate wet oxidation mineralization (Pujo-Pay & Raimbault, 1994) and subtracting phosphate and nitrate concentrations from the total dissolved nitrogen and phosphate concentrations to obtain the correspondent dissolved fraction (DON and DOP concentrations). Detection limits were 0.02 µM for NH_4^+, 0.05 µM for NO_3^-, 0.01 µM for NO_2^- , 0.05 µM for PO_4^{3-}, 0.02 µM for DON and 0.02 µM for DOP.

For the analysis of the abundance of heterotrophic bacteria via flow cytometry, 1.98 mL were subsampled from each tank in Eppendorf tubes. Samples were fixed with 200 µL of a mix of glutaraldehyde (25%) and pluronic acid (10%) at a 100:10 dilution for 15 min at room temperature and then flash-frozen in liquid nitrogen and stored at -80°C until analysis. Samples were analysed at the PRECYM Flow Cytometry Platform at the Mediterranean Institute of Oceanography in Marseille, France. Prior to analysis, samples were stained with 2 µL SYBR Green I at 1/10x of the stock solution (10,000x Thermo Fischer Scientific, France) and then measured using a CytoFLEX flow cytometer (Beckman Coulter, France) fitted with violet (405 nm), blue (488 nm) and red (638 nm) lasers. Trucount TM beads (BD Biosciences, France) were used to determine the analysed volume. Additionally, fluoresbrite 2 µm latex beads (Polysciences Inc., Warrington, PA, USA) were added as internal size standards. Samples were run at low speed (10-30 µL min^{-1}) and heterotrophic bacteria were identified in a plot of side scatter (SSC) versus green fluorescence.

In addition to the measurements described above which were done in collaboration with Alba Filella and the help of a student, I collected water to measure bulk N_2 fixation and C fixation rates and single-cell rates of N_2 fixation, NO_3^-- and NH_4^+- assimilation on day 0, day 2 and day 4. Prior to each sampling, the tanks were carefully homogenized with the custom-made stirrer to minimise adhesion to the walls of the tanks and sedimentation.

I-2.3 Bulk rate measurements

To determine community N_2 fixation rates, the dissolved method was applied (Klawonn et al., 2015; Mohr et al., 2010). Two weeks prior to the experiment, ^{15}N enriched seawater was prepared in 600 mL Schott bottles equipped with a septum cap by filtration (0.2 µm) of water collected from the Mediterranean Sea and subsequent adjustment of the pH to the three target scenarios by bubbling with CO_2. 6 mL of $^{15}N_2$ gas was added to every bottle with a gas-tight syringe. Bottles were shaken vigorously for one minute and then stored horizontally at 4°C until usage during the experiment.

During the actual experiment, 30 mL of enriched seawater was siphoned into 300 mL polycarbonate bottles and 30 µL of $NaH^{13}CO_3$ (0.1 M) was pipetted into the bottles prior to sampling. Bottles were then filled directly from the minicosms until overflowing, avoiding air bubbles, and sealed gas-tight with a septum cap. The bottles were then incubated for 24 h in water baths set to the target temperature of the respective treatment, receiving the same light:dark cycle as the minicosms. After the incubation, bottles were inverted several times and 12 mL of the sample were removed with a pipette and transferred to an Exetainer® for the determination of the ^{15}N enrichment in the water via membrane inlet mass spectrometry (MIMS). Afterwards, the whole water sample was filtered onto precombusted (450°C, 4 h) GF/F filters (Advantec GF-075, nominal pore size 0.3 µm) with a peristaltic pump. Filters were then placed into Eppendorf tubes and stored at -20°C until analysis via isotope ratio mass spectrometry (IRMS).

Bulk N_2 fixation rates were calculated after Montoya et al. (1996) as following:

$$N_2\ fixation\ rate = \frac{(A_{PN}^{final} - A_{PN}^{t=0})}{(A_{N_2} - A_{PN}^{t=0})} * \frac{[PN]}{\Delta t} \quad (1)$$

Where A = atom% ^{15}N in the particulate organic nitrogen (*PN*) at the end (*final*) or beginning ($t = 0$) of the incubation or in the dissolved N2 pool (N_2).

Bulk C fixation rates were calculated after Collos (1987) as follows:

$$V_C\ (t) = \frac{(^{13}C_{final} - ^{13}C_{initial})}{(DIC - ^{13}C_{initial}) * t} \quad (2)$$

V_C is the specific uptake rate (C uptake per unit particulate C per unit time), $^{13}C_{final}$ is the concentration of ^{13}C in atom% in the sample after incubation, $^{13}C_{initial}$ is the concentration of ^{13}C in atom% at the beginning of the incubation, hence the natural abundance of ^{13}C in the

sample, DIC – $^{13}C_{initial}$ is the concentration of ^{13}C in the dissolved phase, and t is the incubation time. To obtain the transport rate of C normalised to biomass, $V_C(t)$ is then multiplied by the concentration of particulate organic carbon (POC).

I-2.4 Single-cell rates of N_2 fixation, nitrate and ammonium assimilation

Single-cell analysis was performed on only one sample per treatment, which was collected from either 1A, 2B or 3C, respectively.

For the determination of single-cell N_2 fixation rates, 6 mL of ^{15}N enriched sea water, prepared as explained in the previous subsection, was added to 60 mL glass vials, which were then filled with water from the respective minicosm until overflowing. Vials were sealed gas-tight, inverted 30 times to mix the sample and placed into the incubators matching the respective scenario for 24 hours. Samples where then filtered onto 25 mm polycarbonate filters (0.2 µm pore-size) with a peristaltic pump. Filters were preserved with 2 mL of a 4 % paraformaldehyde solution and stored at -20°C until analysis via nanometer-scale secondary ion mass spectrometry (NanoSIMS).

For NO_3^- and NH_4^+ assimilation rates, water was collected from the minicosms in 60 mL glass vials before noon every other day over the course of the experiment. $Na^{15}NO_3$ or $^{15}NH_4Cl$, respectively, was added to a final concentration of 0.03 µM and vials were inverted 30 times before being placed in the incubators. Samples for the analysis of NO_3^- uptake were incubated for 3-4 hours, while the incubation of samples enriched with ^{15}N-NH_4 was terminated after 2 hours. The incubation covered the period of maximum daylight, when phytoplankton is thought to be most active in terms of nitrogen assimilation (Cochlan et al., 1991). Incubations were terminated by filtration of the whole sample onto 25 mm polycarbonate filters (same as for N_2 fixation rates) with a peristaltic pump. Filters were handled in the same way as described for N_2 fixation rates.

As a first step for NanoSIMS analysis, regions of interest (ROIs) had to be marked on the filters with a laser dissection microscope (Leica LMD 7000). Healthy cells, detected by active fluorescence of Chlorophyll *a* and phycoerythrin, were identified and marked. At least 20 cells per filter were analysed. A 10 mm piece was cut out of the filter, mounted onto an aluminium stub equipped with a conductive graphite sticky tape layer and sputtered with 30 nm gold with a Cressington 108 auto Sputter Coater (Watford, United Kingdom) to ensure conductivity. Filters were then placed into the NanoSIMS for the analysis of ^{15}N and ^{13}C

enrichment with a Cameca NanoSIMS 50 L (Cameca, Paris, France). In brief (after Nuñez et al., 2018), material is eroded from the filter surface under high vacuum (usually 10^{-10} bar) by implantation with a caesium primary ion beam (Figure I-2). The emitted material consists of secondary ions, which are accelerated into a mass spectrometer (MS), where they are analysed according to their mass. Due to the coaxial setup of the NanoSIMS, the secondary ions must have the opposite charge from the primary ions to enable extraction to the mass spectrometer (Mueller et al., 2013). With the NanoSIMS method, ion maps of the filter can be created and the isotopic composition of single cells can be identified. Every third sample is a natural abundance sample without tracer, hence a known ratio of heavy to light isotopes (in my case $^{13}C/^{12}C$ and $^{15}N/^{14}N$) to monitor the measurement. The lateral resolution of the NanoSIMS is up to 50 nm. The precision of the measurements was calculated as three times the standard deviation of natural abundance samples and was 0.001 atom% for ^{13}C and 0.0003 atom% for ^{15}N. Images of secondary electrons ($^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$, $^{31}P^-$) were recorded simultaneously using mass detectors equipped with electron multipliers (Hamamatsu) for the ions. The mass resolving power was adjusted to be sufficient to suppress interferences at all masses, allowing e.g. the separation of $^{13}C^-$ from interfering ions such as $^{12}C^1H^-$. Prior to the analysis, sample areas of 50×50 µm were sputtered for 10 – 15 min with 600 pA to erode the gold, clean the surface and reach the steady state of secondary ion formation. The primary ion beam current during the analysis was 1 pA. The scanning parameters were 512×512 pixels for areas of 45 to 49 µm, with a dwell time of 250 µs per pixel. 60 planes were analysed and accumulated using the software LOOK@NanoSIMS (Polerecky et al., 2012). The planes were checked for inconsistencies and the drift was corrected. Regions of interest (ROIs) were defined semi automatically based on ^{31}P as the primary template by applying the interactive thresholding function of the software. Each ROI represented a single cell. In those cases, where the cells were located right next to each other or were slightly overlapping, the ROIs were defined manually. Isotope enrichment (^{13}C, ^{15}N) in atom% was obtained from ion counts accumulated over all ROI pixels and over all selected planes by the software.

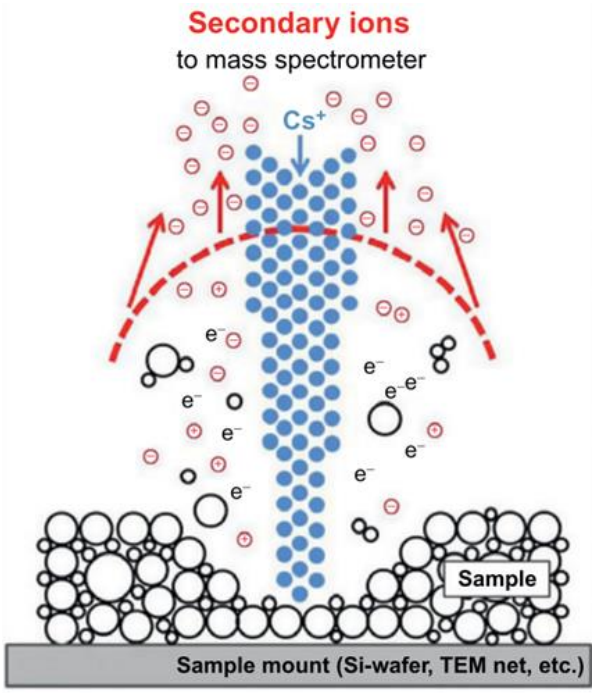

Figure I-2: *Schematic of the nanoSIMS measurement. Figure from Mueller et al. (2013).*

Single cell rates of N_2 fixation were calculated in the same way as bulk rates as explained in the previous section, but for a single cell in fmol cell^{-1} h^{-1}.

NO_3^- and NH_4^+ uptake rates were calculated following the same principal after Dugdale and Wilkerson (1986) as:

$$V_N\ (t) = \frac{15N_{xs,\ sample}}{\left(15N_{xs,\ pool} - F\right) * \mathrm{t}} \tag{3}$$

Where V_N (t) is the specific uptake rate (N taken up per unit particulate N per unit time), $^{15}N_{xs,\ sample}$ is the atom% excess in the sample ($^{15}N_{xs,\ sample} = {}^{15}N_s - F$ with $^{15}N_s$ being the atom% ^{15}N in the sample), $^{15}N_{xs,\ pool}$ is the atom% ^{15}N in the initially labelled fraction, F is the natural abundance of ^{15}N and t is the incubation time. NH_4^+ uptake rates were calculated assuming a NH_4^+ concentration of 0.02 µM, as concentrations were below detection limit at all times during the experiment.

The volumetric rate ρ_N (t) (N uptake in concentration units) was then calculated using V (t) and the PON concentration of the respective cell:

$$\rho_N\ (t) = V(t) * PON\ (t) \tag{4}$$

The PON content of each cell was estimated from its volume, which was obtained from nanoSIMS analysis and the POC content assuming a C:N ratio of 5.

To test for significant differences of uptake rates between the treatments, a Kruskal-Wallis-test was applied using the “kruskal.test()” function from the package “stats” in the software R (R Core Team, 2023). Subsequently, a Wilcoxon Rank Sum test was done using the function “pairwise.wilcox.test()” from the package “stats” (R Core Team, 2023) for pairwise comparisons.

I-3 Results

Diazotroph cultures were added to the tanks on day -1, and while *Crocosphaera watsonii* cells continued growing, *Trichodesmium* sp. cells could not be found via microscopy on day 0 anymore. Therefore, results presented here show the response of *C. watsonii* to the different climate change scenarios only.

Salinity in the tanks stayed constant between 38.8 and 39.1 during the experiment. The temperature in the tanks was quite stable over the course of the experiment and was close to the target scenarios (Figure I-3). The pH was more variable in the beginning of the experiment due to mixing processes with air during the filling of the tanks, but stabilized after the addition of NaOH on day -1. At the start of the experiment on day 0, the pH clearly differed between the three scenarios and was close to the respective target values (Figure I-3). The pH was well controlled by the IKS system over the course of the experiment and stayed relatively constant at the targeted values.

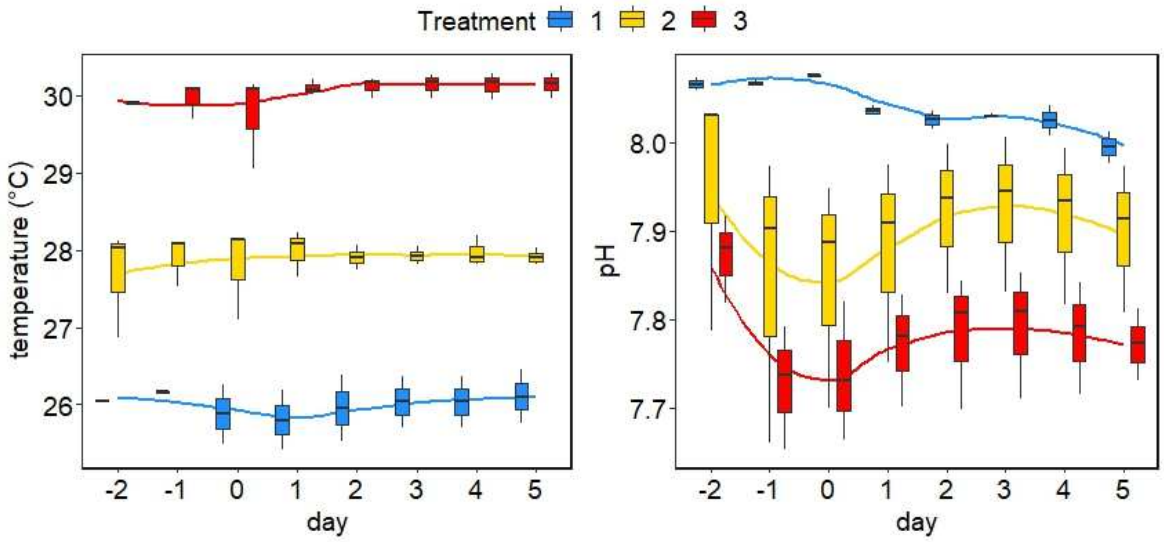

Figure I-3: *temperature and pH over the course of the experiment. The colours of the bars represent the three different treatments. Blue is the control treatment, yellow is the intermediate treatment and red is the extreme treatment.*

Phosphate concentrations stayed constant around 20 µM in all minicosms of the control and extreme treatments and minicosms a and b of the intermediate treatment during the experiment. The increase of phosphate from day -2 to day 0 results from the addition of the diazotroph cultures after the measurement of nutrient concentrations on day -2. The medium added with the cultures contained about 25 µM of phosphate. However, phosphate

concentrations were around 28 µM in the intermediate treatment in minicosm b and minicosm c (only on day 3) over the course of the experiment (Figure I-4). Ammonium concentrations remained below the detection limit (0.02 µM) in all minicosms (data not shown). Nitrate concentrations were more variable, with a tendency to an increase after day 2 (Figure I-4).

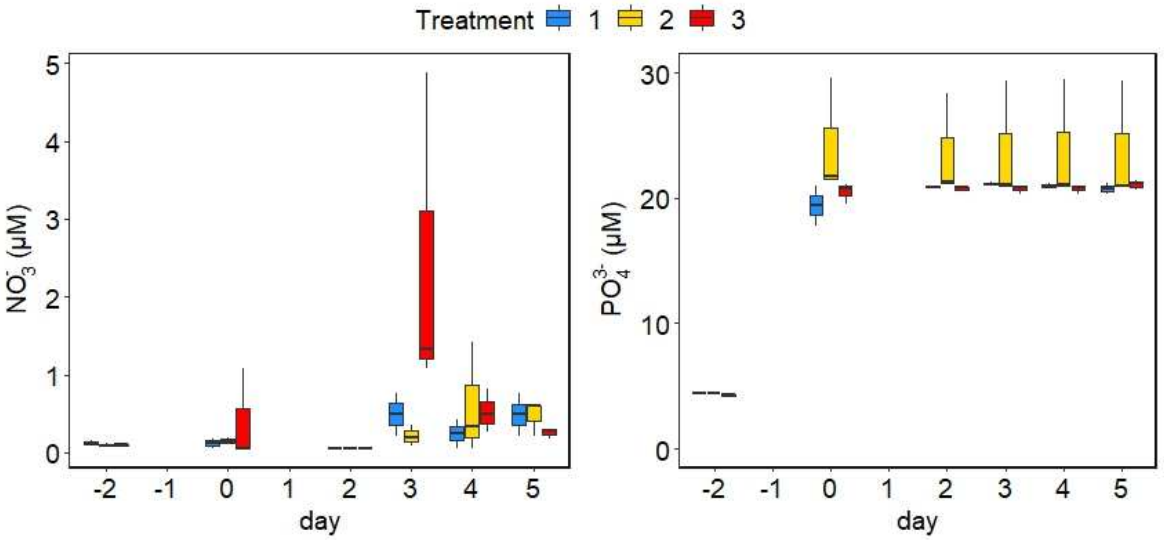

Figure I-4: *Phosphate and nitrate concentrations over the course of the experiment. The colours of the bars represent the three different treatments. Blue is the control treatment, yellow is the intermediate treatment and red is the extreme treatment.*

The abundance of *C. watsonii* increased in all treatments from day -2 until day 0 (Figure I-5). The increase was stronger in the control and high temperature scenarios than in the intermediate one. However, while cell abundance reached a plateau between day 0 and day 2 in the control and high temperature treatments and dropped after day 2, cell abundance continued to increase in the intermediate treatment until day 4. The decrease of cells was strongest in the high temperature treatment.

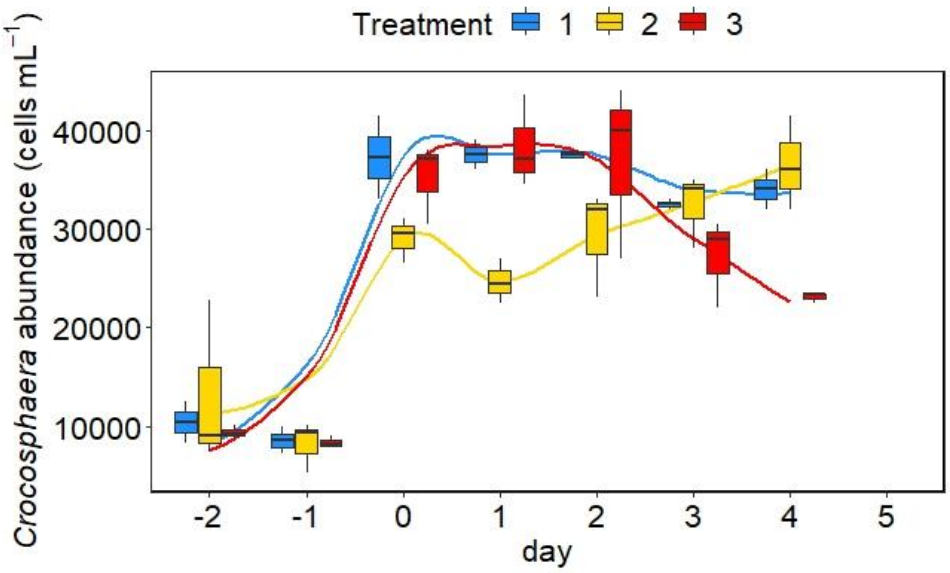

Figure I-5: *Abundance of Crocosphaera watsonii cells over the course of the experiment. The colours of the bars represent the three different treatments. Blue is the control treatment, yellow is the intermediate treatment and red is the extreme treatment.*

The abundance of bacteria increased in the first two days after filling of the tanks and stayed relatively constant around $7.5*10^6$ cells ml^{-1} over the course of the experiment (Figure I-6). A slightly higher abundance of bacteria was observed in the extreme treatment.

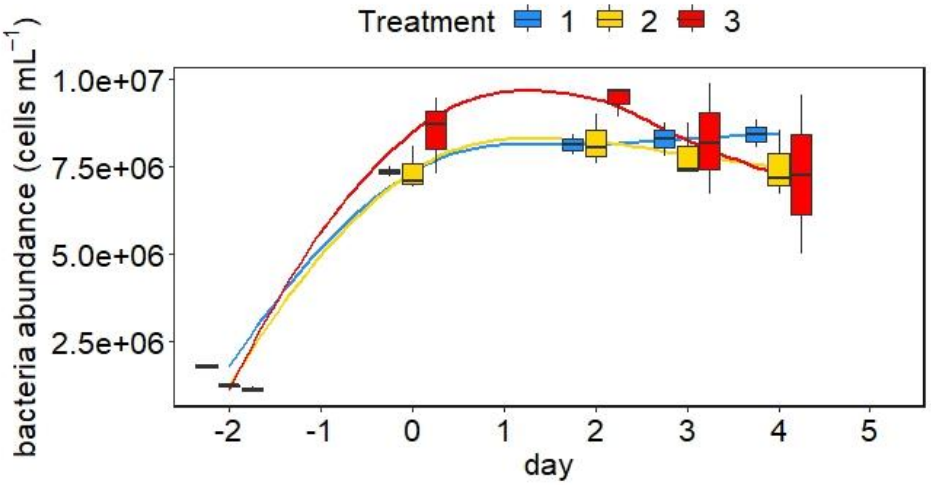

Figure I-6: *abundance of heterotrophic bacteria over the course of the experiment The colours of the bars represent the three different treatments. Blue is the control treatment, yellow is the intermediate treatment and red is the extreme treatment.*

The C:N ratio in *C. watsonii* cells increased over the course of the experiment in all treatments (Figure I-7). At the start of the experiment, C:N ratios were highest in the control treatment (around 9), but ratios aligned between treatments from day 2 onwards to values between 11 and 14.

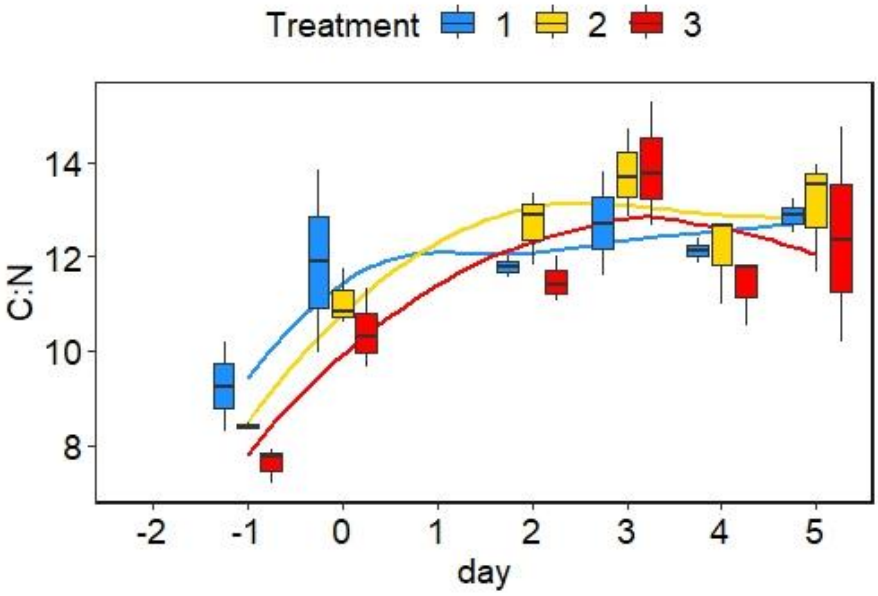

Figure I-7: *C:N ratios of C-watsonii over the course of the experiment. Colour code as in Figure I-1.*

Bulk N_2 and C fixation rates were maximal in the intermediate treatment on day 2, mirroring the behaviour of cell abundance (Figure I-8). Both rates were highest in the intermediate treatment at all three sampling points, while the rates were lowest in the control treatment at the beginning of the experiment (day 0). On days two and four, N_2 fixation rates were lowest

in the extreme temperature treatment, but C fixation rates were still lowest in the control treatment on day two.

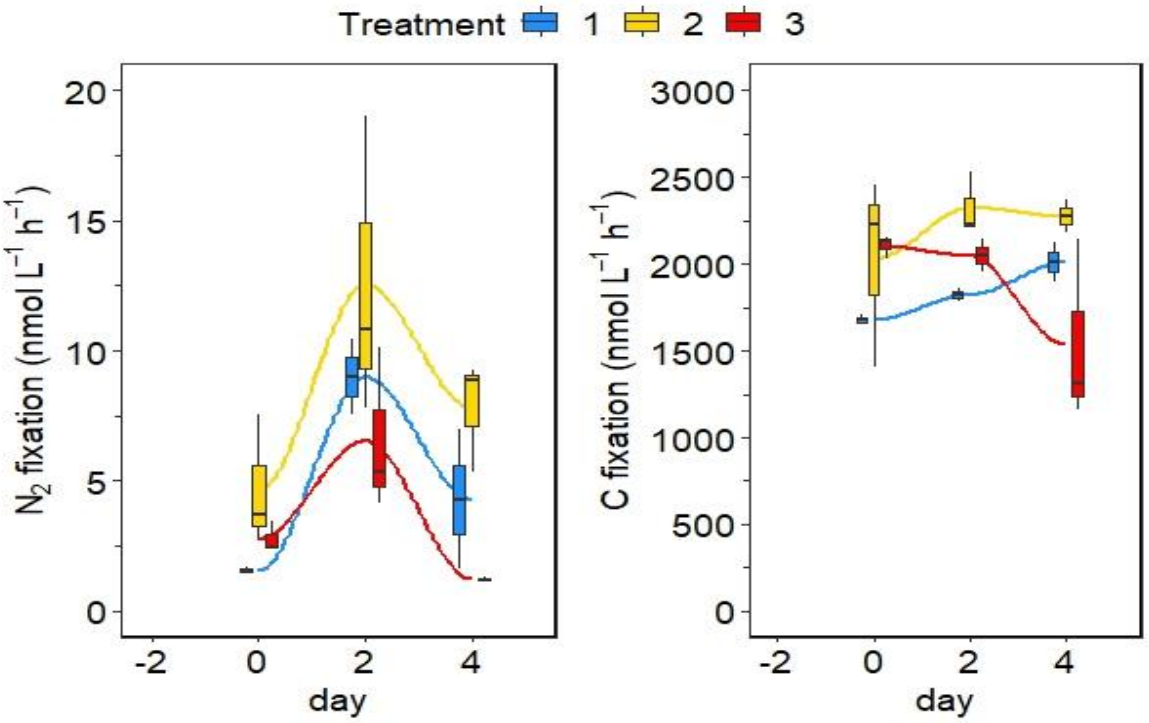

Figure I-8: *Bulk N_2 and C fixation rates at the three sampling points. Colour code as in Figure I-1.*

The variability of uptake rates between different cells within the community was high (Figure I-9). The precision of the measurements as calculated from the standard deviation of natural abundance samples (n = 100) was 0.001 atm% for ^{13}C and 0.003 for ^{15}N. Cell-specific N_2 fixation rates in principal showed the same pattern as the bulk rates, but trends were less pronounced in the cell-specific rates due to the high inter-community variability. While both NO_3^- and NH_4^+ uptake rates were quite similar between treatments, N_2 fixation rates gradually increased during the experiment and were highest in the intermediate treatment. In contrast to the bulk rates, per cell N_2 fixation rates did not drop after day 2. Unexpectedly, cell-specific NO_3^- uptake rates were up to 10-fold higher than cell-specific NH_4^+ uptake rates (Figure I-9). Almost no DIN was assimilated by the diazotrophs on day 4, which was also applicable to N_2 fixation in the control and high temperature treatments. However, N_2 fixation in the intermediate treatment was still elevated compared to the other sampling days and treatments in day 4. A Mann-Whitney-U test was performed on the determined rates and results are shown in Table A-1 in the appendix. There was no clear or consistent pattern of the differences of N uptake rates between treatments. Except from

day 0, cell-specific N_2 fixation rates in the intermediate treatment were significantly higher than in the other treatments ($p < 0.01$), while the extreme treatment did not significantly differ from the control. For NO_3^- uptake, rates in the extreme treatment were significantly different from both the control and the intermediate treatment on day 0 ($p < 0.01$) and rates were lowest on day 4 in the intermediate treatment. NH_4^+ uptake rates on the other hand were significantly higher in the control treatment than in the climate change scenarios on day 0 ($p < 0.01$), but significantly higher in the extreme treatment than in the control on day 2 ($p = 0.006$).

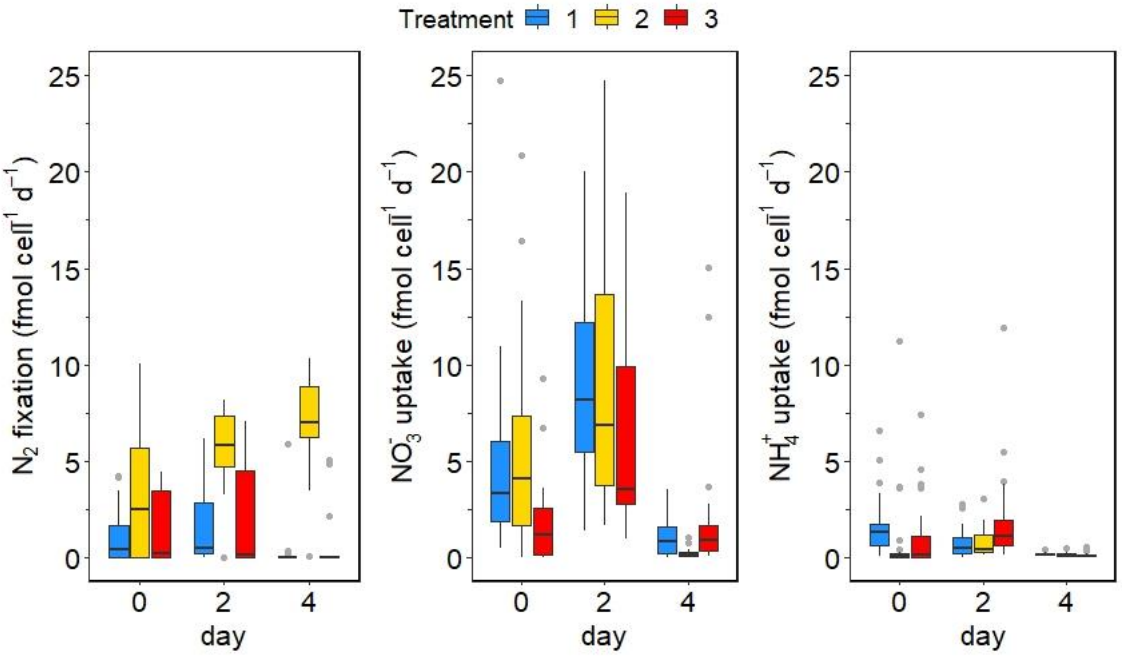

Figure I-9: *cell-specific rates in fmol cell $^{-1}$ day $^{-1}$ for N_2 fixation, NO_3^- assimilation and NH_4^+ assimilation (from left to right). Outliers are shown as grey dots.*

I-4 Discussion

The experiment presented here is one of the few available studies on the response of diazotrophs to a combined alteration of controlling variables, here simultaneous changes of temperature and pH. In addition, we worked with larger volumes simulating more natural conditions compared to previous studies, who experiment with semi-continuous cultures in small volumes (e.g. Fu et al., 2007, 2008; Garcia, Fu, & Hutchins, 2013; Hutchins et al., 2007; Kranz et al., 2010; Levitan et al., 2007). As *Trichodesmium* sp. cells had all died by day 0, the competition between the two diazotrophs could not be investigated. The following discussion therefore focuses on the short-term response of N_2 fixation and the N-metabolism of *Crocosphaera watsonii* WH8501 to increasing temperature and decreasing pH.

Conditions stayed quite constant over the course of the experiment and matched the targeted scenarios. Except from phosphorus, nutrient and trace metal concentrations in the minicosms matched the concentrations in the YPCII medium and therefore I am certain that nutrient or trace metal limitations were not an issue. The constant concentrations of phosphorus (Figure I-4) indicate that this nutrient was not depleted by the diazotrophs. Phosphorus concentrations in the intermediate treatment in minicosm b and minicosm c (only on day 3) were higher than in the other tanks (around 28 µM, Figure I-4), indicating even less consumption in these two minicosms. I therefore can also exclude phosphorus limitation as a stressor, as this nutrient was available at high concentrations throughout the experiment.

I-4.1 Changes in the abundance, elemental stoichiometry, C and N_2 fixation of *C. watsonii*

In all treatments, cells started to grow after addition to the minicosms and cell numbers increased enormously from days 0 to 2 (Figure I-5). Interestingly, the development of cell abundance showed a similar pattern in the control and extreme treatments, while cells in the intermediate treatment behaved differently. After day 2, the abundance of diazotrophs decreased rapidly in the extreme treatment and less pronounced in the control treatment, while cells continued growing in the intermediate treatment. This could indicate that conditions in the intermediate treatment were optimal for the growth of *C. watsonii.* Indeed, it has been shown that 28 - 30°C is the optimum growth temperature for this species (Fu et al., 2014). The rapid decline of cell numbers in the extreme treatment after day 2 however implies that a temperature of 30°C in combination with a lower pH had some kind of

repressing effect on *C. watsonii*. While an increasing temperature up to 30°C alone does not seem to reduce the growth of this diazotroph (Fu et al., 2014), the combined alteration of temperature and pH in our experiment seems to have stressed cells to some extent.

The pattern of cell abundance was mirrored by the response of N_2 fixation rates. Rates were highest in the intermediate treatment and showed a similar behaviour between the control and extreme treatments (Figures I-8, I-9). Somehow, cell-specific N_2 fixation rates continued to increase after day 2, while bulk rates showed a slight decrease towards the end of the experiment. Nevertheless, rates in the intermediate treatment were significantly higher compared to the control and extreme treatments on days 2 and 4 (Table A-1). N_2 fixation in *C. watsonii* was enhanced by an increase in pCO_2 in our experiment, but only if temperature did not exceed 28°C. The development of C fixation rates over the course of the experiment was similar to that of N_2 fixation, with highest rates in the intermediate treatment and a sharp decline in the extreme treatment after day 2. Unlike N_2 fixation rates, C fixation rates in the control treatment did not decrease after day 2 but continued to increase. Growth and activity of *C. watsonii* did not seem to be enhanced by an increasing pCO_2, if temperature exceeded 28°C, but rather the opposite was the case.

From these observations, it can be concluded that temperature seems to have a greater impact on the growth and activity of *C. watsonii* than does pH, at least within the ranges tested in this study. Temperature in the control treatment was below the optimum for this species, while temperature in the extreme treatment tackled the upper limit for growth of *C. watsonii* (Fu et al., 2014). 28°C was the optimum temperature for growth as well as C and N_2 fixation of *C. watsonii* in our study and the slightly reduced pH in this scenario had no negative effect on the diazotroph, as cells continued growing steadily. Still, the rapid decline of cell numbers as well as C and N_2 fixation rates in the extreme treatment seems to confirm the hypothesis presented above that the combination of elevated temperature and reduced pH was stressful for *C. watsonii*. Contradictory to our results, Levitan et al. (2010) observed that high pCO_2 elevated N_2 fixation rates independently of temperature (25°C or 31°C) in *Trichodesmium* sp.. This points to a species-specific response of N_2 fixation to climate change conditions and argues against general assumptions in global ecosystem models. However, it is of course difficult to draw conclusions from only two experiments and more research on these issues is needed. Our results give hints that a combined alteration of pCO_2 and temperature possibly affects *C. watsonii* differently than changes of a single stressor alone. Still, more studies are

needed to fully understand the consequences of environmental changes on this species and other diazotrophs.

I expected changes of the elemental stoichiometry of *C. watsonii* alongside changes in N and C uptake. Indeed, the C:N ratio of *C. watsonii* increased from day -1 to day 0 from values between 8 and 10 to values around 12 to 14, which is much higher than the natural C:N ratio of *C. watsonii* that fluctuates between 5 and 9 (Dron et al., 2012). C:N ratios were highest in the control treatment at the beginning of the experiment, but ratios were highly variable between treatments and sampling days (Figure I-7). There was no clear difference of the C:N ratios of *C. watsonii* between the treatments. Similarly, in the study of Gradoville et al. (2014), the C:N ratio of *C. watsonii* fluctuated between 5.5 and 22 over the diel cycle, independent of different pCO_2 levels tested. The authors report a stimulating effect of elevated pCO_2 on growth, PN and PC production. Studies investigating the effect of elevated pCO_2 combined with increased temperature showed no change or clear effect on the C:N ratios of *Trichodesmium* spp. (Eichner et al., 2014; Hutchins et al., 2007; Levitan et al., 2010). Opposite to these observations, another study reported a slight increase of C:N ratios for *Trichodesmium* spp. under elevated pCO_2 (Levitan et al., 2007). As there is no consistency between studies regarding the response of primary production and N_2 fixation of diazotrophs to climate change, it is not surprising that the diverse results are reflected in unclear changes of elemental stoichiometry. An increase of the C:N ratio can either be the result of increasing C fixation, or reduced N_2 fixation. In our dataset, the increase in cell abundance from day -2 to day 2 is reflected in an increase of POC, while PON of *C. watsonii* increased only slightly (Figure A-1 in the appendix). However, *C. watsonii* might have additionally used an alternative C source than DIC. Elevated C:N ratios have been attributed to the accumulation of organic carbon reserves to fuel N_2 fixation and other cellular processes in the dark by Gradoville et al. (2014). Additionally, in the companion study to the experiment presented here, Filella et al. (submitted) have shown that DOC is used as a C source by *C. watsonii* in response to the climate change scenarios and helps the diazotroph to maintain higher N_2 fixation rates under potentially stressful conditions. While we did not identify a clear effect of elevated pCO_2 and temperature on C and N content of *C. watsonii*, there are hints for the consumption of DOC that are worth further exploration.

Deng et al. (2022) have observed that *C. watsonii* is more abundant in warmer, P-limited areas and this study concludes that the unicellular diazotroph will become more abundant in the future, as P-limitation seems to be relieved by elevated temperature in this species.

Similar observations were made by Yang et al. (2021), who found that *C. watsonii* is well adapted to Fe-limited, warm waters within certain limits and N_2 fixation rates of this species could increase by 91 %, especially in high-latitude areas. While it appears that *C. watsonii* has a great ability to cope with prospected conditions, Deng et al. (2022) also report a decrease of cell size associated with warming, which could have an effect on the sinking velocities of dead biomass and potentially weaken the biological pump. This aspect demands more attention in future studies.

I-4.2 DIN assimilation and potential competition between diazotrophs and non-diazotrophs

Another issue my experiment addressed was the potential competition of diazotrophs and non-diazotrophs for DIN. Therefore, DIN uptake rates were determined to investigate the effect of the two climate change scenarios on the N metabolism of *C. watsonii*. Even though both NO_3^- and NH_4^+ were assimilated by the diazotroph, I did not find a clear switch in N metabolism under the scenarios tested. There was no clear trend in DIN uptake rates neither between treatments, nor between time points during our experiment. Thus, an interesting finding is that while no single-cell N_2 fixation rates in the control and extreme treatments were measureable on day 4, *C. watsonii* consumed NO_3^- at low rates in these two treatments, but not in the intermediate treatment, where N_2 fixation was still high (Figure I-9). This observation might hint at a switch from N_2 fixation to NO_3^- uptake under stressful conditions. However, this interpretation must be treated with caution, as the variability in our single-cell uptake rates was very high. Walworth et al. (2018) have shown that *Trichodesmium* sp. assimilates dissolved organic nitrogen (DON) under elevated pCO_2 conditions. I did not determine DON uptake of *C. watsonii* and accordingly do not have any information on the consumption of DON by this species. However, as Filella et al. (submitted) have shown, DOC supports N_2 fixation of *C. watsonii* under stressful conditions. Therefore, it seems likely that DON could be an alternative N source for this species as well, as suggested by Korth et al. (2012) for the Baltic Sea. .

Surprisingly, NO_3^- uptake rates were higher than NH_4^+ uptake rates (Figure I-9). Even though NH_4^+ is energetically cheaper and preferred over NO_3^- in most cases (Dugdale & Goering, 1967; P. A. Thompson et al., 1989), *C. watsonii* seems to have preferred NO_3^-. However, we cannot rule out the possibility that NH_4^+ was consumed by bacteria instead of

the diazotroph. The abundance of heterotrophic bacteria increased over the first two days of the experiment and then stayed constant (Figure I-6). As the background of nanoSIMS filters was highly enriched in ^{15}N, especially in the ^{15}N-NH_4^+ incubations (Figure A-2) in the appendix), it is possible that a great part of the ^{15}N-NH_4^+ added to our samples was assimilated by bacteria instead of *C. watsonii*. This might be the reason, why I unexpectedly measured higher NO_3^- than NH_4^+ uptake rates.

Dekaezemacker and Bonnet (2011) observed that *C. watsonii* is able to simultaneously fix N_2 and consume both NO_3^- and NH_4^+, with NO_3^- being consumed at lower rates than NH_4^+. The same study reports an inhibition of N_2 fixation in *C. watsonii* WH8501 by a NH_4^+ concentration > 0.2 µM, while no inhibition by NO_3^- was observed. No inhibition of N_2 fixation of *C. watsonii* by NO_3^- was also demonstrated by Großkopf et al. (2012). Masuda et al. (2022) investigated the potential of *C. watsonii* to assimilate DIN and found that both NH_4^+ and NO_3^- were consumed by the diazotroph. In an earlier study, the same authors even found that the growth of *C. watsonii* was limited, if cells were grown in a NH_4^+ free medium, while N_2 fixation rates were independent of NH_4^+ availability at low concentrations (Masuda et al., 2013). Even though no rates were measured by Masuda et al. (2013, 2022), they concluded that the diazotroph is likely to be in competition for DIN with non-diazotrophic phytoplankton, which is also suggested by my results. The larger diazotroph *Trichodesmium* spp. has been shown to reduce its N_2 fixation even in the presence of only 0.5 µM of DIN (Holl & Montoya, 2005), while other studies suggest an inhibition if DIN concentrations exceed several µM (Capone et al., 1990; Mulholland et al., 2001; Ohki et al., 1991) and that *Trichodesmium* spp. is capable of simultaneous N_2 fixation and DIN uptake (Mulholland et al., 1999; Mulholland & Capone, 1999) .

C. watsonii seems to continue fixing N_2 at a constant rate in the presence of DIN. This ability gives the species enormous advantages in N limited regions of the ocean, but also allows it to occur where DIN is available and possibly outcompete other phytoplankton species. Further, it seems that the release of previously fixed N as NH_4^+ or DON by *C. watsonii* is higher, if there is no NH_4^+ present and that assimilated NH_4^+ is not released by the diazotroph (Masuda et al., 2013). These authors further concluded that N-limitation results in a lower retention of fixed N compared to conditions, in which DIN is available. Consequently, *C. watsonii* is likely to be in competition for DIN with non-diazotrophs.

Mulholland and Capone (1999) stress the importance of recycling of N in extremely oligotrophic areas, where N_2 fixation and NH_4^+ uptake are likely tightly coupled in

diazotrophs. The large variability in cell-specific N assimilation rates implies a high capacity of *C. watsonii* to cope well with changes of the environmental conditions. Cells within the community seem to have a diverse potential to switch their N metabolism, with individual cells being able to quickly react to environmental changes such as a DIN pulse. Therefore, once conditions change, these cells could generate high cell densities, while other cells in the community die.

Inter-community variability seems to be a common phenomenon. For example, Berthelot et al. (2019) found large differences between the N uptake of individual cells of *Synechococccus* spp., Klawonn et al. (2019) observed a high variability in NH_4^+ assimilation and C fixation rates of single cells of different phytoplankton species and Eichner et al. (2017) report a high variability of C and N_2 fixation rates between individual organisms in a *Trichodesmium* sp. colony. Furthermore, Delmont (2021) reported the coexistence of diazotrophic and non-diazotrophic *Trichodesmium* spp. and DIN uptake by this species has been demonstrated as well (Goering et al., 1966; Holl & Montoya, 2005). Eichner et al. (2014) investigated the response of *Trichodesmium* sp. to different levels of pCO_2 and growth on NO_3^- and report that while N_2 fixation rates were reduced in cells grown on NO_3^-, production rates were enhanced in these cells, implying that NO_3^- had no negative effect on *Trichodesmium* sp.. In combination with my results, this implies that diazotrophs might have a much higher flexibility with regard to their N metabolism than previously thought, which gives them a huge advantage over non-diazotrophic phytoplankton.

In summary our results imply that diazotrophs are well equipped to cope with the predicted future conditions and could potentially outcompete other phytoplankton species. Especially small unicellular species like *C. watsonii* seem to be rather favoured than suppressed by ocean warming and ocean acidification. The structure of phytoplankton communities is likely to change in a future ocean and the overall consequences for global primary production and C sequestration remain to be resolved. Last but not least, climate change induced changes in N_2 fixation can lead to an alteration of the elemental stoichiometry within the cells of diazotrophs (Jiang et al., 2018). This might have an impact on the feeding behaviour of zooplankton and therefore effects of climate change on phytoplankton are likely to affect also higher trophic levels.

PART II

The Amazon River Plume:
Study site for nitrogen uptake
and phytoplankton community structure

II-1 Introduction

In the first part of this thesis, we have learned that it is not meaningful to solely study effects of climate change on N_2 fixation without taking its importance for and link to the rest of the phytoplankton community into account, if we want to reliably predict the future of global primary production and associated C sequestration. We need to better understand the respective contributions of N_2 fixation and other N assimilation processes to primary production on a local scale in environmental settings differing in their nutrient concentrations, light availability and the intensity of mixing of the water column, to be able to extrapolate our knowledge to the global scale.

Gomes et al. (2018) suggest that river continuums post ideal conditions to study the effect of resource availability on the structure of the phytoplankton community, as the stoichiometry usually changes spatially and, depending on the latitude, also temporally along these continuums. Furthermore, rivers are an important source for nutrients and trace elements that can potentially control production in the ocean (Eppley & Peterson, 1979; Sharples et al., 2017; Smith & Hitchcock, 1994), understanding the controls and mechanisms of global primary production requires understanding biogeochemical processes in estuaries and river plumes. To study the importance of N_2 fixation for primary production relative to other N assimilation processes, we conducted incubation experiments during a cruise from the mouth of the Amazon River, along the river plume to its margins in the Caribbean Sea. The Amazon River is the world's largest river, being responsible for at least 14 % of the global freshwater input to the ocean (Dai & Trenberth, 2002). In the Western Tropical North Atlantic (WTNA), its water discharge accounts for 50 % of the total freshwater input, reaching its peak in May to June with around 200,000 $m^3 s^{-1}$ (Dai & Trenberth, 2002; Nikiema et al., 2007; Oltman, 1968; Smith & Demaster, 1996). It has been shown that the ARP supports high rates of primary production (Subramaniam et al., 2008) and acts as a sink for CO_2 throughout spring and fall, hence is important for C sequestration (Chong et al., 2014; Cooley & Yager, 2006; Ibánhez et al., 2016; Korte et al., 2020; Mu et al., 2021; Subramaniam et al., 2008; Yeung et al., 2012). The N sources supporting this primary production are not yet fully understood. So far, only N_2 fixation has been studied in the Amazon River plume (ARP) and nothing is known about the importance of other N sources for primary production to date. As the ARP is characterised by an intense stratification of the water column, results from our experiments can give hints on the importance of different N-uptake processes and associated CO_2 removal capacity in a future ocean.

II-1.1 The Amazon River and its plume

From February to May, the ARP gets entrained in the North Brazil Current (NBC) and is transported northwards into the Caribbean Sea, but from June to January, the Amazon River plume (ARP) retroflects and turns eastwards with the North Equatorial Counter Current (NECC) (Bourles et al., 1999; Coles et al., 2013; Muller-Karger et al., 1988) (Figure II-1). The huge freshwater lens leads to strong stratification of the water column on the continental shelf (Lentz & Limeburner, 1995). As water mixes more and more with oceanic water along the river-ocean continuum, the plume layer becomes saltier and thicker and can reach up to 23 m beyond the shelf (Weber et al., 2017).

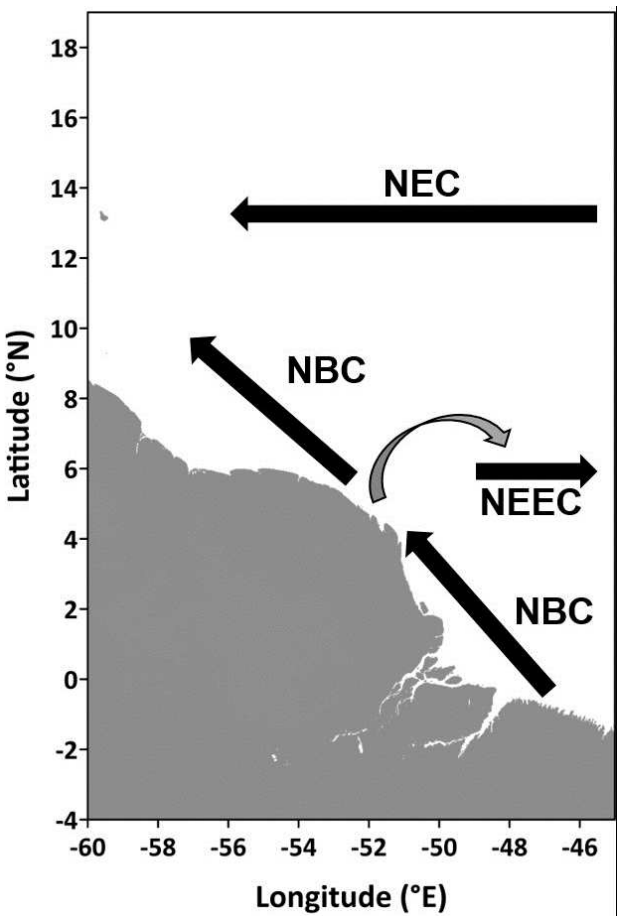

Figure II-1: *Schematic of the surface currents influencing the ARP in the WTNA. Figure after Coles et al. (2013) and Bourles et al. (1999). The grey arrow indicates the retroflection of the NBC, which occurs only between June and January.*

Nutrient concentrations at the Amazon mouth are up to 16 µM nitrate, 144 µM silicate and 0.7 µM phosphate (Demaster & Pope, 1996). While these concentrations are comparable to nutrient concentrations in other large rivers, like for example the Congo River or Mekong River (Grosse et al., 2010; Li & Bush, 2015; Vangriesheim et al., 2009), given the large total discharge volume, the resulting nutrient supply by the Amazon River is much higher (Araujo et al., 2017; Izett & Fennel, 2018; Sharples et al., 2017).

The importance of the Amazon River for the nutrient budget of the continental shelf off Brazil, French Guiana and Suriname has been stressed by previous studies (Santos et al., 2008; Sharples et al., 2017). It structures the phytoplankton community in the WTNA due to its role as a major source for silicate and phosphorus (Araujo et al., 2017; Goes et al., 2014; Weber et al., 2017), but not for nitrogen (Goes et al., 2014; Weber et al., 2017). In oligotrophic areas like the WTNA, NO_3^- and NH_4^+ concentrations are usually below the limit of detection. Though mostly absent in surface waters, nitrate is still the dominant form of dissolved inorganic nitrogen (DIN) in the nearshore and offshore regions of the WTNA including the ARP (Araujo et al. 2017) and the Amazon River itself (Demaster & Pope, 1996). At times, nitrate from subsurface water has been found to reach surface waters on the shelf (Demaster & Pope, 1996; Loick-Wilde et al., 2016) via upwelling and onshore advection (Gibbs, 1982), thus becoming available to phytoplankton in the river plume.

Based on water properties, the plume area can be divided into distinct habitats that are characterised by a specific phytoplankton community (Weber et al., 2019). Habitats with stronger plume influence, i.e. low surface salinity, shallow mixed layer depth (MLD) and Chl *a* maximum and high NO_3^- availability, are mostly dominated by a mixed community of diatoms, cryptophytes and green-water *Synechococcus* spp., while oceanic waters at the plume margins, characterised by high surface salinity, deep MLD and Chl *a* maximum and low NO_3^- availability, are dominated by the diazotroph *Trichodesmium* spp. and the unicellular cyanobacteria *Synechococcus* spp. and *Prochlorococcus* spp. (Araujo et al., 2017; Goes et al., 2014; Weber et al., 2019). In addition, there is the region of intermediate salinity, where nitrogen is mostly undetectable in surface waters, but silicate and phosphate are still available and diatom-diazotroph-associations (DDAs) dominate (Goes et al., 2014; Subramaniam et al., 2008; Weber et al., 2019).The Amazon River water is extremely turbid, as it carries a high sediment and organic material load (Park & Latrubesse, 2015) that causes light limitation of phytoplankton activity. This limitation is strongest at the river mouth, but eases during the mixing of riverine and oceanic water. In the northern areas of the plume,

where waters are much clearer and DDAs dominate, high rates of primary production have been determined (Foster et al., 2007; Subramaniam et al., 2008; Weber et al., 2017).

Globally, nitrogen and phosphorus can both be limiting for marine productivity (Gruber, 2004; Moore et al., 2013). In the ARP, phytoplankton capable of N_2 fixation is not limited by nitrate and can make use of the enhanced concentrations of phosphate, silicate (Goes et al. 2014; Araujo et al. 2017; Weber et al. 2017) and also iron (Bergquist & Boyle, 2006), which is a large advantage compared to other phytoplankton species that depend on nitrate and other bioavailable nitrogen species in the water for growth. The low N:P ratio and high silicate load of the river water (Araujo et al. 2017) fosters N_2 fixing organisms, especially DDAs, in the outer ARP (Carpenter et al., 1999; Foster et al., 2007; Subramaniam et al., 2008; Yeung et al., 2012). Due to the enhanced primary production by DDAs and the resulting uptake of dissolved CO_2, the WTNA acts as a net sink for carbon during most of the year (Cooley & Yager, 2006; Körtzinger, 2003; Subramaniam et al., 2008; Ternon et al., 2000). In a modelling study, Louchard et al. (2021) have shown that the Amazon reduces the release of CO_2 from the WTNA by more than 50 % due to the enhanced primary production it fuels. In times of climate change with rising concentrations of atmospheric CO_2 (Solomon et al., 2009), massive human interventions in the catchment of the Amazon River (Latrubesse et al., 2017), and the large areal extent of the Amazon River plume, it is of special interest to understand the factors controlling primary production and biological processes in the surface waters to set a baseline and improve predictions for the future role of the river plume for the WTNA.

The highly diverse environments along the ARP with a transition from N-rich to N-limited areas make it an ideal study site for the exploration of the interplay between N_2 fixation and other N assimilation processes and the resulting composition of the phytoplankton community. Previous studies in the WTNA have focused mainly on diazotrophs and DDAs in particular. Nevertheless, N_2 fixation does not support all the primary production measured in the WTNA (Subramaniam et al., 2008). The role of different N sources in controlling primary production is not yet fully understood in the ARP, as processes like the assimilation of dissolved inorganic and organic nitrogen have not yet been quantified.

This thesis presents the first measured uptake rates of nitrate (NO_3^-), ammonium (NH_4^+) and amino acids using ^{15}N-labelled substrates in the river-outflow. In combination with carbon uptake rates, we were able to assess the contribution of NO_3^-- and NH_4^+-based production to total primary production in this ecosystem. In addition, we investigated the link between

changes in the N-cycle along the plume axis and the dominant groups of primary producers identified by high precision liquid chromatography (HPLC).

II-1.2 Research Questions and hypotheses

In the second part of this thesis, the nitrogen sources supporting primary production in the ARP are discussed. As previous studies in this region have focused only on N_2 fixation, but this process cannot account for total primary production reported from the ARP, this thesis includes the measurements of nitrogen uptake rates in the plume. I wanted to resolve the following questions:

1.) Which nitrogen sources support primary production in the Amazon River plume in relation to nitrogen fixation?
2.) How does the phytoplankton community structure change in response to changing nutrient concentrations and environmental conditions along the plume?

I was expecting an increased importance of N_2 fixation with increasing distance from the river mouth, as nitrogen becomes more and more limiting, while phosphate is always available from the river or from remineralisation of particles in surface waters (Weber et al., 2017), creating an ideal niche for diazotrophs. In contrast to nitrogen, silicate is supplied at high concentrations by the Amazon and does not seem to be limiting primary production on the shelf (McClain et al., 2001). The high availability of silicate favours diatoms, which are known to preferentially take up NO_3^- as a nitrogen source. Therefore, I assumed that NO_3^- uptake could contribute significantly to primary production in the young plume, where NO_3^- from the river is still available. I anticipated NH_4^+ uptake to support primary production primarily in coastal communities, but also alongside N_2 fixation in the northern plume, as N_2 fixation alone cannot account for the total primary production rates reported from the ARP and diazotrophs are known to release part of the nitrogen they fix as NH_4^+ (Capone et al., 1994; Glibert & Bronk, 1994; Mulholland, Bronk, et al., 2004) as explained in the general introduction of this thesis.

II-2 Material & Methods

II-2.1 Sampling area and hydrography

Water samples were taken between April 12 and May 30 in 2021 during the cruise M174 aboard the RV Meteor, where we followed the plume from the Amazon River mouth up to 16°N (Figure II-2). At five stations, we pursued a Lagrangian sampling strategy and followed the same water mass for up to 48 hours with the help of a drifter. These five stations are hereafter called "drift stations". I applied the habitat definition after Weber et al. (2019), modified for the ARP by Pham et al. (unpublished). According to this definition, my sampling stations could be grouped into six distinct habitats: riverine (RIV), young plume core (YPC), old plume core (OPC), old plume margin (OPM), western plume margin (WPM), modified oceanic sea water (MOSW) and oceanic seawater (OSW). The definition is based on the environmental conditions (sea surface salinity, sea surface temperature, mixed layer depth, depth of the Chl *a* maximum and nitrate availability to surface waters). Dominant phytoplankton groups can be well distinguished between the different habitats, as shown by Weber et al. (2019) and Pham et al. (unpublished).

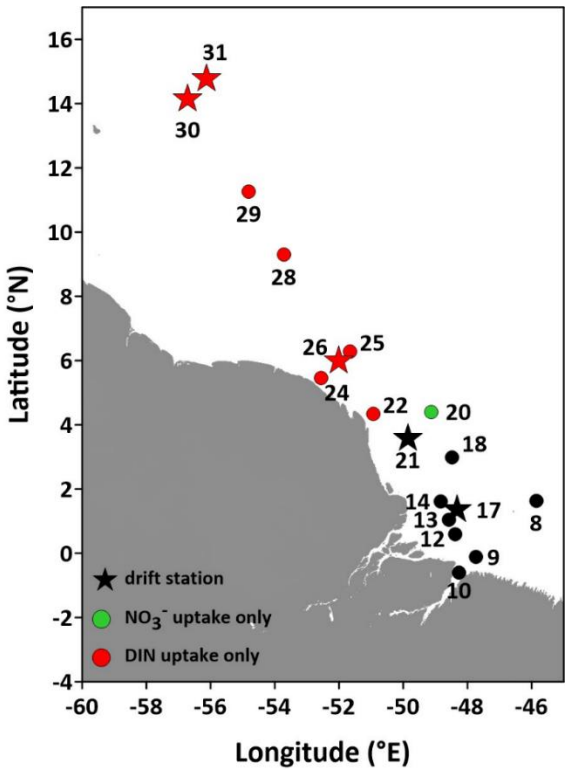

Figure II-2: *Sampled stations during M174. Stars mark the extended stations (N_2 fixation was measured at these stations). At the green station, only nitrate uptake rates were measured. At the red stations only NO_3^- and NH_4^+ uptake rates were determined. At the black stations, both DIN (NO_3^- and NH_4^+) and amino acid uptake rates were measured.*

A Seabird Electronics SBE-911plus CTD-rosette system with 21 Free Flow bottles of 10 L volume was used to measure hydrographic variables and collect water samples. Turbidity and fluorescence were obtained from a D&A OBS-3 turbidity sensor and a WETStar fluorometer, respectively, mounted onto the CTD rosette. Sampling included surface water, the mixed layer depth and the depth of the chlorophyll *a* (Chl *a*) maximum determined from CTD fluorescence profiles. The maximum sampling depth corresponded to the 1% surface irradiance (defined here as the lower boundary of the euphotic zone), which was calculated from CTD profiles. In addition to data from CTD profiles, the structure of the water column was analysed with a Microstructure profiler (MSS) at the drift stations. From these data, the Richardson number was computed to identify mixing and stratification processes.

The lower boundary of the mixed layer depth (MLD) was determined as the maximum of the buoyancy frequency (N^2) calculated from CTD profiles as:

$$N^2(z) = -\frac{g}{\rho} * \frac{\partial \rho}{\partial z} \quad (1)$$

where g is the gravity acceleration, ρ is the density, and $\partial\rho/\partial z$ the partial derivative with respect to depth (z).

In order to compare the calculated mixed layer depth to the plume thickness at those stations that were influenced by the river plume, the bottom of the plume was calculated as the first maximum of the vertical salinity gradient (Weber et al. 2017).

For the analysis of nutrient concentrations, water samples were collected from the CTD rosette and filtered using a 0.2 µm syringe filter. Concentrations were measured on board with a continuous flow analyser (Seal Analytical QuAAtro 39). The detection limit was 0.02 µM for NO_3^-, 0.02 µM for NH_4^+, 0.01 µM for NO_2^-, 0.01 µM for phosphate and 0.03 µM for silicate.

II-2.2 Determination of NO_3^- -, NH_4^+ - and amino acid uptake rates

Water was collected in 1 L or 2.7 L polycarbonate bottles directly from the CTD rosette. Bottle size was chosen according to expected biomass content at the respective station. At every station, one to three depths were sampled in triplicates. To obtain in-situ uptake rates, NO_3^- addition should not exceed 10 % of the ambient concentration (Dugdale and Goering 1967).

NO_3^- and NH_4^+ concentrations were measured on board directly before the start of the incubation experiments and 10% of the ambient concentration was added as $Na^{15}NO_3$ or $^{15}NH_4Cl$, respectively, to determine uptake rates of these nitrogen species. For the determination of carbon uptake rates, 200 µM $NaH^{13}CO_3$ (Sigma Aldrich, 98 atom% ^{13}C) was added to the same bottle. To determine amino acid uptake rates, a self-prepared, dually labelled amino acid mixture was added to the samples to a final concentration of 5 µM of nitrogen (the exact composition is given in Table II-1).Bottles were placed in black gauze bags that matched the percentage of surface light reaching the respective water depths and incubated in transparent deck incubators filled with flowing surface sea water for three to four hours for nitrate uptake measurements or 1 to 2 hours for ammonium and amino acid uptake measurements. The incubation was terminated by filtration of the entire bottle volume (2.7 L) at all stations except the river mouth (filtration volumes were 150 – 250 mL at stations M174-10, -12, -13 and 300 – 620 mL at stations M174-9, -14 and -17) onto precombusted (450°C for 4 hours, prior to the cruise) 25 mm glassfiber filters (Advantec GF-075 in 2021/M174, with a nominal pore-size of 0.3 µm). At the river mouth stations, less water was filtered, as filters clogged quickly due to the high concentrations of particles. Due to the very low filtration volume per filter at stations M174-12 and M174-13, we prepared two filters per replicate bottle at these stations to improve the reliability of our measurements. Filters were dried on board at 50°C for at least 48 hours and then frozen in Eppendorf tubes until analysis in the laboratory at IOW.

For isotope ratio mass spectrometry (IRMS) analysis, dried samples were folded and packed into tin foil capsules and pressed into pellets. The filters were combusted in an elemental analyser (EA) connected to the IRMS via a conflo IV interface. Standard gases were calibrated against values for the PeeDee Belemnite reference for carbon and N_2 for nitrogen (Peterson & Fry, 1987). The δ values were calculated based on the standard delta notation (Peterson and Fry 1987):

$$\delta x\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1000 \quad (2)$$

where *x* (‰) is ^{13}C or ^{15}N and *R* is the ratio of heavy to light isotopes, hence $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample or in the standard, respectively. Calibration material for C and N analysis was Acetanilide (Merck). The analytical precision of the measurement was < ± 0.2 ‰ for $\delta^{15}N$ values in natural abundance samples and enriched samples with $\delta^{15}N$ values of up to 250 ‰. As the variability of measurements increases gradually with increasing tracer enrichment, the precision of the measurement decreases to up to ± 10 ‰ for samples with a high enrichment (i.e. > 250 ‰). This precision was determined from an internal calibration test using standards with $\delta^{15}N$ values between 0 and 4700 ‰ (N 305A, N 305B, Urea 310A, Urea 310B, N 311, C 309A, C 309B). In cases, where the $\delta^{15}N$ value of one replicate differed from the other two replicates by more than 25 %, this replicate was excluded from further analysis and rates were computed from duplicate measurements only. Detection limits for rate measurements were determined after Santoro et al. (2013). In a first step, to account for the detection limit of the mass spectrometer/the elemental analyser, the minimum detectable ^{15}N enrichment in the product pool ($LOD_{product}$), i.e. in our case particulate organic material (PON), is calculated:

$$LOD_{product} = \frac{LOD_{EA}}{100} * [PON] \quad (3)$$

LOD_{EA} is the detection limit of the elemental analyser/the mass spectrometer times 2, i.e. the minimum detectable tracer enrichment in atom% (a 0.2 ‰ enrichment multiplied by 2, which results in a minimum detectable change of 0.00015 atom% or for a 10 ‰ enrichment 0.0073 atom%). *[PON]* is the concentration of PON in the sample in µmol/L.

In a second step, the detection limit of the product pool is used to calculate the minimum detectable uptake rate (LOD_{rate}):

$$LOD_{rate} = \frac{LOD_{product} * [substrate\ pool] + [tracer]}{[tracer]} \quad (4)$$

Where *[substrate pool]* is the ambient concentration of NO_3^- or dissolved inorganic carbon (DIC) at the sampling depth and *[tracer]* is the concentration of the tracer added to the samples in µmol L^{-1}. DIC concentrations for all calculations in this section were obtained

from the formula in Parsons et al. (1984) for surface salinities > 30 and from Druffel et al. (2005) for salinities < 30.

To calculate rates, we first transformed δ values, to atom % and atom % excess. Nitrate uptake rates were then calculated after Dugdale and Wilkerson (1986):

$$V_N\ (t) = \frac{15N_{xs,\ sample}}{(15N_{xs,\ pool} - F) * \mathrm{t}} \quad (5)$$

Where V_N (t) is the specific uptake rate (N taken up per unit particulate N per unit time), $^{15}N_{xs,\ sample}$ is the atom% excess in the sample ($^{15}N_{xs,\ sample} = {}^{15}N_s - F$ with $^{15}N_s$ being the atom% ^{15}N in the sample), $^{15}N_{xs,\ pool}$ is the atom% ^{15}N in the initially labelled fraction, *F* is the natural abundance of ^{15}N and *t* is the incubation time. Rates were calculated assuming a nitrate concentration of 0.001 µM when nitrate concentrations were below detection limit.

The volumetric rate ρ_N (*t*) (N uptake in concentration units) was then calculated using V (*t*) and the δ values of PON at the end of the incubation (PON (*t*)):

$$\rho_N\ (t) = V(t) * PON\ (t) \quad (6)$$

The analysis of stable isotope natural abundance of PON and POC was done at Georgia Tech Institute of Technology (Atlanta, USA). These values are a necessary reference for the natural stable isotope composition of particulate organic material (POM) for the calculation of nitrate and carbon uptake rates.

Calculation of carbon uptake rates is the same as that of nitrate uptake after Collos (1987) as follows:

$$V_C\ (t) = \frac{(^{13}C_{final} - {}^{13}C_{initial})}{(DIC - {}^{13}C_{initial}) * t} \quad (7)$$

V_C is the specific uptake rate (C uptake per unit particulate C per unit time), $^{13}C_{final}$ is the concentration of ^{13}C in atom% in the sample after incubation, $^{13}C_{initial}$ is the concentration of ^{13}C in atom% at the beginning of the incubation, hence the natural abundance of ^{13}C in the sample, DIC – $^{13}C_{initial}$ is the concentration of ^{13}C in the dissolved phase, and *t* is the incubation time.

Rates are reported as mean plus standard deviation of the triplicates collected at each sampled depth. Additionally, results of the rate measurements were integrated trapezoidal over the depth of the euphotic zone (from the surface to the 1 % surface light level for all

cruises) and these integrated rates were then normalized by that depth to account for differences in the depth of the euphotic zone and reach comparable rates between stations. Rates were scaled to daily rates by multiplying by 12 hours, as phytoplankton activity is coupled to the daylight period.

Table II-1: *Overview of the amino acids included in the self-prepared dually labelled amino acid mixture.*

amino acid	% in final mix	g in 1 g final mix	µmol in 1 g final mix
Alanine	7	0.07	785.72
Arginine	7	0.07	401.84
Aspartic acid	10	0.1	751.26
glutamic acid	10	0.1	679.67
glycine	6	0.06	799.25
histidine	2	0.02	128.90
isoleucine	4	0.04	304.95
leucine	10	0.1	762.37
lysine	14	0.14	957.66
methionine	1	0.01	67.02
phenylalanine	4	0.04	242.15
proline	7	0.07	608.01
serine	4	0.04	380.63
threonine	5	0.05	419.75
tyrosine	4	0.04	220.76
valine	5	0.05	426.80

Uptake rates were calculated after Dugdale and Wilkerson (1986) and are reported as mean plus standard deviation of the triplicates collected at each sampling depth. Sampling depths included the surface, the Chl *a* maximum and the base of the euphotic zone, i.e. the 1% light level. The rates were integrated trapezoidal over the depth of the euphotic zone.

By calculating potentially possible primary production rates from NO_3^- or NH_4^+ uptake rates using the C:N ratios measured in the particulate organic material, I estimated the contribution of NO_3^- and NH_4^+ to total primary production. I decided not to use the Redfield ratio of 6.6 for this conversion, as C:N ratios in our samples deviated from this ratio and were quite variable depending on the phytoplankton community composition along the plume axis. Calculations were done with the actual C:N ratio of the samples to account for this

variability. In addition, I calculated the F-ratio from NO_3^-, NH_4^+ and N_2 fixation rates as (NO_3^- uptake rate + N_2 fixation rate/(NO_3^- uptake rate + N_2 fixation rate + NH_4^+ uptake rate) to get an estimate of the contributions of new and regenerated production to total production (Eppley & Peterson, 1979).

II-2.3 Determination of N_2 fixation rates

The dissolved seawater method after Mohr et al. (2010) was used to determine N_2 fixation rates at the extended stations. Water samples were taken directly from the CTD rosette from three sampling depths in triplicates.

^{15}N-enriched seawater was prepared one day before the actual start of the incubation experiment. On the first day at the extended stations, water samples were taken from the sampling depths chosen for N_2 fixation rate measurements. The water was sterile filtered through 0.2 µm filters and distributed into 600 mL Schott bottles via siphoning with Tygon tubing. Schott glass bottles were sealed gas tight and 6 mL of $^{15}N_2$ gas was added to the Schott bottles with a gas tight syringe and bottles were shaken for 1 minute and then stored horizontally at 5°C for at least 24 h. During this time, the Schott bottles were shaken at least three more times for 1 minute.

On the second day at the drift station, water sampling for incubation experiments was conducted in 4.6 L polycarbonate bottles directly from the CTD rosette. The same three depths as on day one were sampled in triplicates. At the river mouth (station 17), 2.7 L polycarbonate bottles were used for the incubation, because of higher particle concentration in the water. Schott bottles, that contained no bubble any more, were opened and 460 mL of ^{15}N-enriched seawater (270 mL at the river mouth station) was siphoned to the bottom of the polycarbonate bottle. Polycarbonate bottles were then sealed gas tight and 100 µL of $NaH^{13}CO_3$ solution (0.1 M) were added to measure primary production rates alongside with N_2 fixation rates. Then the bottles were shaken 30 times and incubated for 24 h in on-deck incubators as described above. The incubation was terminated by filtration with a peristaltic pump. Right when bottles were opened for filtration, a 12 mL water sample was taken from the incubation bottle with a gas tight syringe connected to Tygon tubing and transferred to a 12 mL Exetainer, to determine the final concentration of $^{15}N_2$ in the water. To preserve the sample, 100 µL of a saturated $ZnCl_2$ solution was added to the sample (Klawonn et al., 2015). Samples were stored at 5°C until analysis via membrane inlet mass spectrometry

(MIMS) at the Mediterranean Institute of Oceanography (MIO) in Marseille, France in August 2021.

N_2 fixation rates were calculated after Montoya et al. (1996) and Mohr et al. (2010) as:

$$N_2\ fixation\ rate = \frac{A_{PN}^{final} - A_{PN}^{t=0}}{A_{N_2} - A_{PN}^{t=0}} * \frac{[PN]}{\Delta t} \quad (8)$$

where A = atom% ^{15}N in the particulate organic nitrogen (*PN*) at the end (*final*) or beginning ($t = 0$) of the incubation or in the dissolved N_2 pool (N_2).

II-2.4 Phytoplankton community composition by HPLC

Samples for phytoplankton pigment analysis were collected in opaque sampling bottles (2.3 L) from the CTD rosette and were immediately filtered onto Whatman GF/F filters. Filters were stored in liquid nitrogen until analysis at the NASA Ocean Ecology Laboratory at the Goddard Space Flight Center as described in Van Heukelem & Thomas (2001). The phytoplankton community composition was investigated via high-performance liquid chromatography (HPLC). Certain pigments have been shown to be specific for some phytoplankton taxa and can therefore be used to characterize the phytoplankton community composition (Hirata et al., 2008; Li et al., 2002; Uitz et al., 2006; Vidussi et al., 2001).

The contribution of each phytoplankton group to total Chl *a* was estimated using the CHEMTAX program after Mackey et al. (1996). Pigments included in the analysis are: divinyl chlorophyll a, divinyl chlorophyll b, chlorophyll b, chlorophyll c1, c2 and c3, peridinin, 19-butanyloxyfucoxanthin, fucoxanthin, neoxanthin, violaxanthin, gyroxanthin, 19-hexanoyloxyfucoxanthin, alloxanthin, zeaxanthin and lutein. Ratios of pigments to total Chl *a* used for the computation were taken from literature values summarized and published by Wright (2017) based on Jeffrey & Wright (2005) and Zapata et al. (2005). Results from this calculation are reported as 8 phytoplankton groups: colonial cyanobacteria (freshwater and marine colonial species, e.g. *Nodularia* sp., *Aphanizomenon* sp., *Anabaena* sp., *Trichodesmium* sp.), the unicellular cyanobacteria *Synechococcus* sp. and *Prochloroccous* sp., prasinophytes (mixture of species), cryptophytes (mixture of species), diatoms (mixture of species), haptophytes (mixture of species) and dinoflagellates (mixture of species). The phytoplankton groups included in the analysis were chosen based on available references of previous analysis of the phytoplankton community composition in the ARP (Goes et al.,

2014; Gomes et al., 2018; Wood, 1966). Different ratios were used for samples within the plume and the surface versus samples from below the plume and at greater depth at the oceanic stations, as colonial cyanobacteria are supposed to be present in surface waters only (A. Subramaniam, pers. comm.).

The relative contribution of each phytoplankton group calculated here should be considered as an approximation rather than an accurate quantification, as some phytoplankton groups share pigments and therefore can be over- or underestimated by this approach.

II-2.5 Statistical analysis

All rates as well as means and standard deviations from triplicate measurements were calculated in Microsoft Excel.

A Kruskal-Wallis-test was conducted to identify significant differences between uptake rates in the habitats with the statistics software R (version 4.2.3), using the function "kruskal.test()" from the package "stats" (R Core Team, 2023). Subsequently, a Dunn test with a Benjamini-Hochberg correction for pairwise comparisons (Benjamini & Hochberg, 1995) was done in the software R using the function "dunnTest()" from the package "FSA" (Ogle et al., 2023) to identify the habitats where rates were significantly different.

To identify the most important variables controlling N uptake and primary production in the ARP, a principal component analysis (PCA) was performed with the software R (version 4.2.3) using the function "PCA()" from the FactoMineR (version 2.7) package (Sebastien et al., 2008). To make variables comparable, data were standardised by subtracting the mean and dividing by the standard deviation of each variable. For the PCA analysis, the dataset was split into surface samples and samples from within the plume versus samples from below the plume in order to distinguish controlling variables along the plume gradient from those below the plume.

II-3 Results

II-3.1 Environmental conditions

We followed the ARP until about 16°N and encountered a change of the sea surface salinity (SSS) inside the plume from 0.47 at the river mouth to 33.62 at the northernmost station (Figure II-2), accompanied by a decrease of sea surface temperature (SST) from up to 29.23°C at the mouth down to 26.51°C at the northernmost station. In general, the plume water could be identified by low SSS, mostly high SST and declining turbidity from 9.7 NTU at the river mouth to 0.1 NTU in the northern plume (Table II-2). The river plume was a distinct water masses identified by its higher temperature and lower salinity (Figure II-3). The strong stratification caused by the low density waters is obvious from high Richardson numbers (Figure II-4). We saw that the surface layer hardly mixes despite the strong tidal influence close to the river mouth. As the plume travels northwards, this effect is reduced and the stratification becomes weaker (Volker Mohrholz, pers. comm.).

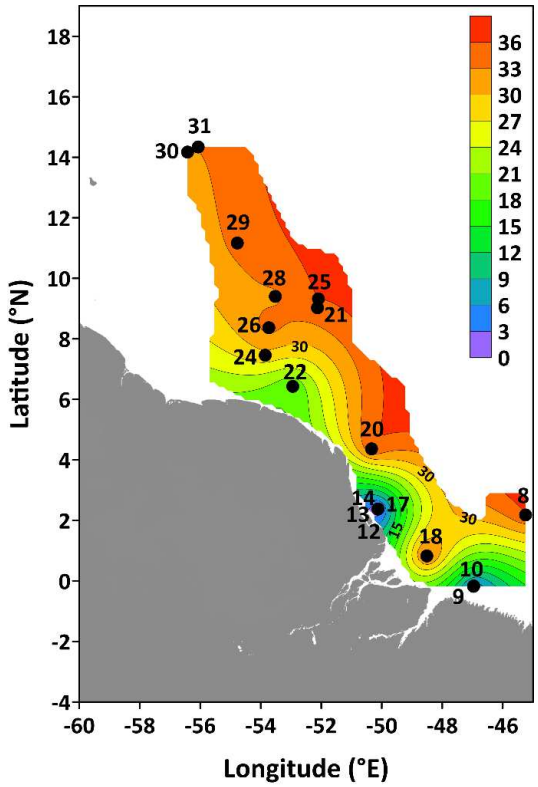

Figure II-2: *Sea surface salinity (SSS) shown in Lagrangian coordinates to achieve a synoptic structure of the river plume, as stations are transformed from a grid fixed in space to a grid fixed in time. Therefore, we can see the strucuture of the plume, as if we had sampled all stations at the same time point, which in this case is the last sampling day of the cruise. The computation of Lagrangian coordinates was conducted by Joachim Dippner.*

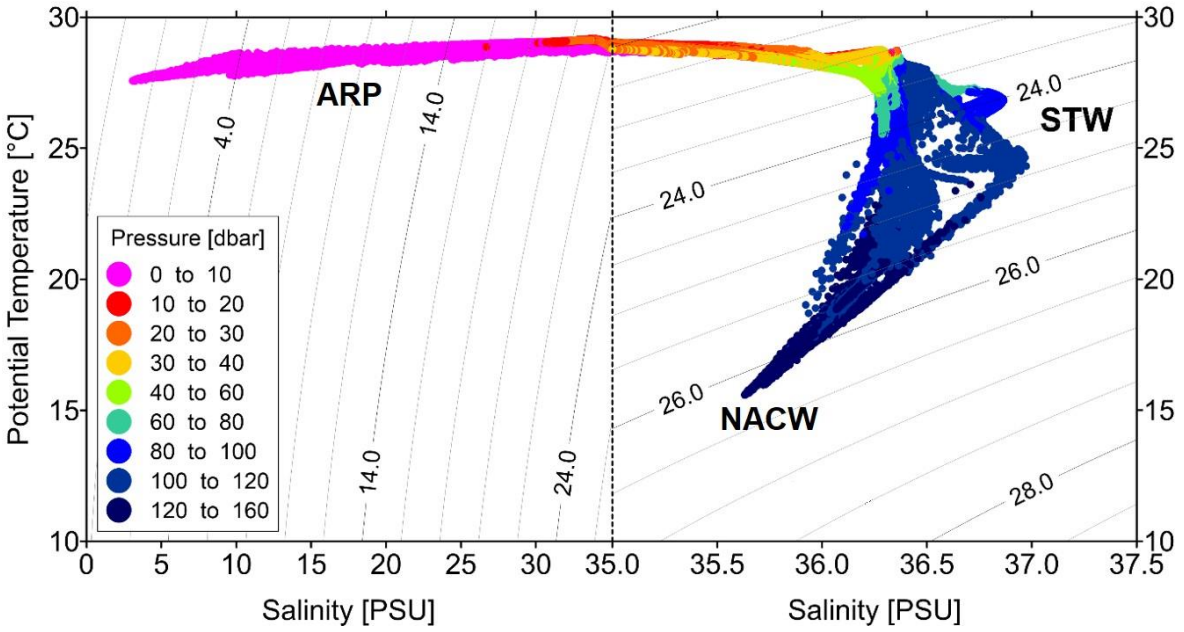

Figure II-3: *Temperature vs salinity from CTD profiles of all stations sampled during cruise M174. The colour indicates the density. The plume ("ARP") can be clearly distinguished from other water masses by its low density. "NACW" is North Atlantic Central Water and "STW" is Subtropical Water. Plot done by Volker Mohrholz.*

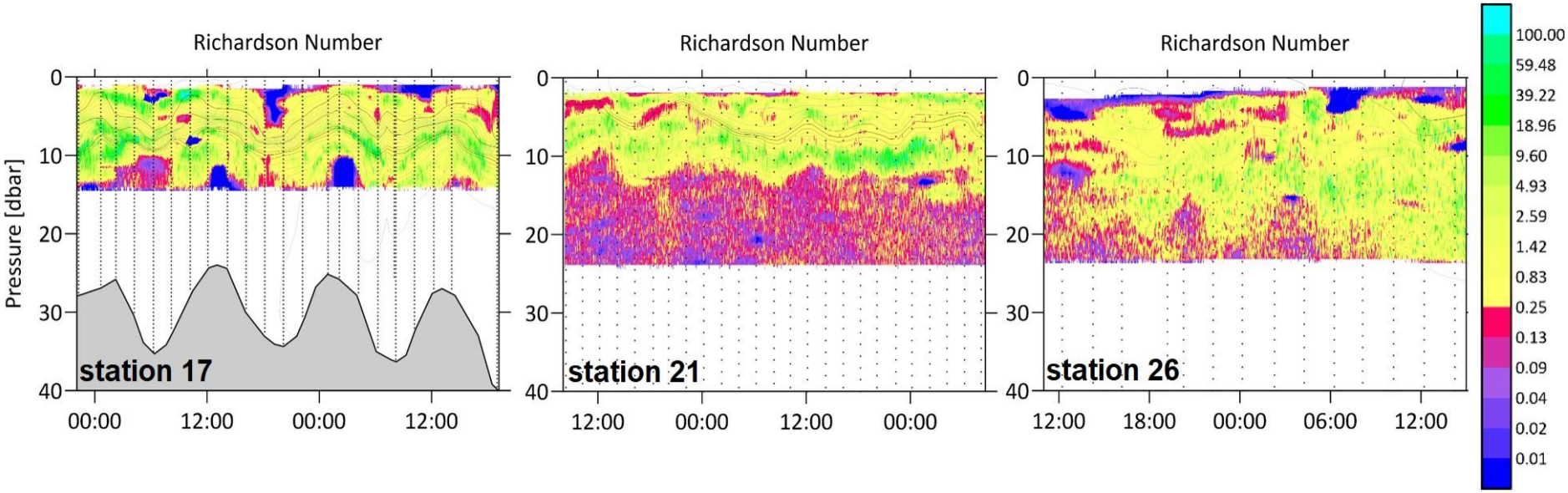

Figure II-4: *The Richardson number was computed from data obtained with the Microstructure profiler (MSS) for three of the five drift stations (stations 17, 21 and 26 from left to right). The number depends on the ratio of the buoyancy over the vertical shear. If the Richardson number is below 0.25, turbulence dominates, i.e. low numbers indicate high mixing, while high numbers indicate no mixing between water layers. The grey area in the panel for station 17 represents the sea floor and the shape originates from the strong tidal impact at this station during the 48 h of sampling. Calculations and figure were done by Volker Mohrholz.*

Table II-2: *ranges of surface nutrient concentrations, surface nutrient ratios, surface turbidity, surface Chl a concentrations from HPLC data and the depth of the nitracline and mixed layer (MLD) for the different habitats. Turbidity is given in nephelometric turbidity unit (NTU). The number of stations per habitat is given in the habitat column (n).*

habitat (n)	**NO_3^- (µM)**	**NH_4^+ (µM)**	**PO_4^{3-} (µM)**	**$Si(OH)_4$ (µM)**	**DIN:DIP**	**$Si(OH)_4$: NO_3^-**	**turbidity (NTU)**	**Chl *a* (mg m^{-3})**	**nitracline (m)**	**MLD (m)**
RIV (6)	10.23 – 18.44	0.32 – 1.22	0.31 – 0.90	127.29 – 167.23	11.07 – 18.92	8.19 – 16.53	2.90 – 9.7	0.31 – 3.32	0	4 – 9.5
YPC (2)	0.50 – 0.93	0 49 – 2.09	0.14 – 0 25	8.35 – 32.66	4.90 – 17.06	16.17 – 60.68	0.13 – 0.45	0.28 – 5.79	6 - 12	5.5 - 12
OPC (1)	0.41	0.28	0.17	27.79	4.14	68.02	0.23	1.05	40	4.5
OPM (2)	0.08	0.07 – 0.28	0.03 – 0.05	4.20 – 6.32	5.27 – 13.36	31.62 – 81.66	0.09 – 0.1	0.20 – 0.35	180	16 - 24
WPM (2)	0.45 – 1.00	0.19 - 0.55	0.08 - 0.09	2.30 - 5.73	6.95 - 20.65	2.30 - 12.86	0.07 - 0.09	0.11 - 0.17	80 - 100	10 - 75
MOSW (1)	0.04	0.07	0.02	3.13	5.10	82.29	0.10	0.18	180	17
OSW (4)	0.07 – 1.44	0.04 – 0.55	0.04 – 0.13	0.85 – 7.37	1.78 – 13.30	0.84 – 113.45	0.07 – 0.1	0.08 – 0.52	100 - 180	10 - 90

The mixed layer depth (MLD) increased with distance from the river mouth from about 4 m to 90 m in the OSW habitat (Table II-2) and corresponded to the base of the plume layer at all stations except station 26 (WPM habitat, base of the plume at 8 m depth) and those of the OSW habitat, where the plume was absent. The two stations in the WPM habitat differed largely in their MLD, which was defined at 10 m and 75 m for stations 22 and 26, respectively.

Nutrient concentrations in surface waters were highest at the Amazon River mouth (RIV habitat, Table II-2), with surface NO_3^- concentrations of 10.1 - 18.4 µM, NH_4^+ concentrations between 0.32 µM and 1.22 µM, PO_4^{3-} concentrations between 0.3 µM and 0.9 µM and silicate concentrations of 142.6 – 167.2 µM (Table II-2, Figure II-5). In the YPC and OPC habitats PO_4^{3-} and silicate concentrations were still elevated compared to the habitats with less plume influence, while NO_3^- was already depleted to concentrations below 2 µM. NH_4^+ concentrations were in the same range as in the RIV habitat. While NO_3^- and PO_4^{3-} concentrations were mostly below 0.1 µM throughout the euphotic zone at stations beyond the shelf (WPM, OPM, MOSW, OSW habitats), silicate concentrations between 0.57 and 8.36 µM were measured there. NH_4^+ concentrations in surface waters ranged between 0.28 µM and 1.01 µM in surface waters beyond the shelf and low concentrations between 0.1 µM and 0.2 µM were detectable throughout the euphotic zone in the northern plume (OPM and MOSW habitats). Silicate to NO_3^- ratios were always above the Redfield-Brzezinski ratio of 1.04 in surface waters and therefore it can be concluded that silicate was not limiting (Brzezinski, 1985) throughout the ARP. There was only one exception to this statement, which was station 20 (OSW habitat).

DIN:DIP ratios were very high in the RIV habitat (Table II-2) and decreased rapidly in the YPC and OPC habitats. In the WPM habitat DIN:DIP ratios were slightly higher again. In the OPM, MOSW and OSW habitats DIN:DIP ratios were lowest, with the exception of station 20, where a ratio of 11.03 was determined in surface waters.

The nitracline was found between 6 m and 40 m depth in the YPC and OPC habitats, between 80 m and 100 m depth in the WPM habitat and between 100 m and 180 m in the OPM, MOSW and OSW habitats. In the RIV habitat, NO_3^- concentrations were highest in surface waters (Table II-2).

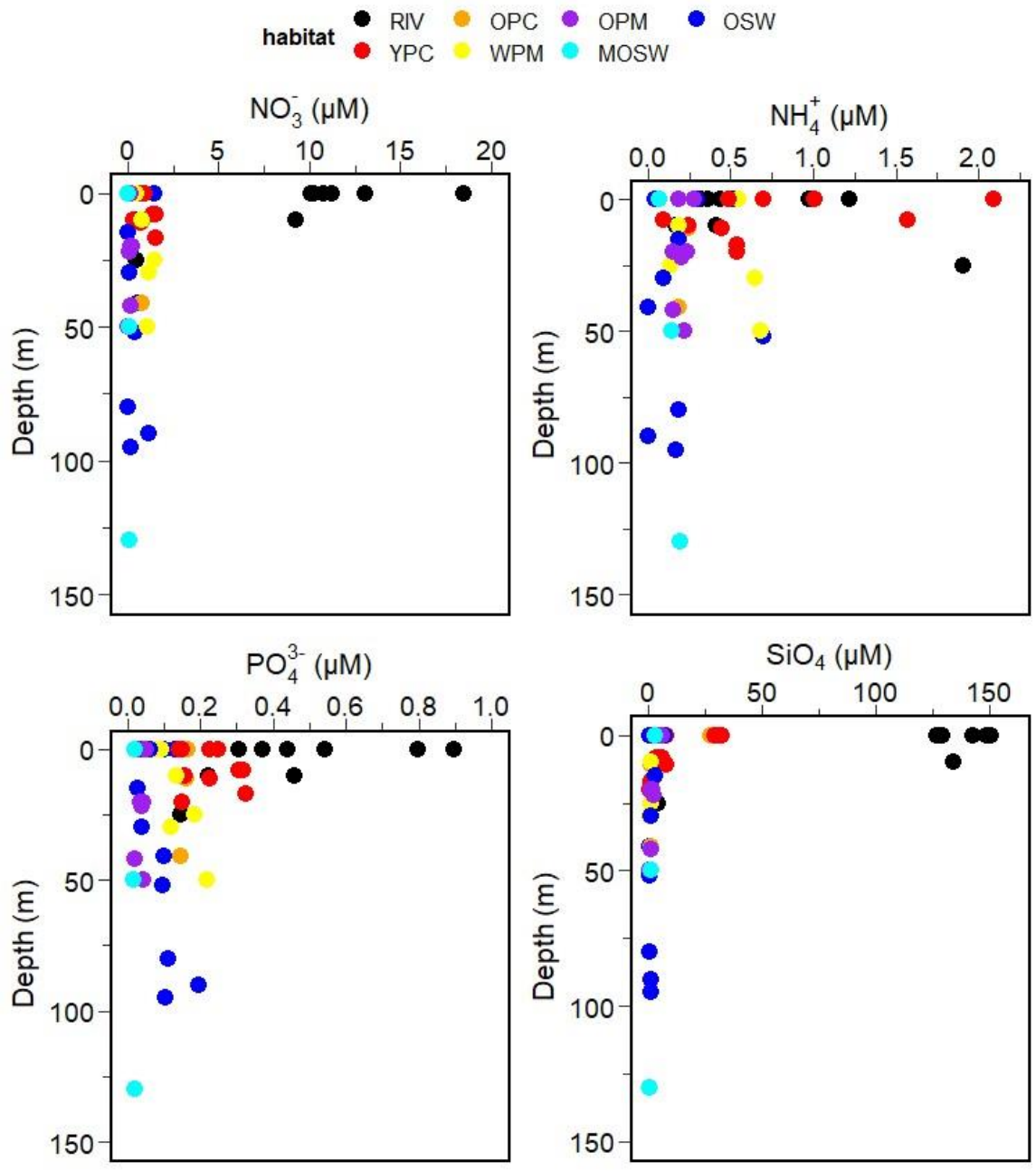

Figure II-5: *Depth profiles of nutrient concentrations from M174 at the sampling stations and depths. Colours indicate the respective habitat. RIV is the riverine habitat (black), YPC is the young plume core (red), OPC is the old plume core (orange), WPM is the western plume margin (yellow), OPM is the old plume margin (purple), MOSW is the modified oceanic sea water (cyan), OSW is the oceanic sea water (blue).*

II-3.2 Nitrogen assimilation and primary production rates

All rates were above the detection limit, which ranged between 0.001 to 0.577 nmol L^{-1} h^{-1}. All rates are in-situ uptake rates (< 30 % tracer addition), except from surface NH_4^+ uptake rates at stations 28 and 31 as well as the 1 % light level at stations 26 and 28 (tracer additions > 30 % of the ambient NH_4^+ concentration). Depth profiles of uptake rates are shown in the appendix (Figure A-3). Uptake rates were highest at the surface at all stations. Rates were integrated between the surface and the 1 % light level and normalised to the depth of the euphotic zone at the respective station to account for the differences in the maximum water depth sampled and reduce the bias resulting from greater volume used to calculate the integrated rates. Averaged primary production was quite similar between the different habitats, except from low rates in the OPC and OSW habitats (Figure II-6). NH_4^+ uptake rates were always higher than nitrate and amino acid uptake rates (Figure II-7). We measured the highest NO_3^- uptake rate in the YPC habitat at station 24 (888.7 µmol N m^{-3} d^{-1}), highest amino acid uptake rates in the RIV habitat at station 14 (931.7 µmol N m^{-3} d^{-1}) and highest NH_4^+ uptake rates in the RIV habitat at the same station (5556.1 µmol N m^{-3} d^{-1}). All N uptake rates were high at two stations in the RIV habitat (stations 14 and 17). All average N uptake rates were highest in the RIV or YPC habitats and lowest at the MOSW and OSW stations (Table II-3). N_2 fixation rates were lowest in the RIV habitat (10.6 µmol m^{-2} d^{-1}) and increased constantly towards the northernmost stations. In the OPM and MOSW habitats, high fixation rates were measured (424.2 µmol m^{-2} $d^{-1}$and 379.9 µmol m^{-2} d^{-1} for stations 30 and 31, respectively).

As the difference between uptake rates along the gradient of plume influence should be tested, the Kruskal-Wallis test was performed on volumetric rates inside the plume layer (or surface layer at OSW stations) only. The Kruskal-Wallis test indicated significant differences in the N uptake and primary production rates between the habitats ($p < 0.05$, Figure A-4). The Dunn test was applied to identify the habitats that differed significantly in their uptake rates. Results are summarized in Table A-2 in the appendix. While no significant differences in NO_3^- uptake rates were detected, NH_4^+ uptake rates in the YPC habitat were significantly higher than in all other habitats. N_2 fixation rates in the RI habitat were significantly lower compared to the OPC, OPM and WPM habitats and amino acid uptake rates were significantly higher in the OPC habitat than in the RIV and OWS habitat. Primary production rates in the OSW habitat were significantly lower than rates in the RI, YPC and OPM habitats, but no significant difference was detected between the other habitats. Primary

production was supported by N_2 fixation to maximal 33 % at all drift stations, while NO_3^- and NH_4^+ uptake rates supported up to 73 % and 82 % of primary production, respectively (Table II-3, Figure A-5).

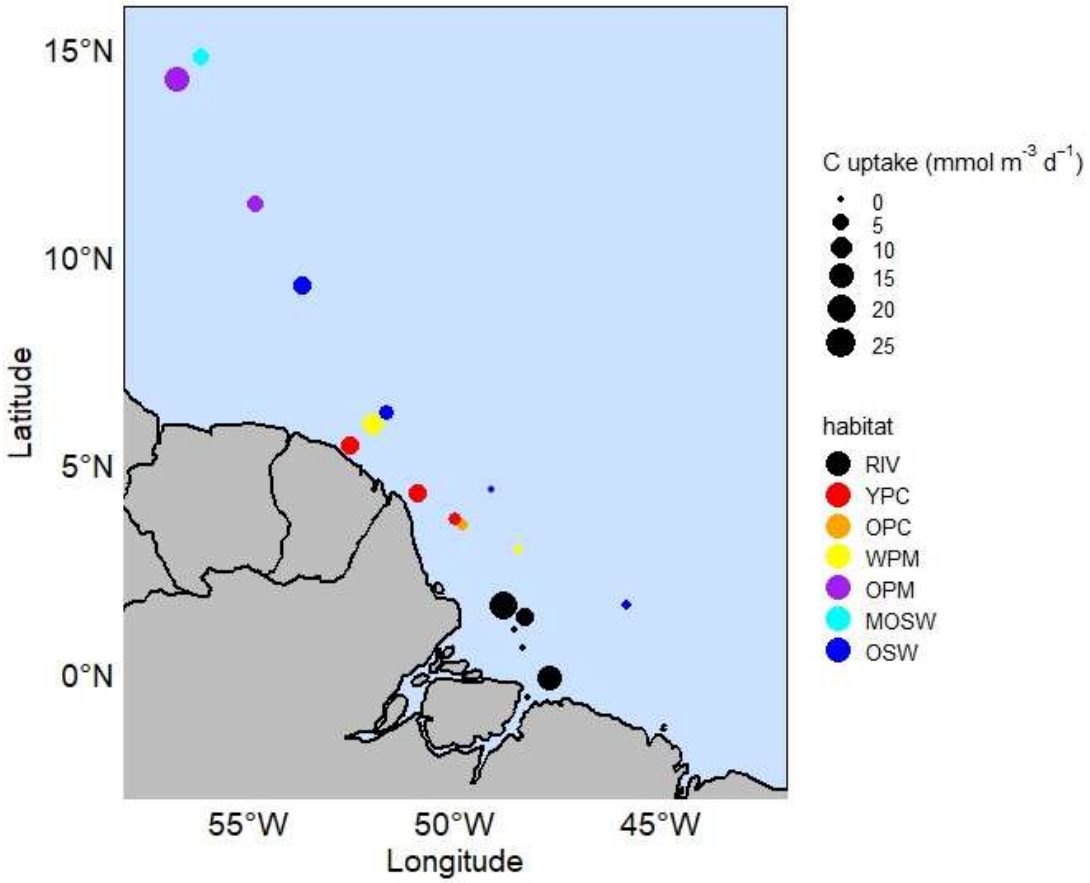

Figure II-6: *Primary production rates at the sampled stations integrated between the surface and the 1 % light level (in mmol m^{-3} d^{-1}). Rates are normalised to the depth of the euphotic zone. Colours indicate the respective defined habitat for the station and the size of the circles is representative of the magnitude of the rate.*

Table II-3: *mean N uptake and N_2 fixation rates and primary production ("PP") supported by NO_3^-, NH_4^+ (mean ± standard deviation) and N_2 fixation (only one station per habitat sampled) in the different habitats. Rates are integrated between the surface and the 1 % light level and normalised to the depth of the euphotic zone- "n" gives the number of stations per habitat. "NA" is not available." N_2 fix." is N_2 fixation.*

habitat	mean NO_3^- uptake (µmol N m^{-3} d^{-1})	mean NH_4^+ uptake (µmol N m^{-3} d^{-1})	mean amino acid uptake (µmol N m^{-3} d^{-1})	N_2 fixation rate (µmol N m^{-3} d^{-1})	mean PP (mmol N m^{-3} d^{-1})	PP supported by NO_3^- (%)	PP supported by NH_4^+ (%)	PP supported by N_2 fix.$^+$ (%)
RIV (n = 6)	194.6 ± 104	1501.9 ± 2043.6	289 ± 310.45	0.4	7.6 ± 9.7	9 ± 12	25 ± 44	0.02
YPC (n = 6)	473.6 ± 447.1	2479 ± 1162.3	153.3 (n = 1)	n.a.	5.2 ± 2.3	44 ± 62	24 ± 9	n.a.
OPC (n = 6)	151.4	278.6	397.4	2.3	1.5	75	12	15
WPM (n = 6)	191.6 ± 116.9	709.2 ± 312	71.8 (n = 1)	7.2	5 ± 4.8	73 ± 47	82 ± 7	33
OPM (n = 6)	135.4 ± 84.1	618.8 ± 424.2	NA	8.5	9.9 ± 7.6	11 ± 0.3	77 ± 23	20
MOSW (n = 6)	43.7	NA	NA	2.8	5.2	7	88	19
OSW (n = 6)	46.8 ± 48.6	426.9 ± 267.2	NA	n.a.	1.2 ± 1.5	14 ± 15	100 ± 66	n.a.

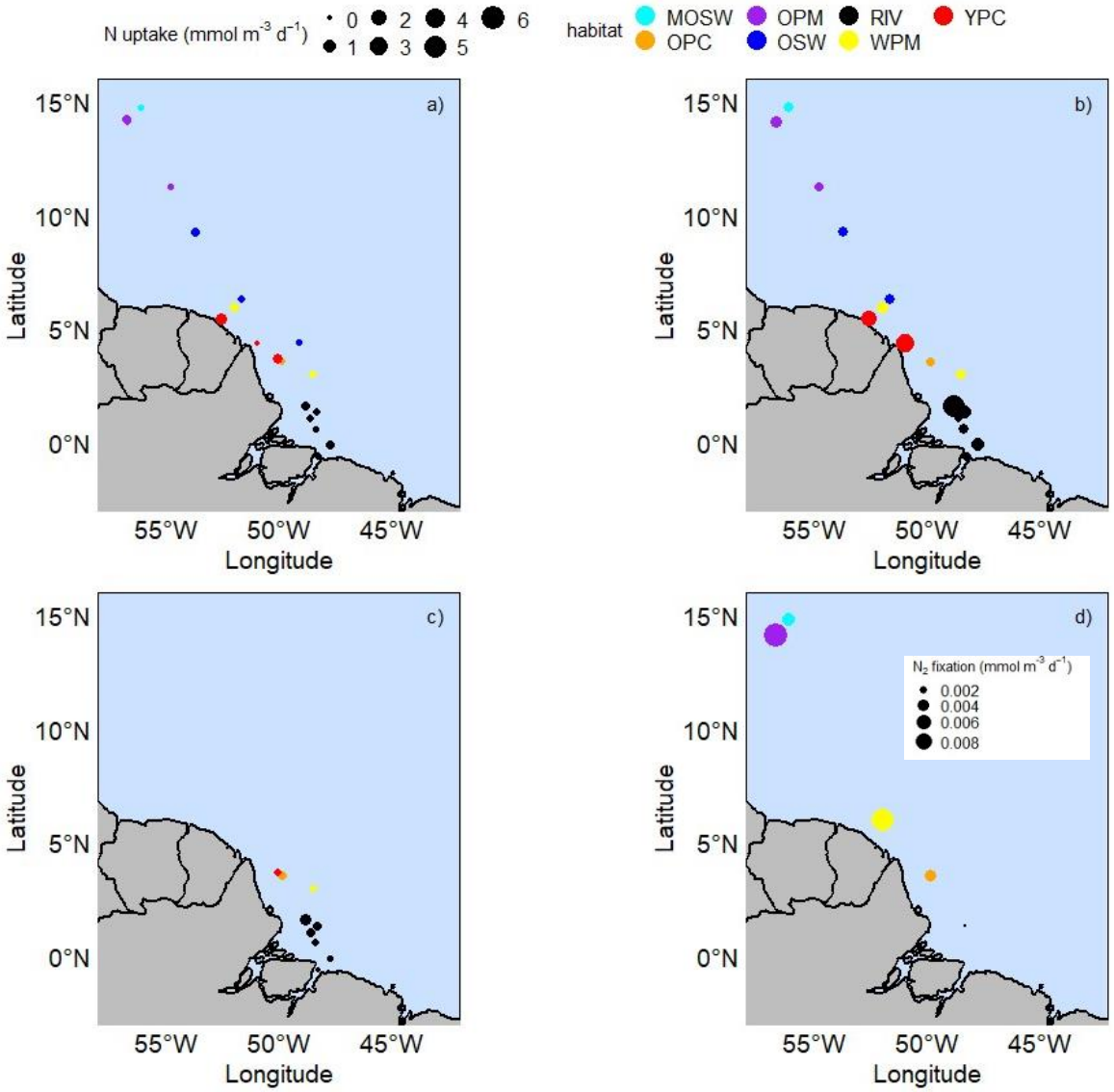

Figure II-7: *Nitrogen uptake rates integrated over the depth of the euphotic zone (surface to 1 % light level) and normalised to the euphotic zone depth. Colours indicate the respective defined habitat for the station and the size of the circles is representative of the magnitude of the nitrogen uptake rate. a) NO_3^- uptake rates, b) NH_4^+ uptake rates, c) amino acid uptake rates and d) N_2 fixation rates. Rates are given in mmol m^{-3} d^{-1}. Note the different scale for N_2 fixation rates.*

Uptake ratios of DIC to DIN were mostly below the average POC:PON ratio (i.e. 8.9) of my samples (Figure II-8). Only at stations in the OPM and MOSW habitats, and few sampling points in the OSW and WPM habitat, the ratio of uptake rates was above the POC:PON ratio.

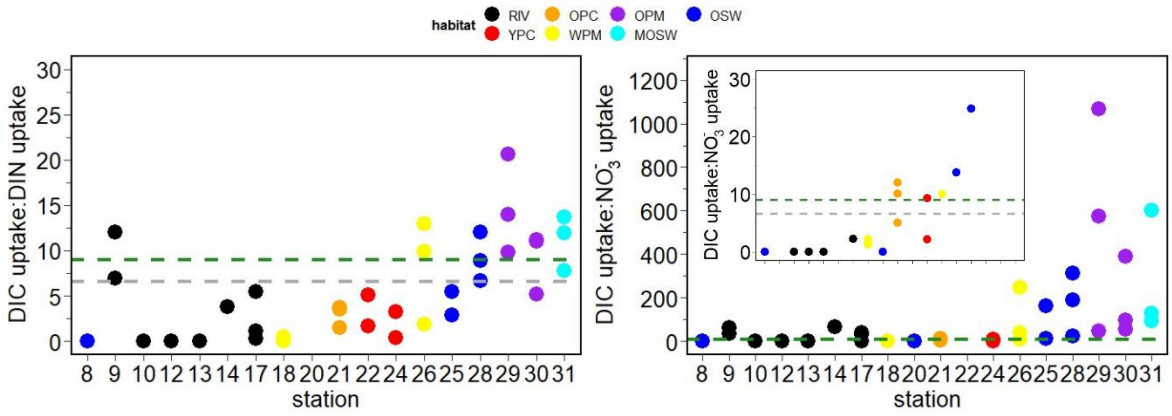

Figure II-8: *DIC to DIN uptake ratios at the sampled stations. The colours indicate the different habitats. The grey dashed line marks the Redfield ratio of 6.6, while the green dashed line marks the average POC:PON ratio of my samples, which was 8.9. The left figure shows the ratios of DIC to total DIN uptake rates, while the right figure shows ratios of DIC to NO_3^- uptake rates only. The small inlay in the right figure shows ratios below 30 only for reasons of a better overview.*

The f-ratio was very low (0.06 – 0.39) throughout the sampled stations, hence the fraction of new production (NO_3^- uptake + N_2 fixation) was small (Figure II-9). This points to a high potential for recycling of nitrogen within the euphotic zone. The highest values calculated from depth integrated rates (0.29 – 0.39) were found at stations 10, 13, 24 and 28, while surface f-ratios were highest (0.29 – 0.39) at stations 13, 18, 21 and 24. High f-ratios not necessarily corresponded to the stations with the highest NO_3^- uptake rates, as NO_3^- uptake was rather low at stations 10, 13 and 18.

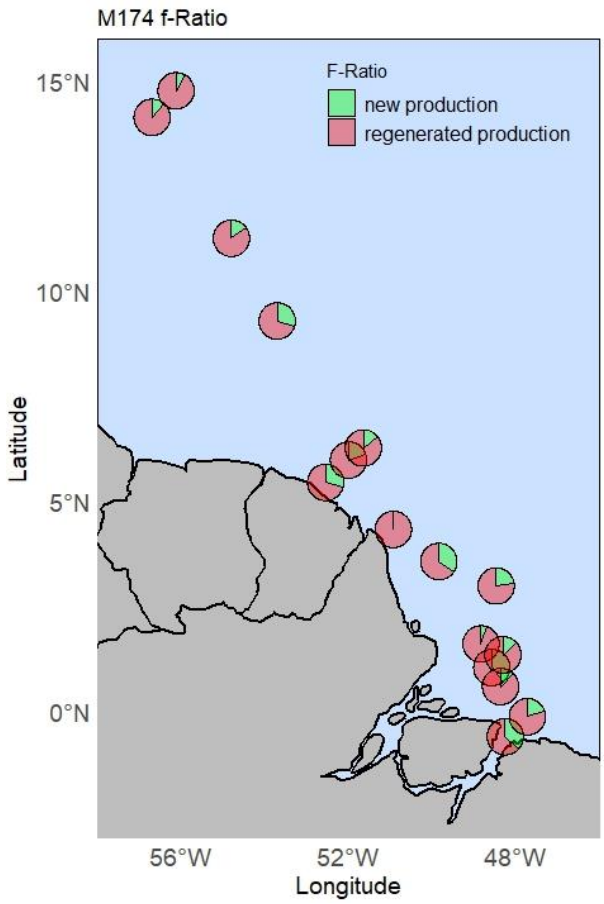

Figure II-9: *Map showing the F-ratio at the sampled stations calculated from integrated N uptake rates. The fraction of NO_3^- uptake (F) is shown in green, while 1-F, the recycled production represented by NH_4^+ uptake, is shown in red.*

The contribution of NO_3^- and NH_4^+ uptake to total primary production changed along the plume (Tab. II-3, Figure A-5 in the appendix). On average, NO_3^- uptake contributed up to 75 % of total primary production in the YPC, OPC and WPM habitats, but was of minor importance in the RI habitat and in the northern plume (OPM, MOSW, OSW habitats). On contrast, NH_4^+ uptake contributed up to 100 % of total primary production at the WPM, OPM, MOSW and OSW habitats, but much less at the RIV, YPC and OPC habitats. The estimated contribution of N_2 fixation to primary production was maximal in the WPM and MOSW habitats with 33 and 19 %, respectively (Table II-3).

II-3.3 Phytoplankton community

At the river mouth (RI habitat), total Chl *a* and phytoplankton pigment concentrations were low. Diatoms and colonial cyanobacteria dominated the stations closest to the mouths of the Amazon and Pará rivers, while cryptophytes were the dominant group at stations further from the mouth (stations 9 and 17), where total Chl *a* concentrations at the surface increased to 0.8 mg/m³. Dinoflagellates and prasinophytes also contributed to the phytoplankton community at these low salinity stations, albeit at lower concentrations than diatoms (Figure II-10). Diatoms were clearly the dominant phytoplankton group in surface waters at stations close to the coast in the YPC and OPC habitats, while haptophytes and *Synechococcus* spp. dominated surface waters in the OPM, WPM and OSW habitats, with the exception of stations 28 and 30, where colonial cyanobacteria represented a major fraction of the phytoplankton community.

The spatial distribution of the phytoplankton community composition changed not only along the plume axis, but also vertically (Figure II-10). The OSW, MOSW, WPM and OPM stations were characterized by a dominance of *Synechococcus* sp. and *Prochlorococcus* sp., alongside with haptophytes throughout the water column. The contribution of haptophytes increased with increasing depth.

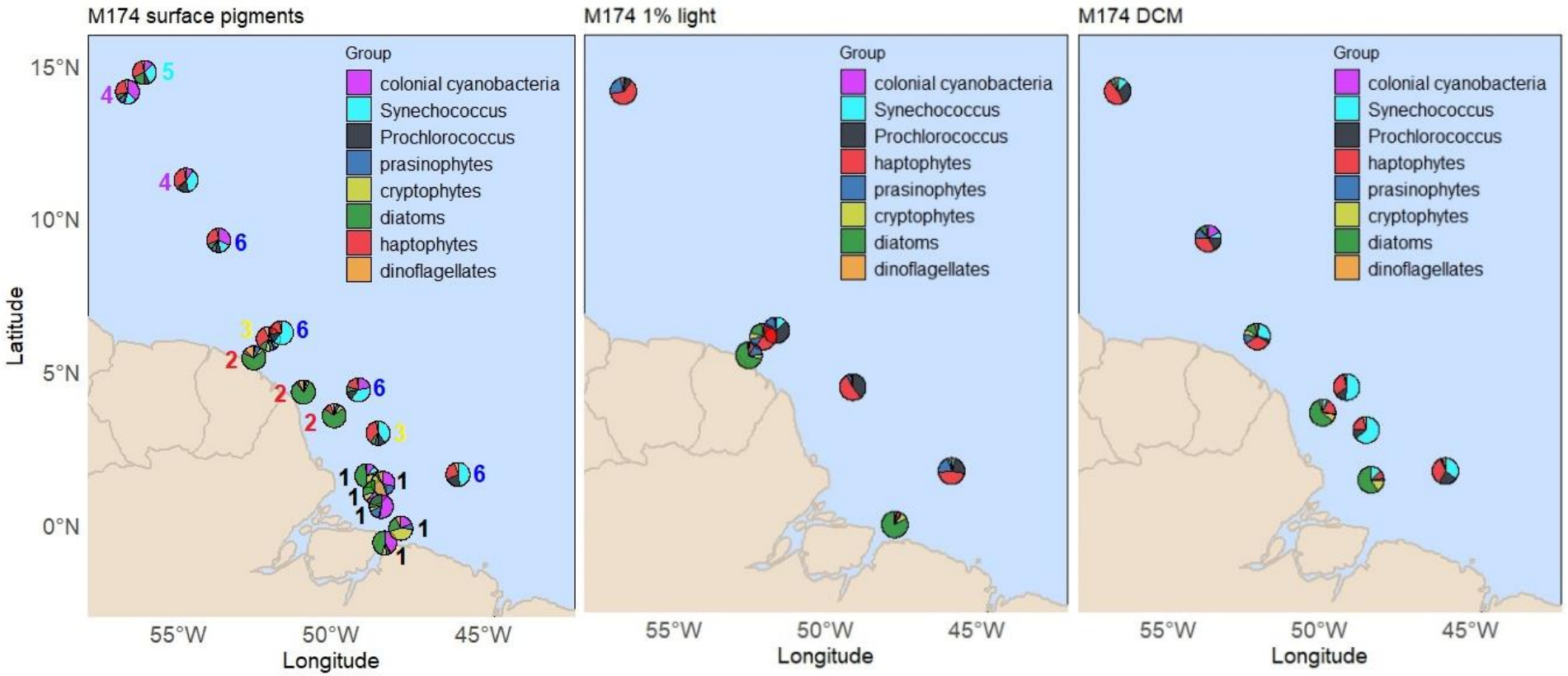

Figure II-10: *Maps of the spatial and vertical composition of the phytoplankton community calculated with CHEMTAX for surface samples, the deep chlorophyll maximum (DCM) and the base of the euphotic zone (the 1 % light level). The numbers in the left panel indicate the respective habitat each station is attributed to and the colour of the numbers represents the colours attributed to each habitat in the Figures II-5 to II-8. 1 = RIV habitat, 2 = YPC habitat, 3 = WPM habitat, 4 = OPM habitat, 5 = MOSW habitat and 6 = OSW habitat.*

Linear regression analysis was performed to identify correlations between phytoplankton groups and N-uptake rates. Only significant correlations are shown here (Figure II-11). Diatom and dinoflagellate abundance was positively correlated to the uptake rates of all three N-species investigated, while cryptophytes did not show significant correlations with NO_3^- uptake rates. The only significant positive correlation for *Synechococcus* sp. was found with NH_4^+ uptake.

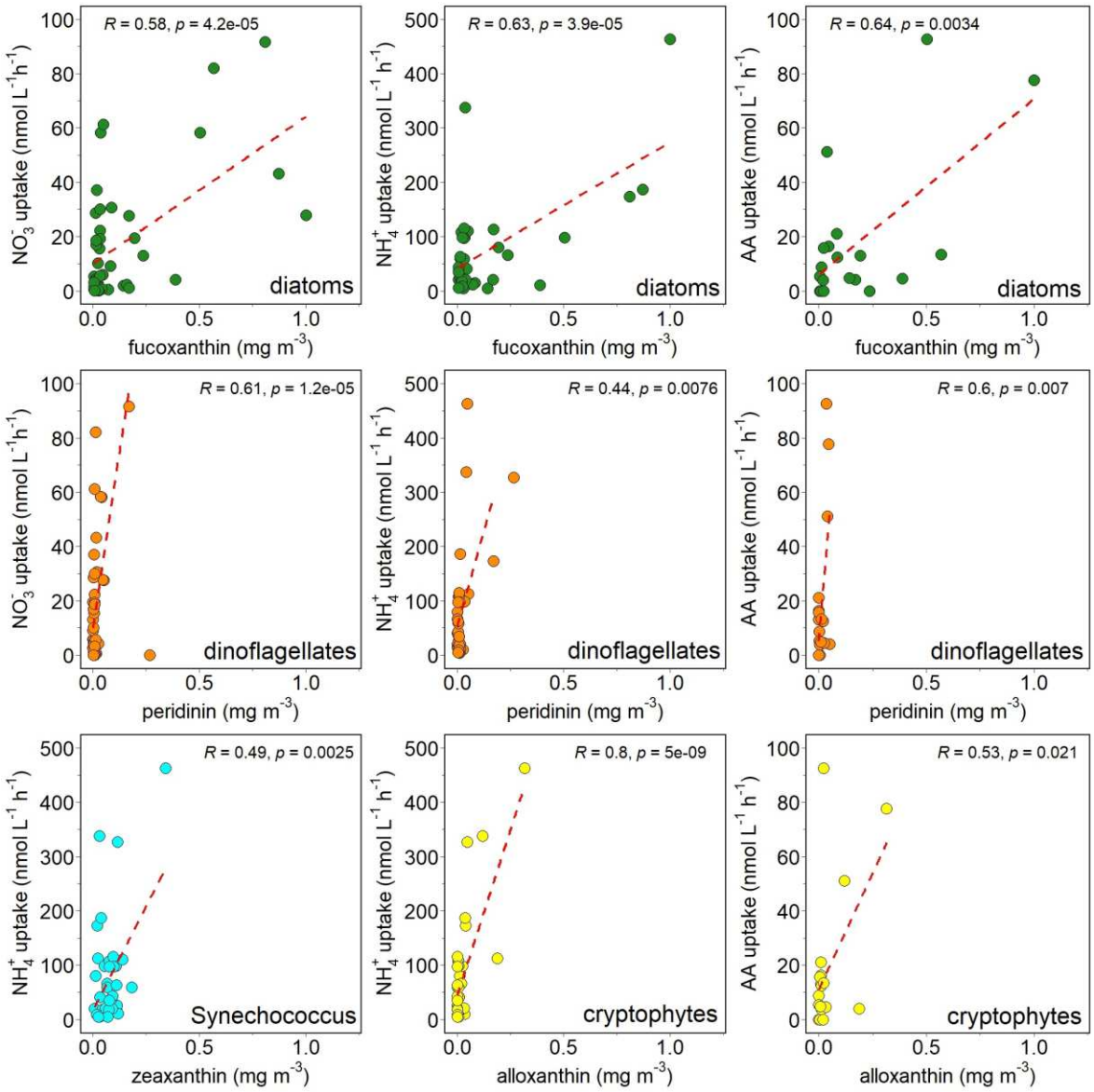

Figure II-11: *Linear regression analysis for phytoplankton groups and N-uptake rates. Only significant correlations are shown. The colours refer to the respective phytoplankton groups: diatom abundance is shown in green, dinoflagellate abundance in orange,* Synechococcus *sp. abundance in cyan and cryptophyte abundance in yellow.*

To identify relationships of environmental variables and DIN uptake rates along the plume axis, a PCA analysis was conducted with data from the plume layer and surface samples from the OSW habitat (Figure II-12). The first PC consisted mainly of nutrient concentrations, salinity, temperature, the plume thickness and nitracline depth, as well as the oceanic phytoplankton groups (mainly *Synechococcus* spp., *Prochlorococcus* spp. and haptophytes) and contributed 38.86 % to the explained variance in the dataset. The second PC included DIN and amino acid uptake and primary production rates as well as colonial cyanobacteria, diatoms, dinoflagellates and total Chl *a*. The explained variance of the second PC was 16.44 % (Figure II-12). Turbidity contributed equally to both PC1 and PC2. DIN uptake and primary production rates were positively correlated to dinoflagellates, diatoms and total Chl *a*. In particular, NO_3^- and amino acid uptake rates were positively correlated to dinoflagellates, while NH_4^+ uptake rates were associated with diatoms. All variables seemed to be more or less equally important to explain the variance in the dataset and we could not identify a clear controlling factor on the DIN uptake rates or primary production. PAR seemed to play a minor role in explaining the variance of our data within the plume, but it should be noted that PAR data is not available for three of the six stations sampled at the river mouth. The PAR sensor did not work well at these stations, probably due to high concentration of particles in the water. N_2 fixation played a minor role to explain the variance within the plume and was positively correlated to salinity, the plume thickness and the nitracline depth, as well as the abundance of haptophytes, *Synechococcus* spp. and *Prochlorococcus* spp., but not to the abundance of colonial cyanobacteria like *Trichodesmium* spp.. Nutrient concentrations were negatively correlated with salinity and rather positively with turbidity and in the case of NH_4^+ also with temperature within the plume.

A second PCA analysis was conducted on samples from below the plume and the OSW stations (excluding surface samples) to compare conditions and relationships within the plume or surface layer to the rest of the euphotic zone below the plume (Figure II-12). The first PC explained 26.39 % of the variance in the dataset and consisted mainly of phosphate, nitrite and silicate concentrations, the nitracline depth and plume thickness, the abundance of haptophytes and dinoflagellates, as well as amino acid uptake and turbidity. The second PC explained 14.36 % of the variance and consisted mainly of NO3- and PO43-, the abundance of prasinophytes, cryptophytes, diatoms and dinoflagellates and amino acid uptake. Amino acid uptake contributed equally to both PC1 and PC2. N_2 fixation and amino acid uptake were more important below the plume than within it, while DIN uptake

contributed much less to the explained variance. N_2 fixation was associated with total Chl *a* concentrations, while amino acid uptake was positively correlated to the abundance of dinoflagellates, as observed within the plume. The abundance of *Synechococcus* spp. played a minor role below the plume. NO_3^- and PO_4^{3-} concentrations were weakly positively correlated to salinity below the plume, in contrast to within the plume. Below the plume, NH_4^+ concentrations were positively correlated to nitrite concentrations and silicate concentrations were positively correlated to turbidity.

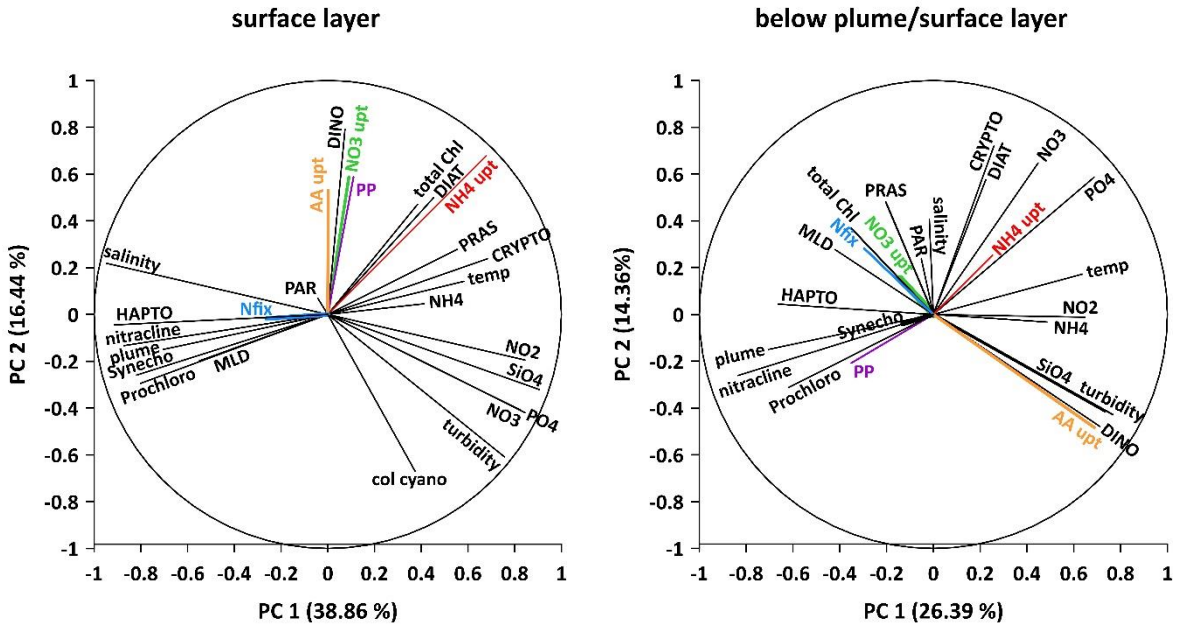

Figure II-12: *PCA analysis for values within the plume/at the surface of OSW stations (left) and below the plume (right), respectively. "PP" is primary production (in purple), "AA upt" is amino acid uptake (in orange), "NO3 upt" is nitrate uptake (in green), "NH4 upt" is ammonium uptake (in red) and "Nfix" is N_2 fixation (in blue). "MLD" is the mixed layer depth, "plume" refers to the plume thickness, "PAR" is photosynthetically active radiation, "DINO" are dinoflagellates, "DIAT" are diatoms, "PRAS" are prasinophytes, "CRYPTO" are cryptophytes, "col cyano" are colonial cyanobacteria, "Synecho" is* Synechococcus *spp., "Prochloro" is* Prochlorococcus *spp., "HAPTO" are haptophytes.*

II-4 Discussion

The results show a change of environmental conditions along the plume axis, as the influence of the ARP decreases towards the north, resulting in a distinct separation of phytoplankton communities. However, these differences were not reflected clearly in the dominant nitrogen source used by phytoplankton. My observations will be discussed in more detail in the following.

II-4.1 Characterisation of environmental conditions and phytoplankton communities in the different habitats

Environmental conditions (stratification, turbidity, nutrient concentrations)

The RI habitat was characterized by high concentrations of DIN, PO_4^{3-} and silicate and high turbidity (Table II-2, Figure II-5). DIN:DIP ratios were relatively close to the Redfield ratio, which is a special feature of the Amazon River compared to other large rivers that mostly have elevated DIN:DIP ratios far above the Redfield ratio (Turner et al., 2003). As a result of the strong stratification, there was no mixing between the surface and the deeper water layers, as obvious from the low Richardson numbers below the halocline (Figure II-4). Only within the surface layer, higher Richardson numbers were determined, pointing to mixing within the plume layer, probably as a result of the strong tidal impact at the river mouth (V. Mohrholz, pers. comm.). Accordingly, tight coupling of production and recycling processes exits within the surface layer. Similar to the RIV habitat, nutrient concentrations in the YPC habitat were still elevated, although NO_3^- concentrations had decreased drastically (Figure II-5), implying high uptake of this N species in the estuary. As a result, DIN:DIP ratios were much higher than in the RIV habitat. Turbidity was lower in this habitat compared to the RI habitat, providing better light conditions for phytoplankton productivity. The OPC habitat showed similar conditions as the YPC habitat. In the other habitats, stratification by the plume was reduced and deeper MLDs were found. DIN concentrations were low, but above detection limit in surface waters. PO_4^{3-} and silicate concentrations were low as well and turbidity had decreased to values below 0.1 NTU in all of these habitats. While light limitation was alleviated, nutrient limitation was the greater challenge for phytoplankton communities in these oligotrophic conditions.

Phytoplankton community composition

In response to changing environmental conditions, the phytoplankton community composition differed clearly between the defined habitats with higher vs lower plume influence, resulting in a coastal as opposed to an oceanic phytoplankton community. As is obvious from their globally distinct distribution, phytoplankton groups differ not only in their preferred range of environmental conditions, like salinity, temperature, light availability and nutrient demands, but also in their N uptake kinetics (Glibert et al., 2016). Additionally, different taxonomic groups pursue disparate trophic modes, which enable them to occupy distinct niches. The characteristics for the phytoplankton groups included in the HPLC analysis are summarized in Table II-4. Clear relationships between environmental variables and systematic phytoplankton groups are difficult to find in literature. The groups identified with an HPLC analysis contain many different species and therefore, the variety within these groups is very large and non-specific at the group level. This is especially true for cryptophytes, prasinophytes and haptophytes and stresses the importance to include species-specific adaptations and preferences in global ecosystem models to predict future changes.

Turbidity and light limitation

Turbidity contributed significantly to the explained variance in the dataset, both within and below the plume (Figure II-12). This shows that the ARP has a tremendous impact on light availability, which can lead to limitation of phytoplankton activity. Recently, Latasa et al. (2023) have shown that diatoms and *Synechococcus* spp. prefer shallow layers with high irradiance, while *Prochlorococcus* spp. and certain haptophyte groups preferentially occur in deeper layers with low light intensities. Dinoflagellates, green algae (including prasinophytes) and haptophytes were predominantly detected in water layers with intermediate irradiance. Cryptophytes seem to be very flexible in their tolerance of rapid and large fluctuations in light intensities (Mendes et al., 2023). While the observed distribution pattern is also applicable to the vertical distribution of phytoplankton groups in the ARP (Figure II-10), the spatial distribution of phytoplankton groups along the turbidity gradient of the plume does not always reflect this pattern. Cryptophytes seem to be able to cope well with the low light availability in the RI habitat and therefore may have an advantage over other species. In less turbid areas cryptophytes seems to be outcompeted by diatoms or haptophytes. Interestingly, *Synechococcus* spp. can be split into green-light specialists that dominate in warm, green, equatorial waters and blue-light specialists that dominate in

oligotrophic areas (Grébert et al., 2018), which probably explains why this group is present at high abundance in both offshore waters and waters with an intermediate salinity.

Diatoms were a dominant group in the RIV habitat, but were more abundant in the YPC habitat, where turbidity had decreased. This pattern shows their preference for high light intensities. As I did not determine phytoplankton at the species level, I cannot tell if the diatom species composition differed between the RIV and YPC habitats. Possibly, the diatom species in the RIV habitat are freshwater species and differ in their light preferences from oceanic taxa. The preference of dinoflagellates and haptophytes for higher irradiance was consistently reflected in their distribution, as these groups occurred only at less turbid stations. Prasinophytes however did not show a clear dependence on light availability, as they were ubiquitous along the plume axis, albeit at low concentrations.

Nutrient uptake strategies

It is likely the phytoplankton groups' preferences for nutrient availability that lead to a separation of a coastal phytoplankton community consisting of diatoms, dinoflagellates, cryptophytes and freshwater colonial cyanobacteria occurring and an oceanic community consisting of haptophytes, *Synechococcus* spp., *Prochlorococcus* spp. and oceanic colonial cyanobacteria in the other habitats (Figure II-10). Oceanic phytoplankton groups were positively correlated with salinity, while the coastal groups were correlated positively with salinity in the PCA analysis (Figure II-12). These correlations reflect the association of the coastal community with more nutrient rich plume waters as opposed to the more oceanic and oligotrophic waters. Latasa et al. (2023) found that haptophytes and *Prochlorococcus* spp. dominated the phytoplankton biomass in the oligotrophic areas in the tropical and subtropical ocean, comprising 35 to 36 % of total biomass. Diatoms were least abundant in the areas investigated with a contribution of only 1.6 % to total phytoplankton biomass. As diatoms are r-strategists with high growth rates but rather low nutrient uptake efficiencies, this group prefers habitats with a low stability (Litchman, 2007; Margalef, 1978), i.e. a rather mixed water column with high nutrient concentrations (Li, 2002). On contrast, K-strategists like haptophytes (Alexander et al., 2015; Litchman, 2007) are favoured by habitats with a high and persisting stability of the water column (Parry, 1981). As a result of this K-strategy, haptophytes can cope well with more nutrient-depleted conditions, as their nutrient affinity is higher than that of diatoms (Alexander et al., 2015). Mixotrophy is another trait that can help phytoplankton to prevail in environments with low nutrient availability. This feeding mode has been observed in cryptophytes (Unrein et al., 2014; Yoo et al., 2017), as well as

haptophytes (Jones et al., 1993; Unrein et al., 2014), prasinophytes (Lewis & McCourt, 2004) and dinoflagellates (Jeong et al., 2010; Smayda, 1997).

Table II-4: *Preferences for different environmental variables of the phytoplankton groups included in my study. "n.a." indicates that information is not available.*

group	S	T	water column	irra-diance	preferred N-source	[DIN]	[P]	$[Si(OH)_4]$	trophic mode	reference
diatoms	coastal	optimum 20°C	mixed	low	NO_3^-	high	low	high	autotrophic	Probyn and Painting (1985), Alexander et al. (2015), Margalef (1978), Li (2002)
dinoflagellates	coastal	above 20°C	stratified	species-specific	NH_4^+	low/high	low/high	/	autotrophic, mixotrophic, heterotrophic	Olofsson et al. (2019), Jeong et al. (2010) and references therein, Litchman et al. (2006), Smayda (1997), Heaney & Eppley (1981)
cryptophytes	coastal	polar, temperat e, tropic	n.a.	high and low, very flexible	reduced N forms	rather high	n.a.	/	autotrophic, mixotrophic	Mendes et al. (2023), Richardson (2022), Yoo et al. (2017), Unrein et al. (2014), Berg et al. (2003)
prasinophytes	coastal to open ocean	polar to tropical	n.a.	high	n.a.	rather high	n.a.	/	autrotrophic, mixotrophic	Litchman et al. (2006), Lewis and McCourt (2004), Dos Santos et al. (2017), Shi et al. (2009), Wu et al. (2014), Romari et al. (2004)
haptophytes	open ocean	20 – 30°C	rather stratified	high/low	n.a.	low	low	/	autotrophic, heterotrophic, mixotrophic	Endo et al. (2018), Litchman et al. (2007), Edvardsen et al. (2016), Jones et al. (1993), Unrein et al. (2014), Alexander et al. (2015), Latasa et al. (2023)
Synechococcus	coastal to open ocean	polar to tropical	rather mixed	high	NO_3^-	rather high	low	/	autotrophic	Partensky et al. (1999), Moore et al. (2002), Bertilsson et al. (2003) and Heldal et al. (2003)

group	S	T	water column	irra-diance	preferred N-source	[DIN]	[P]	[$Si(OH)_4$]	trophic mode	reference
Prochlorococcus	open ocean	(sub)-tropical	rather stratified	high/low	NH_4^+	low	low	/	autotrophic	Partensky et al. (1999), Moore et al. (1998), Bertilsson et al. (2003), Heldal et al. (2003)
DDAs	~30-35 PSU	(sub)-tropical	rather stratified	n.a.	N_2	low	high	high	autotrophic	Carpenter et al.(1999), Scharek et al. (1999), Foster et al. (2012)
freshwater (colonial) cyanobacteria	fresh-water, estuaries	temperat e to tropical	n.a.	n.a.	DIN, N_2	rather low	high or low	/	autotrophic	Callieri et al. (2012), Ferber et al. (2004), Spröber et al. (2003)
oceanic colonial cyanobacteria (*Trichodesmium* spp.)	oceanic	20-34°C	n.a.	rather high	N_2	low	low	/	autotrophic	Capone et al. (1997), Breitbarth et al. (2007)

A surprising finding was that the coastal phytoplankton groups did not show a clear correlation with nutrients from the river, except from prasinophytes and cryptophytes that seem to depend on NH_4^+ concentrations (Figure II-12). Gomes et al. (2018) have shown that cryptophytes occurred in low salinity waters of the ARP, where DIN was available at low concentrations. On contrast, Jiang et al. (2015) observed a dominance of cryptophytes in stratified oligotrophic waters at the margins of the Changjiang River plume, which was also observed by Pham et al. (in prep.) for other cruises in the ARP region, but not during my study. It seems that this group is highly variable in its nutrient uptake efficiency and able to cope with a wide range not only of light intensities but also nutrient concentrations. Even though the PCA analysis did not indicate a significant correlation of diatoms with silicate concentrations, the dependence of diatoms on silicate was obvious in their restriction to coastal habitats with high silicate availability. In agreement with my findings, Endo et al. (2018) could show that the abundance of diatoms was not correlated with nutrient concentrations. The authors explained the lack of correlation between diatoms and NO_3^- concentrations with a combination of the large intracellular pool this group is known to possess (Karp-Boss et al., 1996) and rapid uptake of NO_3^-, whenever it becomes available. Dinoflagellates, which can actively regulate their position in the water column and have also been shown to be mixotrophic (Jeong et al., 2010; Stoecker, 1999) or heterotrophic (Gomez, 2012), did not show a positive correlation with nutrient concentrations. Even though the preference of the coastal community is reflected in their occurrence in the more nutrient-rich areas of the ARP, some groups seem to additionally profit from mixotrophic and heterotrophic feeding modes.

Interestingly, colonial cyanobacteria were rather correlated positively with turbidity and nutrient concentrations, even though these correlations were only weak. Except from stations 28 and 30, colonial cyanobacteria were mainly found in the RI habitat and therefore the correlation of this group with turbidity is probably mainly due to the high abundance of freshwater cyanobacteria in the mouth region of the Amazon. Several freshwater cyanobacteria taxa have been identified in the Amazon river (Genuário et al., 2017) and estuary (Cunha de Oliveira et al., 2019). However, the correlation could also imply that oceanic colonial cyanobacteria like *Trichodesmium* spp. rely on nutrients delivered by the ARP, presumably on PO_4^{3-} in the first place, while the other oceanic phytoplankton groups are mostly independent of nutrients from the plume and can cope well with oligotrophic conditions. It has been shown by previous studies (Goes et al., 2014; Weber et al., 2017) that phosphate released during the remineralisation of particles fuels productivity in the northern

ARP. This regeneration of phosphate within the river plume additionally supports the productivity of phytoplankton in the more oceanic habitats. A special case is the MOSW habitat, where DDAs were found during our cruise. The diatom host in this symbiosis profits from riverine silicate, which is still available and likely also the phosphate released from particles in the northern plume.

The oceanic phytoplankton groups *Prochlorococcus* spp. *Synechococcus* spp. and haptophytes were positively correlated with salinity and negatively with nutrient concentrations (Fig. II-11, II-12). On contrast to my findings, Endo et al. (2018) found that the abundance of haptophytes was positively correlated with concentrations of NO_3^-, PO_4^{3-} and silicate. As haptophytes can be heterotrophic or mixotrophic (Jones et al., 1993; Unrein et al., 2014), the missing correlation of this group with nutrient concentrations in my study can possibly be explained by their feeding mode. Haptophytes in the ARP occurred in rather nutrient-deplete areas, where mixotrophy probably helps them to sustain their growth. They were not part of the coastal phytoplankton community thriving in nutrient-rich waters of the plume and seem to be outcompeted there. Farias et al. (2022) identified the vertical structure of the water column as a main driver for phytoplankton community dynamics in the Western Tropical Atlantic south of the Amazon River mouth. The explanation for this finding seems to be the difference in nutrient availability. They found mainly a vertical change in phytoplankton species composition, with a dominant role of *Prochlorococcus* sp. at the deep chlorophyll maximum (DCM), where nutrient concentrations were higher than at the surface. Additionally, a shallow nutricline and MLD promoted higher phytoplankton biomass in this study. This is in agreement with my findings, as total Chl *a* concentrations were negatively correlated with the depth of the nitracline and the MLD in the plume layer, thus indicating higher total phytoplankton biomass at stations with a shallow nitracline and MLD. Furthermore, as in the study of Farias et al. (2022), picocyanobacteria, in our case *Synechococcus* spp., were the main phytoplankton group in nutrient poor surface waters with a low N:P ratio. However, the nitracline in our study was located much deeper than in the study of Farias et al. (2022). Accordingly, nutrient concentrations below the plume were generally low. Still, we see the same pattern of the vertical distribution of the two picocyanobacteria. It has been shown that picocyanobacteria have very low P requirements compared to other species and therefore are well adapted to environments with low nutrient availability (Bertilsson et al., 2003; Heldal et al., 2003). In the oligotrophic Gulf of Mexico, *Prochlorococcus* spp. is the group that relies most on recycled NH_4^+, while *Synechococcus* spp. efficiently exploited NO_3^- at low concentrations, which is assumed to result from

nitrification in the euphotic zone (Yingling et al., 2022). It is well established that small phytoplankton groups are often dominant in oligotrophic regions, as they have higher nutrient uptake efficiencies than larger phytoplankton cells (Aksnes & Egge, 1991; Chisholm, 1992; Fiksen et al., 2013; Hein et al., 1995; Lindemann et al., 2016) and it is likely that this advantage will promote the growth of these groups in the future ocean.

It becomes obvious that coastal phytoplankton groups thrive in the more nutrient rich, but light limited habitats of the ARP, while the oceanic phytoplankton groups prefer the oligotrophic habitats where light availability is high. Diatoms clearly dominate, where nutrient concentrations are elevated and light limitation is reduced. Thanks to their tolerance of low light intensities, cryptophytes profit from high nutrient concentrations in areas where light limitation suppresses high abundance of diatoms. Prasinophytes and dinoflagellates co-occur with other phytoplankton groups at rather low abundance and presumably rely on mixotrophy as a feeding mode, as more dominant groups likely consume the greatest fraction of nutrients. Haptophytes are well adapted to nutrient depleted areas in the ARP and together with picocyanobacteria dominate the oligotrophic habitats. *Synechococcus* spp. and *Prochlorococcus* spp. clearly differ in their vertical distribution, with the latter group preferring deeper waters with lower light intensities. Marine colonial cyanobacteria (*Trichodesmium* spp.) and DDAs could be found only at individual stations in the oligotrophic northern ARP.

II-4.2 Primary production and nitrogen uptake in the coastal and oceanic phytoplankton communities

Primary production

As sampling was conducted along the full axis of the ARP, turbidity was highly variable between stations, leading to a huge divergence of the euphotic zone depth (the 1 % light level). To account for the differences in sampling depths and the resulting bias of greater integration volume on the resulting uptake rate, all uptake rates were normalised to the depth of the euphotic zone at the respective station. Without the normalisation, primary production rates appear to be highest in the OPM and MOSW habitats, lowest at the WPM habitat and quite uniform in all other habitats, which is only a result of the greater water depth used for integration in the OPM, MOSW and OSW habitats. After the normalisation, primary production rates were elevated at three stations of the highly turbid RIV habitat compared to

the other habitats (Figure II-6), which fits the volumetric uptake rates and was not obvious in the depth integrated rates without normalisation. When regarding normalised rates, primary production was lower than in the RIV habitat and quite uniform in all other habitats, with two exceptions. Firstly, the OPM habitat, where higher rates of 15.25 mmol m^{-3} d^{-1} were measured at station 30 and secondly station 26 in the WPM habitat with rates of 9.73 mmol m^{-3} d^{-1}. The high primary production at these two stations is likely fuelled by N_2 fixation, which will be discussed below. NO_3^- and PO_4^{3-} availability is higher compared to more northern areas of the plume, while turbidity has decreased, resulting in high phytoplankton productivity. In the OSW and MOSW habitats, nutrient concentrations are generally low and primary production rates are reduced compared to the OPM, WPM, YPC and RIV habitats.

At three of the six stations in the RIV habitat, the high turbidity prevented primary production. Surprisingly, light availability was sufficient to allow for phytoplankton activity at the three less turbid stations (9, 14, 17) in the RIV habitat. Sufficient light seems to be available at the very surface and rapidly declines to almost darkness within the mixed layer. While the plume causes a strong stratification, the Amazon estuary is also impacted by the tides and a complex interaction of currents, which lead to mixing processes within the surface layer (Volker Mohrholz, pers. comm.). Phytoplankton probably profits from this mixing, as it circulates through the euphotic zone to areas, where photosynthesis is possible. The coastal phytoplankton community seems to profit from the high nutrient concentrations and is responsible for quite high primary production rates. Clearly, diatoms have a major share in the primary production, as they were the most abundant group in most of the RIV and YPC habitats. With few exceptions, primary production rates from the ARP are much higher than in other river plumes or productive regions with average rates per habitat between 67.24 mmol C m^{-2} d^{-1} in the RI habitat and 669.32 mmol C m^{-2} d^{-1} in the MOWS habitat. For instance, production rates in the Eastern Tropical North Pacific of 14.89 – 32.47 mmol C m^{-2} d^{-1} (Painter et al., 2013) of 6.4 – 36.8 mmol C m^{-2} d^{-1} in the Mekong River plume (Voss et al., 2006) and of 32.08 – 67.83 mmol C m^{-2} d^{-1} in the equatorial Pacific (Le Bouteiller et al., 2003) have been reported. This likely demonstrates that phytoplankton profits from the riverine nutrients, even in the habitats with reduced plume influence.

Nitrogen uptake

The Kruskal-Wallis test indicated significant differences between N uptake rates in the surface layer of the different habitats ($p = 0.013$, $p = 0.0003$, $p = 0.00002$ and $p = 0.0063$ for NO_3^-, NH_4^+, amino acid uptake and N_2 fixation rates, respectively). As expected, N_2 fixation rates were very low in the Amazon estuary, where diazotrophs were absent, and increased with decreasing plume influence, reaching very high rates in the OPM (station 30) and WPM (station 26) habitats (Figure II-7). At both these stations, N_2 fixation rates were maximal. At station 30, colonial cyanobacteria contributed 38 % to total Chl *a*, which explains the high diazotrophic activity that fuels primary production. However, at station 26, haptophytes and *Synechococcus* spp. dominated the community. Some haptophyte species have been shown to live in symbiosis with the N_2 fixing unicellular cyanobacteria group A (UCYN-A) (Endo et al., 2018; Mills et al., 2020; Thompson et al., 2014). The high N_2 fixation rates in combination with the dominance of haptophytes at this station hint to the presence of this symbiosis at station 26. The pairwise comparison indicated significant differences between the very low rates in the RIV habitat and the higher rates in the OPC, OPM and WPM habitats. Even though the comparison with the MOSW habitat was not significant, N_2 fixation rates in this habitat were also higher than in the RIV habitat. In conclusion, N_2 fixation is an important nitrogen source for the oceanic phytoplankton community and contributes to primary production in all habitats except the RIV and presumably also YPC habitats, where the coastal phytoplankton community thrives. I did not measure N_2 fixation rates in the OSW habitat in this study. Several studies in the ARP report a high contribution of diazotrophs like *Trichodesmium* sp. and diatom-diazotroph associations (DDAs) to the phytoplankton community in the northern plume and adjacent oceanic areas (Foster et al., 2007; Goes et al., 2014; Gomes et al., 2018; Weber et al., 2017) and high rates of N_2 fixation in the northern plume (Subramaniam et al., 2008). N_2 fixation in the ARP was modelled by Louchard et al. (Louchard et al., 2023), who could show that this process is enhanced by 74 % by the Amazon, mainly driven by the activity of DDAs. Further, it has been postulated that diazotrophs more or less double the amount of new N available in the ARP and this N fuels primary production (Louchard et al., 2021). I therefore believe that N_2 fixation is an important nitrogen source also in the OSW habitat. In the Mekong River plume, high rates of N_2 fixation were determined and this process was identified as an important nitrogen source (Bombar et al., 2010; Grosse et al., 2010). The rates reported from the Mekong River plume by Bombar et al. (2010) are comparable in magnitude to the rates I measured in the ARP, while the rates from surface waters only, reported by Grosse et al. (2010), are about

ten times higher than my rates. The South China Sea undergoes seasonal changes and is highly influenced by the monsoon. The two studies reporting N_2 fixation rates from the Mekong River plume were conducted in different years with variable monsoon influence, which probably explains the large discrepancies in the rates. N_2 fixation rates reported from the ARP were mainly measured during the high discharge period, where the supply of phosphorus and silicate is maximal. Rates during low river discharge are lacking at the moment, but could be different from the ones I determined.

The pairwise comparison indicated no significant differences of NO_3^- uptake rates between the habitats. Highest rates were measured in the RIV and YPC habitats, where turbidity had decreased and NO_3^- was available (Table II-3). In the YPC habitat, only ~ 9 % of the riverine NO_3^- was left. Even though most of the NO_3^- had been consumed in the Amazon estuary, the remaining concentrations were sufficient to support high uptake rates in the YPC habitat. Only ~ 0.4 % of the riverine NO_3^- were left at the northernmost station 31 (MOSW habitat) and NO_3^- uptake rates measured there were the lowest of all habitats. Both the PCA and linear regression analysis showed that mostly the coastal phytoplankton groups (diatoms, dinoflagellates, cryptophytes) were positively correlated to DIN and amino acid uptake rates as well as primary production within the plume.

Amino acid uptake rates were comparable in magnitude to NO_3^- uptake rates and were significantly higher in the RIV and OPC habitats than in the YPC, WPM and OSW habitat, but I did not determine uptake rates further than 5°N and therefore do not have any information on this process in the northern plume. As shown by other studies, the uptake of dissolved organic nitrogen (DON), like amino acids, can contribute significantly to total nitrogen uptake, for example in estuarine systems (Andersson et al., 2006), on the continental shelf off Florida (Wawrik et al., 2009), and in the stratified Mid-Atlantic Bight, where dissolved free amino acids (DFAAs) contributed < 5 %, but still significantly, to total measured nitrogen uptake in surface waters (Bradley et al., 2010). I assume that amino acids originating from recycling processes in the surface layer might contribute to total nitrogen uptake also in the northern ARP, but to which extent remains to be determined.

NH_4^+ uptake rates exceeded NO_3^- and amino acid uptake rates at all sampled stations. Rates in the YPC habitat were significantly higher than in all other habitats. However, highest NH_4^+ uptake rates were determined in the RI habitat, but due to the large variability in uptake rates in this habitat, the differences of uptake rates were not significant. In the OPM, MOSW and OSW habitats, where substrate concentrations were low, NH_4^+ uptake rates were lower

than in the YPC and RI habitats, but still elevated compared to other regions (see below). Interestingly, the highest NH_4^+ uptake rate was determined at station 14, where primary production rates were maximal within the RIV habitat as well and NH_4^+ concentrations were lower than at the other stations in the RIV habitat. This likely reflects high rates of recycling of N alongside high productivity. An overestimation of the rates due to bacterial uptake can be excluded, as bacterial NH_4^+ uptake rates (measured in the dark) were one order of magnitude lower than my uptake rate at this station (N. Choisnard, pers. comm.). Even if bacterial uptake contributed to NH_4^+ uptake rates measured during daytime, the overestimation would be only minor and would not interfere with the interpretations made above.

In summary, DIN and amino acid uptake rates fuelled the high primary production measured at the stations where the coastal phytoplankton community was present. Middelburg and Nieuwenhuize (2000) measured NO_3^- and NH_4^+ uptake rates in six turbid estuaries at the European Atlantic coast. Uptake rates ranged from 5 to 1560 nmol N L^{-1} h^{-1} for NH_4^+ and from 0.25 to 250 nmol N L^{-1} h^{-1} for NO_3^-. My rates in the Amazon estuary were between 5.84 and 58.19 nmol N L^{-1} h^{-1} for NO_3^- and 14.17 to 463.01 nmol N L^{-1} h^{-1} for NH_4^+, so lower than the maximum rates determined by Middelburg and Nieuwenhuize (2000). The authors attributed the high NH_4^+ uptake rates to heterotrophic bacteria. While heterotrophy was also what I expected for the mouth region of the Amazon, I surprisingly determined quite high DIC uptake rates at two of the four river mouth stations, which hints more towards photoautotrophic activity of phytoplankton. Only at these two stations, I also measured higher DIN uptake rates compared to the rest of the mouth region (RI habitat). Middelburg and Nieuwenhuize (2000) identified strong heterotrophic activity in highly turbid estuaries. Heterotrophy was not measured during our cruise, but I cannot exclude that heterotrophic bacteria contributed to the N uptake rates. If DIN uptake rates are converted to theoretical DIC uptake rates assuming the Redfield ratio of 6.6, theoretical DIC uptake rates were higher than actually measured ones at stations 12, 13 and 14 in the RI habitat, which suggests that DIN uptake measured at these stations was not exclusively performed by autotrophic organisms. Mixotrophy has been reported from the Amazon estuary (Pinto et al., 2020), which fits my observations. At these stations, primary production rates were zero and DIN uptake rates were low, implying that heterotrophic or mixotrophic organisms are responsible for N uptake at these three highly turbid stations.

In the Mississippi River plume, NH_4^+ uptake rates of 16.5 to 260 nmol N L^{-1} h^{-1} and NO_3^- uptake rates between 3.2 and 25 nmol N L^{-1} h^{-1} have been measured (Wawrik et al., 2004). Again, my uptake rates from the ARP are generally higher and while Wawrik et al. (2004) observed an increasing trend of NH_4^+ uptake rates towards the Mississippi River delta, it was rather the other way around for the Amazon estuary. However, NH_4^+ uptake in the Amazon estuary was quite variable between the stations, with high uptake rates at two stations and remarkably lower rates at the other RIV stations. Light limitation in the Amazon estuary might be stronger than in the Mississippi delta, preventing high uptake rates in parts of the mouth region. NH_4^+ uptake rates from the northern ARP fall within the upper range of the rates measured in the Mississippi River plume. NO_3^- uptake rates are more similar to the range observed by Wawrik et al. (2004), but at some of our stations in the YPC and OPC habitats rates (up to 91.7 nmol N L^{-1} h^{-1}) exceeded those determined in the Mississippi by far. In agreement with my observations, NH_4^+ uptake rates were higher than NO_3^- uptake rates in the Mississippi River plume, even though NO_3^- concentrations were higher than NH_4^+ concentrations. In addition, in both the ARP and the Mississippi River plume the f-ratio was low, indicating a high probability of recycling processes within the upper layer. These recycling processes seem to prevent nutrient depletion in the productive shelf regions of both river plumes.

It seems that a competition for NH_4^+ between phytoplankton and nitrifiers prevailed at the Amazon River mouth. Nitrification rates were highest (up to 301.6 nmol L^{-1} h^{-1}) at those stations (Choisnard et al., submitted), where DIN uptake rates were low. This shows that nitrifiers win the competition for NH_4^+ over phytoplankton, where light limitation by the river plume prevents phytoplankton productivity. Where light limitation decreases, DIN uptake rates were high and nitrification rates decreased. Bacteria, like nitrifiers, take up NH_4^+ more slowly than phytoplankton (Legendre & Rassoulzadegan, 1995) and therefore get outcompeted, once phytoplankton is no longer restricted by other variables. It seems that NO_3^- originating from nitrification in more turbid areas of the river is taken up by phytoplankton in less turbid areas in the Amazon estuary. In conclusion, nitrification contributes to primary production in the Amazon estuary.

Phytoplankton community and preferred N sources

Nitrogen utilization differed between the coastal and the oceanic phytoplankton communities. An unexpected finding was that N_2 fixation seems to contribute only a minor fraction of the N sources for primary production in the ARP (maximal 33 %), even in the oligotrophic habitats. N_2 fixation by *Trichodesmium* spp. has been shown to fuel primary production via the release of newly fixed nitrogen, as well as carbon fixation by the diazotroph itself (Shiozaki et al., 2018). On the contrary, unicellular diazotrophs like UCYN-A and *Crocosphaera watsonii* contribute much less to primary production, both directly and indirectly (Shiozaki et al., 2018). It seems that the transfer of previously fixed nitrogen to other phytoplankton by *C. watsonii* and *Cyanothece* sp. is only half of that transferred by *Trichodesmium* spp. (Berthelot et al., 2016), which might leave them as a less important nitrogen source in comparison to larger diazotrophs at comparable abundance. Shiozaki et al. (2018) concluded that the importance of N_2 fixation for primary production could largely depend on the composition of the diazotroph community. DIN uptake rates exceeded N_2 fixation rates and alone almost fully supported primary production in all habitats (Figure A-5), implying that N_2 fixation directly was of minor importance for productivity during my study, supporting only 19 and 33 % of primary production at the two station where rates were highest (stations 30 and 26). However, the contribution of nitrogen sources to total primary production based on C:N ratios is only a rough estimate and the magnitude of uptake rates alone speaks for itself. Nevertheless, the role of N_2 fixation should not be underestimated based on these results, as large diazotrophs like DDAs or *Trichodesmium* spp. that have been shown to directly contribute to high rates of primary production in the ARP (Scharek et al., 1999; Subramaniam et al., 2008; Weber et al., 2017) were not very abundant during the time of our cruise. Furthermore, the release of previously fixed nitrogen was not measured here and is not accounted for in the assumption that N_2 fixers contribute only a minor percentage of primary production. High NH_4^+ uptake rates in the OPM, MOSW and OSW habitats support the idea that diazotrophs indirectly support the high productivity in the ARP, but this indirect pathway cannot be quantified without measurements of the release of newly fixed nitrogen and remains a subject of future studies.

In the coastal community dominated by diatoms, both NO_3^- and NH_4^+ uptake rates were highest. However, at station 22 (YPC habitat), where diatoms were also the dominant phytoplankton group, NH_4^+ uptake rates were very high, while NO_3^- uptake rates were low, implying that diatoms efficiently use both these DIN sources, as was confirmed by the

significant positive correlations between diatoms and both NO_3^- and NH_4^+ uptake rates (Figure II-11). The abundance of cryptophytes was positively correlated with NH_4^+ uptake rates as well (Figure II-11). Fittingly, at stations 9 and 17, where NH_4^+ uptake rates were also elevated, cryptophytes dominated the phytoplankton community. It appears that both diatoms and cryptophytes show high NH_4^+ uptake rates at the Amazon River mouth. Berg et al. (2003) investigated the linkage between nitrogen uptake rates and phytoplankton groups during blooming events in the Baltic Sea. In agreement with my results, they found that NO_3^- uptake was associated with diatoms, while NH_4^+ uptake was associated with cryptophytes, as well as dinoflagellates and filamentous cyanobacteria. According to the HPLC analysis, dinoflagellates represented only a minor fraction of the phytoplankton community, but might be underestimated by the HPLC analysis conducted here, as they can have various chloroplasts and therefore be wrongly attributed to another phytoplankton group that shares this pigment (Zapata et al., 2012). Dinoflagellates were detected at stations 9 and 17 (RIV habitat) and stations in the YPC habitat, all of which showed elevated NO_3^- uptake rates. Their abundance correlated positively with both NH_4^+ and NO_3^- and also amino acid uptake rates (Figure II-11). Usually, this group prefers NH_4^+ over NO_3^- (Glibert et al., 2016 and references therein), but Olofsson et al. (2019) observed a higher contribution of dinoflagellates to NO_3^- uptake compared to NH_4^+ uptake. In conclusion, the coastal phytoplankton groups, i.e. dinoflagellates, diatoms and cryptophytes all assimilate DIN and amino acids in the RIV and YPC habitats. This might not be true for other regions, but it seems that phytoplankton in the ARP have acclimated to the overall rather low nitrogen concentrations by high flexibility in their utilization of nitrogen sources.

With decreasing plume influence, the contribution of the coastal phytoplankton groups decreased and that of cyanobacteria and haptophytes increased (Figure II-10). At the same time, DIN uptake rates were reduced and N_2 fixation rates increased. *Synechococcus* sp. was highly abundant at stations with intermediate and high salinity (WPM, OPM, MOSW habitats). Similar observations were reported from the Mississippi River plume by Wawrik et al. (2004) and Wawrik & Paul (2004): *Synechococcus* sp. numerically dominated the plume and was highly abundant at intermediate salinities of 30.8 to 31.5, but further offshore diatoms were dominant, which is contradictory to my results. At the most offshore plume stations, a high abundance of *Prochlorococcus* sp. was observed, coinciding with the highest NH_4^+ uptake rates (Wawrik et al., 2004). The abundance of *Prochlorococcus* spp. increased in my study as the plume travelled northwards, with maxima in abundance at the 1 % light level at OSW stations, where it was the dominant group together with haptophytes. On

contrast to the Mississippi plume, NH_4^+ uptake rates were lower in the offshore OSW habitat compared to the YPC and RI habitats and there was no correlation between *Prochlorococcus* spp. abundance and NH_4^+ uptake rates, even though this group is known to assimilate NH_4^+ (Moore et al., 2002). The common vertical distribution of the two picocyanobacteria with *Synechococcus* spp. dominating the upper layers and *Prochlorococcus* spp. dominating deeper layers (Partensky et al., 1999) was obvious in the ARP. *Synechococcus* spp. and haptophytes co-dominated surface waters in the oceanic communities. A positive correlation with NH_4^+ uptake rates was found for *Synechococcus* spp. abundance (Figure II-11), while haptophytes did not show any correlation with N uptake rates, implying that this group most likely performs a mixotrophic lifestyle in the plume. As haptophytes are known to graze on picocyanobacteria (Frias-Lopez et al., 2009), their co-occurrence with this group can likely be explained by their feeding mode.

Diatoms dominated the most productive inshore stations in the Mississippi plume (Wawrik et al., 2004), which is only partly in agreement with my findings, as cryptophytes alongside diatoms represented a major fraction of the community at two stations with high primary production rates. At these stations, the highest NO_3^- uptake rates were determined, while NH_4^+ uptake rates were also elevated (and higher than NO_3^- uptake rates) in both my study and the one of Wawrik et al. (2004). Wawrik et al. (2009) linked diatoms to NH_4^+ uptake, but both NO_3^- and urea were also used by this phytoplankton group, which fits the results presented here. Ratios of the uptake of DIC to DIN were below the ratio of POC:PON most stations (Figure II-8), which indicates that the focus of phytoplankton uptake lies more on N than DIC. This is especially true for the YPC and OPC habitats, where diatoms dominated the phytoplankton community. Diatoms are known to have a large internal NO_3^- pool that even increases under low light conditions in some species (Needoba & Harrison, 2004).

The small phytoplankton species *Synechococcus* sp., seems to be more flexible in its nitrogen source, using both organic and inorganic nitrogen substrates (Berthelot et al., 2021; Wawrik et al., 2009). This finding probably explains why *Synechococcus* sp. is so abundant in vast areas of the ARP and other regions, while diatoms are more restricted to the habitats with sufficient DIN concentrations. Weber et al. (2021) also observed a shift from a system where NO_3^- was the dominant nitrogen source close to the coast in the Mekong River plume to a diazotroph dominated phytoplankton community in offshore waters, where N_2 fixation was more important, which I could also show for the ARP.

At stations 28 (OSW) and 30 (OPM), the HPLC analysis indicated that colonial cyanobacteria (including *Trichodesmium* sp.) accounted for a greater fraction of the phytoplankton community compared to the other stations in the northern plume (Figure II-10). Interestingly, NO_3^- uptake rates were higher at these two stations than at the other stations of the OPM, MOSW and OSW habitats (Figure II-7). N_2 fixers are known to use DIN and potentially compete with non-diazotrophic phytoplankton for nitrogen (Holl & Montoya, 2005; Masuda et al., 2013), which might be the case here. At other stations in the northern plume, small non-diazotrophic picocyanobacteria like *Synechococcus* spp. and *Prochlorococcus* spp. seem to be more dominant together with haptophytes, sometimes along with DDAs, as indicated by the significant contribution of diatoms to the phytoplankton community at station 31 (MOSW habitat). At these stations, NO_3^- uptake seems to be of minor importance, while NH_4^+ uptake rates are high. This points to the importance of recycling of newly fixed nitrogen in the surface layer. As demonstrated by Berthelot et al. (2019), NO_3^- uptake accounts for a very small fraction of total nitrogen uptake in picocyanobacteria, while regenerated nitrogen (i.e. NH_4^+ and urea) are predominantly consumed, which fits my observations.

II-4.3 The importance of N-recycling

Traditionally, primary production can be distinguished in new and regenerated production (Dugdale & Goering, 1967). New production is supported by NO_3^- uptake and N_2 fixation, whereas regenerated production is fuelled by the uptake of regenerated nitrogen sources, i.e. NH_4^+ and dissolved organic nitrogen (DON). New production is associated with higher export of carbon to the deep ocean via the biological pump. The *f*-ratio ($f = NO_3^-$ uptake/(NO_3^- uptake + NH_4^+ uptake) can be used to calculate the contribution of new and recycled production to total production (Dugdale & Goering, 1967; Eppley & Peterson, 1979). In a system like the ARP it does not seem appropriate to apply this concept of new and regenerated production, as nitrogen is both consumed and recycled quickly within the euphotic zone. The strong stratification by the plume mostly prevents mixing with deeper, nutrient rich water and we assume that the biomass is predominantly recycled within the surface layer. Therefore, not all NO_3^- in the surface layer is new nitrogen, but can also originate from nitrification and should accordingly be considered as "regenerated NO_3^-". Several studies have shown that nitrification takes place in surface waters (Smith et al., 2014; Wan et al., 2018; Wankel et al., 2007) and plays a significant role in providing NO_3^- for

primary production, e.g. for the oligotrophic South Pacific Gyre (Raimbault & Garcia, 2008), the equatorial Pacific (Raimbault et al., 1999), the Mediterranean Sea (Diaz & Raimbault, 2000) and the Atlantic (Fernández & Raimbault, 2007). All of these studies discuss the overestimation of new production due to the presence of regenerated NO_3^-. Choisnard et al. (submitted) determined nitrification rates in the ARP, showing that nitrification takes place at high to moderate rates in the surface layer in the RI and YPC habitats. Accordingly, it is reasonable to assume that regenerated NO_3^- contributes to primary production in these habitats.

Even though the traditional concept is only applicable with caution, I calculated the *f*-ratio to obtain a rough estimate of the importance of new and recycled production for total primary production. The *f*-ratio at my sampling stations was generally very low (0.06 to 0.39), implying a high potential for regenerated production, especially in the northern plume (>60 %, Fig. II-9) and the percentage of primary production supported by NH_4^+ was higher in the habitats with reduced plume influence (Table II-2, Fig. A-5), alongside with higher NH_4^+ uptake rates in these habitats (Fig. II-7). These findings point to an important role of recycled production in supporting total primary production, especially in the northern ARP, where phosphate is recycled in surface waters as well. In addition, the ratios of DIC to NO_3^- uptake versus DIC to total DIN uptake (Figure II-8) show that NO_3^- uptake is not an important N source for primary production at most stations, as ratios of DIC: NO_3^- uptake are far above the average ratio of POC:PON of 8.9. The ratio of DIC uptake to total DIN uptake on the other hand is below the average ratio of POC:PON at most stations in the RI, YPC, OPC and WPM habitats, which indicates that NH_4^+, but not NO_3^- uptake is most important for phytoplankton productivity, supporting the hypothesis that mostly regenerated production fuels productivity.

However, NO_3^- uptake might be underestimated due to the inhibitory effect of NH_4^+ on this process. Especially close to the Amazon River mouth, up to 2 µM of NH_4^+ were determined in surface waters. Several studies have shown that even low concentrations of NH_4^+ can reduce NO_3^- uptake (Cochlan & Harrison, 1991; Glibert et al., 2016 and references therein; L'Helguen et al., 2008; Paasche & Kristiansen, 1982). In my study, NO_3^- uptake rates showed no negative correlation to NH_4^+ concentrations (Figure A-6 in the appendix), as it was observed in the studies cited above. Still, the potential underestimation of NO_3^- uptake rates due to inhibitory effects of ambient NH_4^+ might explain the mismatch between stations with a higher *f*-ratio and high NO_3^- assimilation rates, as I observed at stations 14 and 17.

Early measurements in the Amazon estuary summarized in McClain et al. (2001) showed that the supply of nitrogen from the external sources alone cannot account for the primary production determined on the Amazon shelf. Therefore, the authors come to the conclusion that recycling within the water column is necessary to explain the high productivity. Similarly, Wawrik et al. (2004) established the idea that recycled production is important in the Mississippi River plume and production is supported by the regeneration of NH_4^+. Their study reports high rates of NH_4^+ regeneration, which exceed NH_4^+ uptake by 1.7 to 5.7-fold within the plume. Regenerated production dominates in the offshore Mississippi plume, while NO_3^- driven new production is dominant in the coastal plume, which is consistent with my findings. Moreover, Farias et al. (2022) observed a gradient of increasing new production from the coast to offshore waters south of the Amazon mouth and concluded that recycled production was important in the western tropical Atlantic. Other studies, like Shilova et al. (2017) and Berthelot et al. (2021) reported that urea and also NH_4^+ were dominant nitrogen sources in oligotrophic areas. Here, I did not determine urea uptake rates and have only limited information on amino acid uptake, but still my findings and results of these previous studies suggest an important role of recycled nitrogen sources in the ARP, as has been shown for other regions. I assume that N_2 fixation in combination with recycling of nitrogen within the surface layer supports productivity in the habitats with low or no plume influence, where N_2 fixation rates were high. Besides, even the habitats with higher plume influence show quite high NH_4^+ uptake rates and rather low *f*-ratios. Recycling of nitrogen therefore seems to be important throughout the whole ARP region. In alignment with this conclusion, the model of Louchard et al. (2023) shows that the recycling of organic nitrogen in the ARP is the most important N source to the WTNA. Even though NH_4^+ uptake rates at three stations (26, 28, 31) are potential rates, I am certain that the rates accurately reflect high recycling in the ARP, because primary production rates are high and not fully fuelled by N_2 fixation or NO_3^- uptake. Moreover, Olofsson et al. (2019), had similar findings and could show that NH_4^+ is consumed and produced continuously. They stated that this might be the reason, why uptake rates can be high while concentrations of this nutrient are very low, like in the MOSW and OSW habitats.

II-4.4 Environmental controls of productivity and importance of different phytoplankton groups for CO_2 fixation in the ARP

No clear environmental controlling factor on phytoplankton community composition and nitrogen uptake rates could be identified, although some stick out and impact specific habitats or groups. The analysis rather shows that the changing environmental conditions along the plume axis all together shape specific phytoplankton communities and determine nitrogen utilization and recycling processes. Light limitation caused by the high turbidity of the Amazon River water is clearly a major control. NO_3^- uptake rates were highest at intermediate salinities in the YPC and OPC habitats, where nutrients from the Amazon were still available and a decrease in turbidity had alleviated light limitation. This is a typical finding also in the Mississippi River plume (Wawrik et al., 2004 and references therein). Furthermore, NH_4^+ uptake rates were high throughout the plume area. In relation to N_2 fixation, DIN assimilation seems to be more important for primary production in the ARP. Nevertheless, my results hint towards the role of N_2 fixation as an indirect support of productivity via the release of previously fixed nitrogen. Phytoplankton within the plume seems to rely on a combination of nutrients from the river and from recycling within the surface layer, while phytoplankton below the plume can potentially profit from nutrients originating from deeper, nutrient rich water, although we did not see upwelling during our cruise. Single nutrient concentrations do not seem to be limiting growth and productivity within the plume, as phytoplankton groups change according to their requirements. Therefore, it is nicely documented how the phytoplankton community along the ARP relies on recycling of nitrogen within the surface layer near the river mouth followed by a regime where the plankton is sustained by NO_3^- from the Amazon and by N_2 fixation and nitrogen regeneration in the far northern areas of the plume (Figure II-13). It could be shown here that recycling of nitrogen within the surface layer is most important for the productivity in the ARP. Haptophytes and picocyanobacteria, which dominated the oceanic phytoplankton community and largely rely on this recycling, have been shown to be responsible for up to 38 % and > 60 % of primary production in the subtropical and tropical North East Atlantic, respectively (Jardillier et al., 2010). Obviously, both groups significantly contribute to CO_2 fixation and besides their small size likely are important for carbon export, for example via the formation of aggregates (Deng et al., 2015; Guidi et al., 2016; Richardson & Jackson, 2007). However, it is unclear at the moment, how this export can be related to actual carbon sequestration, hence CO_2 storage in the deep ocean (Guidi et al., 2016). As aggregates can

be of the same size as those of larger phytoplankton (Deng et al., 2015) and especially diatoms are associated with high rates of carbon export (Guidi et al., 2016; Sancetta et al., 1991; Scharek et al., 1999), it is likely that this holds true for small phytoplankton as well. Still, this idea can only be confirmed by future studies. Anyways, it is difficult to assess the contribution of each phytoplankton group and even different nitrogen uptake processes to carbon sequestration. Stratification and the high potential for recycling of biomass within the surface layer of the ARP might prevent export of particles out of the euphotic zone in vast areas of the plume. If biomass is recycled over and over within the surface layer, there is no actually new CO_2 fixation, as the biomass stock stays more or less constant. On the other hand, nitrogen that enters the phytoplankton biomass stock via N_2 fixation directly or the release of previously fixed nitrogen can add to an increase of CO_2 fixation by phytoplankton. Clearly, the traditional concept of new and regenerated production (Dugdale & Goering, 1967) is challenged here and the distinction between these two processes is not straight forward, especially with regard to carbon sequestration. More research on the carbon sequestration potential of different phytoplankton groups is needed, in order to imbed new results into global models and predict the future of primary production and the ocean's storage capacity for CO_2 in the changing ocean.

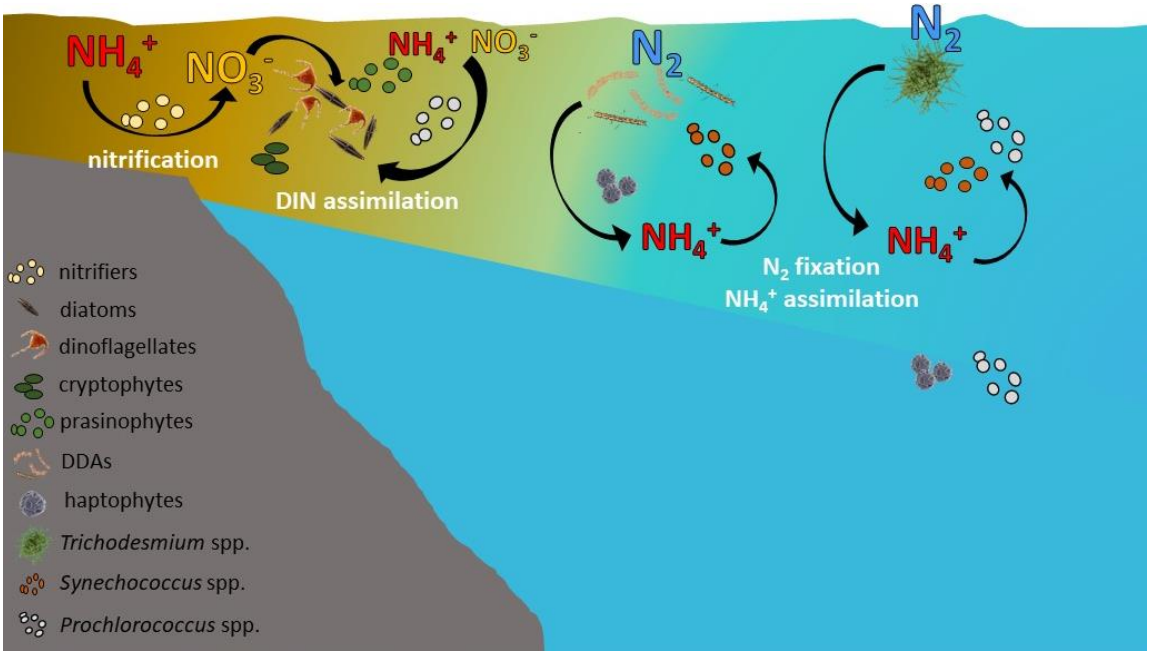

Figure II-13: *Schematic summary of the changing nitrogen sources and phytoplankton communities along the Amazon River plume.*

II-4.5 The future of the ARP

The only N-cycling and primary production measurements available from the Amazon shelf region are from the late 90's (Demaster & Pope, 1996; Smith & Demaster, 1996) and recent measurements from the ARP are lacking. Since then, the Amazon catchment area has been undergoing huge changes in terms of deforestation, damming of tributaries, wild fires and land reclamation (Latrubesse et al., 2017; Martinelli et al., 2010; Ometto et al., 2011). My results provide a baseline for nitrogen uptake processes in the ARP and are a first step towards a better understanding of the impact of the river water in the vicinity of the mouth and on the shelf. By follow up cruises our knowledge on this issue will be improved and we will be able to make more reliable predictions for the future of the WTNA, e.g. as a CO_2 sink and nutrient source.

Ongoing deforestation and increased agricultural land use (Ometto et al., 2011) in the Amazon rainforest change the nutrient load of the river. These changes predominantly lead to increases in the DIN:DIP ratio in the Amazon, as N is used in fertilizer in excess of P (Glibert et al., 2014; Sutton et al., 2013). This could impact primary production and N-cycling in the estuary and consequently the river plume (Wang et al., 2019). If primary production would increase in response to elevated N inputs, this might ultimately cause a P-limitation for the phytoplankton community. Diatoms are poor competitors for P compared to dinoflagellates (Egge, 1998) and accordingly flagellate abundance might increase, while the abundance of diatoms would decrease, leading to a change in community structure. Another possible scenario could be a decrease in P-export to the northern plume as a consequence of enhanced productivity in the young ARP, which could ultimately lead to P-limitation in diazotroph dominated areas of the plume. If productivity in the Amazon estuary and young plume is enhanced tremendously, a resulting P-limitation could ultimately lead to decreased DIN uptake on the shelf and higher DIN concentrations then might reach the northern ARP and could potentially reduce N_2 fixation, as also suggested by Gomes et al. (2018). Gomes et al. (2018) compared the phytoplankton community composition from the ARP with the Changjiang plume, which has a high N:P ratio when it enters the East China Sea. The authors document an absence of *Trichodesmium* spp. and DDAs in waters influenced by the Changjiang plume and attribute this lack of diazotrophs to the high N:P ratio. Instead, prochlorophytes dominate the offshore waters. A main conclusion from their study is that the N:P ratio of the freshwater supply determines the composition of the phytoplankton community, with high ratios favouring diatoms and prochlorophytes and low

ratios favouring diazotrophs. The Changjiang plume catchment area has experienced huge anthropogenic changes like intense fertilizer usage in agriculture (Beusen et al., 2016; Zhang et al., 2007), whereas the Amazon catchment area is still mainly dominated by forest land (Aufdenkampe et al., 2007). The situation in the East China Sea allows a glimpse at what the future of the ARP might look like, if deforestation and population growth continue. If higher N:P ratios lead to enhanced productivity of phytoplankton on the shelf, nutrients from the river might be depleted more rapidly and less phosphate and silicate would be exported from the estuary. The niche for diatoms along the plume and DDAs in the northern ARP might vanish as a consequence and carbon export could then decrease. Further studies are needed to monitor the N:P load of the ARP and evaluate the consequences of potential changes. Swann et al. (2015) report that deforestation could cause an elongation of the dry season over the Amazon and a potential reduction of rainfall. A decrease in the discharge volume of the Amazon River would result in reduced nutrient supply, which probably would affect the productivity in the WTNA negatively.

Another challenge the ARP is faced with is ocean warming and acidification. Laufkötter et al. (2015) investigated drivers and uncertainties of future global primary production in marine ecosystem models. They identified temperature and nutrient concentrations as equally important drivers for changes in global primary production, as alterations of these variables are enhanced phytoplankton growth, increased nutrient limitation and a decrease in phytoplankton biomass. In the first chapter of this thesis it was shown that ocean warming might alleviate P-limitation of diazotrophs (Deng et al., 2022; Jiang et al., 2018). While N_2 fixation in the ARP could profit from such an alleviation that possibly counteracts a decreasing P export from the river, other phytoplankton groups in the ARP might experience a rather negative effect of nutrient limitation in general. For example, Aranguen-Gassis et al. (2019) postulated that diatoms can adapt to high temperatures only under N-replete conditions and the models of Bopp et al. (2005) and Alvain et al. (2013) suggest that diatom dominance is reduced under warmer, more stagnant conditions due to enhanced nutrient limitation. Further, it has recently been shown that silicate limitation might become even more severe due to ocean acidification, as a decrease in pH slows down the rate at which silicate dissolves in the water during remineralisation of diatom biomass and a resulting increase in the Si:N ratio in exported particles (Taucher et al., 2022). It is forecasted that the abundance of diatoms will decrease by 13 – 26 % by the year 2200 due to silicate limitation (Taucher et al., 2022). Higher temperatures and CO_2 concentrations have been shown to result in increasing growth rates and elevated nitrate requirements of a large diatom species

(Qu et al., 2018). Likely, this finding is valid for other phytoplankton groups as well and an enhanced nutrient demand in response to ocean warming and an increase in pCO_2 adds up to reduced nutrient availability caused by stronger stratification. As a result, productivity of species adapted to nutrient-replete conditions, like diatoms could decrease. Diatoms are often associated with high rates of primary production and it is unclear, if a decline of the production rates of this group can be compensated by other taxa. For the ARP, the model of Louchard et al. (2023) shows that when nutrient delivery by the river is switched off, small phytoplankton and *Trichodesmium* spp. outcompete DDAs in the ARP, probably partly resulting from silicate limitation. This is in agreement with the idea that warming favours small phytoplankton over larger species, which is for example supported by the studies of Morán et al. (2010) and Agawin et al. (2000). They found that phytoplankton size decreases with increasing temperature, implying that global warming will likely result in a larger contribution of picophytoplankton to phytoplankton biomass. This could result in a reduced potential for C sequestration, as sinking rate depends on cell size. The same result could arise from a possible increase of the abundance of dinoflagellates in a warmer ocean (Kibler et al., 2015), as dinoflagellates are less likely to sink out of the euphotic zone due to their motility. However, as discussed in the previous section, the role for different phytoplankton groups in C sequestration is unclear at present.

General Conclusion – Implications from this thesis

In a changing ocean, it is more important than ever to fill our gaps of knowledge. It has become obvious that research is still facing many challenges in predicting the effect of climate change and anthropogenic impacts on the marine nitrogen cycle and the consequences for oceanic productivity (Hutchins & Capone, 2022). Same holds true for phytoplankton diversity. Future conditions have been postulated to favour small species over larger ones. My results support the idea that the abundance of unicellular diazotrophs like *Crocosphaera watsonii* is likely to increase in the future. Altogether, the results from the climate change experiment show that *C. watsonii* has the ability to acclimate to environmental changes on short time scales of weeks to few months. The species shows a high inter-community variability of N assimilation pathways, which enables individual cells to quickly react to changes, like for example a DIN pulse, and potentially start a new bloom. The diazotroph investigated here is highly likely to positively adapt to conditions expected in a future ocean with warmer temperatures and a higher pCO_2. As a result of stronger stratification of the surface ocean due to warming, nutrient inputs from deeper waters will probably be reduced, creating an ideal niche for N_2 fixers, as long as phosphate and iron are available in sufficient concentrations. Diazotrophs, like *C. watsonii* could outcompete non-diazotrophic phytoplankton, especially in areas where N is already limiting productivity at present conditions. As warming often seems to result in a decrease of cell size, sinking velocities might be altered and the amount of C exported to the deep ocean could be reduced. How the potentially stimulating effect of climate change on phytoplankton growth and a smaller cell size could counteract each other in terms of CO_2 storage on a global scale cannot be predicted at this stage.

On a local scale, I could show that recycling of N within the surface layer alongside N_2 fixation is important to sustain productivity in the ARP as a highly stratified system. These conditions likely reflect how primary production in a warmer, acidified ocean might look like. As shown in the first part of this thesis and earlier studies, diazotrophs like *C. watsonii* are capable of simultaneous N_2 fixation and DIN uptake and recycling of N has been suggested to help diazotrophs sustain their productivity in extremely oligotrophic areas. Even though I did not determine the abundance of *C. watsonii* in the ARP, unicellular non-diazotrophic cyanobacteria were dominant in the nutrient-depleted areas of the plume. *C. watsonii* likely has ideal prerequisites to thrive in the ARP alongside other diazotrophs and unicellular species in the future. As a significant primary producer, this species could be

important to sustain productivity in the ARP and other comparable systems, if other diazotrophs like DDAs should vanish from the plume due to nutrient limitation. If highly productive estuaries like the Amazon mouth region suffer from anthropogenic impacts and climate change in terms of a reduced productivity, this will inevitably have consequences for the global oceans, as the export of riverine nutrients largely depends on the biogeochemical processes in the estuaries. Rivers supply nutrients to the ocean that are important to sustain global primary production, but coastal ecosystems like the Amazon and its plume are highly vulnerable to anthropogenic impacts and alterations of the nutrient ratios in the river water could have more severe consequences than can be foreseen now. I have discussed here the adaptations of phytoplankton groups to the respective conditions in their habitats and it is obvious that river plumes like the ARP provide ideal conditions to support a highly diverse community. It is unclear, how fragile this diversity is, if conditions in the river plume change and it needs to be investigated in the future, how much the different groups rely on each other's presence and what effect a change of community composition will have for global primary production. A model of Dutkiewicz et al. (2021) suggests that changes in the planktonic community will be key determinants of the global change of ecology an biogeochemistry in the future ocean. They found that a shift in phytoplankton size classes (mostly a replacement of larger species by smaller ones) could compensate for a reduction of primary production in terms of export flux. However, the authors stress that their model results should not be seen as a forecast for biogeochemistry or ecology in the future ocean, but rather explore processes and feedbacks that could be tested in field or laboratory studies.

While it seems that most diazotrophs are likely to cope well with future conditions, this might not hold true for other phytoplankton species. The abundance of diatoms for example is thought to decrease under intensified nutrient limitation. How such changes in phytoplankton community structure will affect the ability of the ocean to act as a CO_2 sink remains to be evaluated by future studies. To improve global models of primary production, it is necessary to include different phytoplankton groups and broaden the niche for diazotrophs, as N_2 fixation is not restricted to areas where DIN is absent. Furthermore, it could be demonstrated in this thesis that experiments investigating multiple stressors are needed, as the response of diazotrophy and phytoplankton communities can be different, if several variables change simultaneously compared to only a single stressor that is altered and a realistic climate change scenario includes a combination of changing variables.

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Submitted manuscripts and grants

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Manuscripts that include data presented in this thesis that have been submitted to peer reviewed journals, but are not part of this thesis:

1.) Umbricht, J., Burmeister, C., Dippner, J. W., Liskow, I., Montoya, J. P., Subramaniam, A., Voss, M.: **Nitrate uptake and primary production along the Amazon River plume continuum**. Submitted to *Journal of Geophysical Research: Biogeosciences* on 26th June 2023.

   The manuscript contains NO_3^- uptake and primary production rates and HPLC data from the M174 cruise, which are also presented in this thesis, and is currently under review.

2.) Filella, A., Umbricht, J., Klett, A., Vogts, A., Vannier, T., Grosso, O., Voss, M., Riemann, L., Benavides, M.: **DOM offsets the detrimental effects of climate change in the nitrogen fixing cyanobacterium *Crocosphaera***. Submitted to *Limnology & Oceanography Letters* on 11th May 2023.

   The manuscript presents results from the joint climate change experiment, including the general setup and experimental procedure as well as growth rates and per cell N_2 fixation rates of *C. watsonii*. The manuscript is currently under review after revision.

Appendix

Table A-1: *p-values obtained from the Mann-Whitney-U pairwise comparison for cell-specific uptake rates determined in the climate change experiment. "n.s." is not significant*

day 0 NO_3^- uptake	treatment 1	treatment 2		Day 0 NH_4^+- uptake	treatment 1	treatment 2		Day 0 N_2 fixation	treatment 1	treatment 2
treatment 2	n.s.	/		treatment 2	< 0.001	/		treatment 2	n.s.	/
treatment 3	0.009	0.003		treatment 3	0.003	n.s.		treatment 3	n.s.	n.s.
day 2 NO_3^- uptake	treatment 1	treatment 2		Day 2 NH_4^+- uptake	treatment 1	treatment 2		Day 2 N_2 fixation	treatment 1	treatment 2
Treatment 2	n.s.	n.s.		treatment 2	n.s.	/		treatment 2	0.004	/
Treatment 3	n.s.	n.s.		treatment 3	0.006	n.s.		treatment 3	n.s.	0.005
day 4 NO_3^- uptake	treatment 1	treatment 2		Day 4 NH_4^+- uptake	treatment 1	treatment 2		Day 4 N_2 fixation	treatment 1	treatment 2
treatment 2	< 0.001	/		treatment 2	n.s.	/		treatment 2	< 0.001	
treatment 3	n.s.	< 0.001		treatment 3	n.s.	n.s.		treatment 3	n.s.	< 0.001

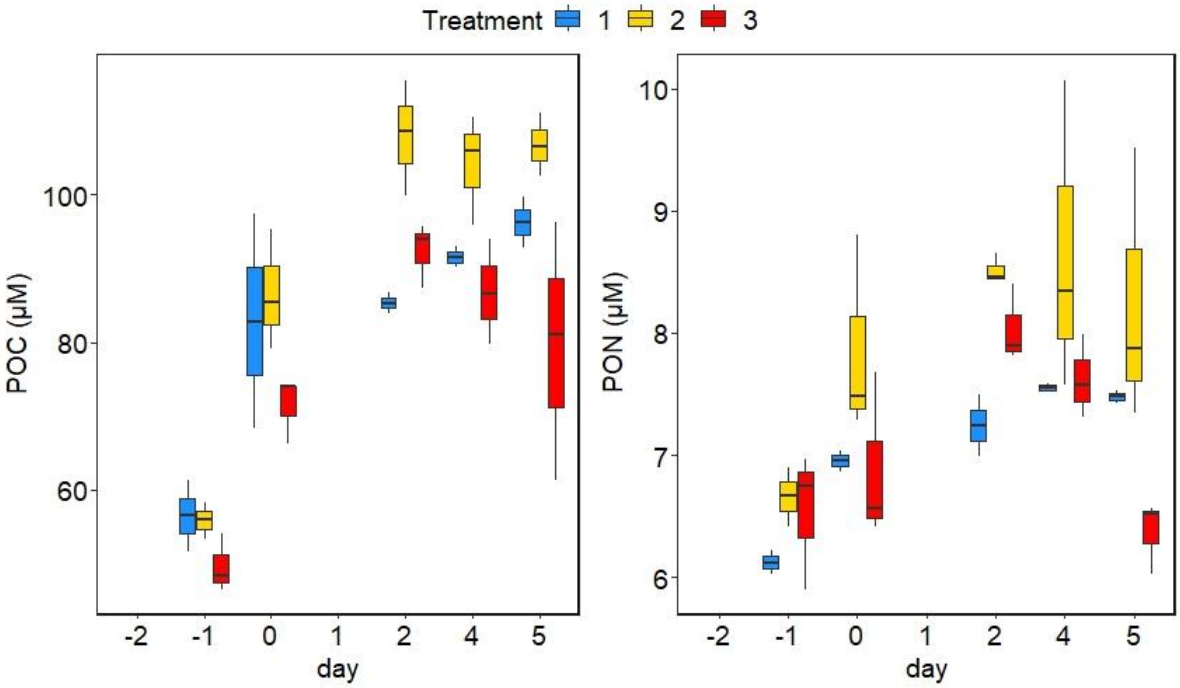

Figure A-1: *POC (left) and PON (right) concentrations from the different treatments over the course of the climate change experiment. Note that there are no samples from days -2, 1 and 3.*

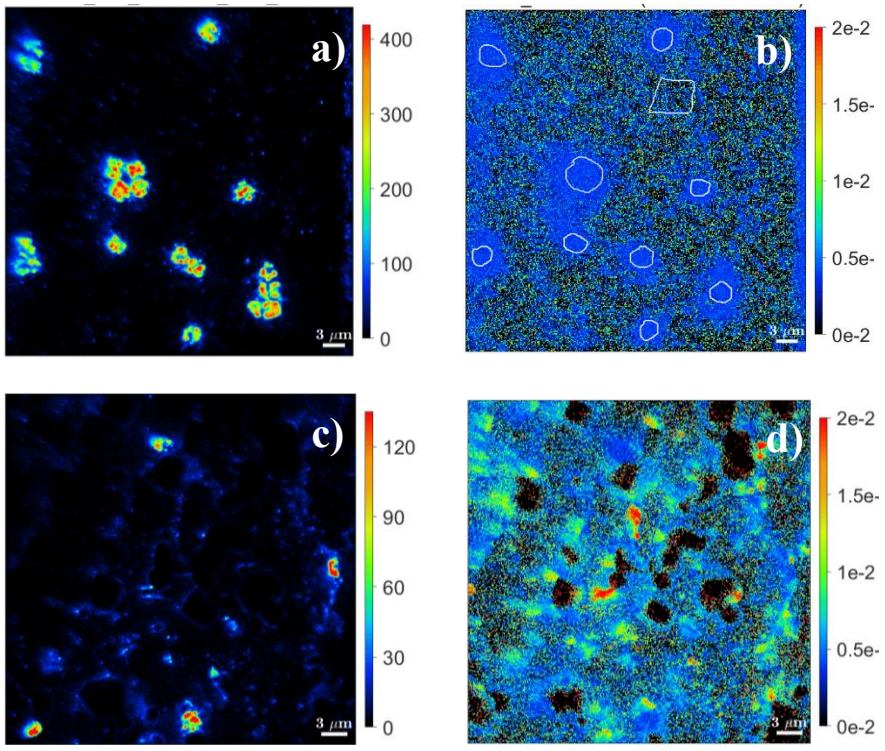

Figure A-2: *Images from nanoSIMS analysis. A) shows the P content of natural abundance samples used to identify cells, b) is the $^{12}C^{15}N/(^{12}C^{15}N + ^{12}C^{14}N)$ of the same sample as shown in a), c) is the P content of a sample enriched with $^{15}N\text{-}NH_4^+$, d) is the $^{12}C^{15}N/(^{12}C^{15}N + ^{12}C^{14}N)$ of the same sample as shown in c).*

Table A-2: *Results from the Dunn test with Benjamini-Hochberg correction for the pairwise comparison of uptake rates between the different habitats. "NA" is not available. Significantly different comparisons are marked in bold.*

habitats compared	p-value NO_3^- uptake	p-value NH_4^+ uptake	p-value amino acid uptake	p-value N_2 fixation	p-value primary production
MOSW - OPC	0.33	0.60	NA	0.61	0.31
MOSW – OPM	0.99	0.79	NA	0.29	1.00
OPC – OPM	0.35	0.64	NA	0.56	0.13
MOSW – OSW	1.00	0.68	NA	NA	0.12
OPC – OSW	0.24	0.72	**0.002**	NA	0.91
OPM – OSW	0.91	0.69	NA	NA	0.01
MOSW – RI	0.30	0.62	NA	0.12	0.98
OPC - RI	0.94	0.11	0.57	**0.03**	0.13
OPM – RI	0.31	0.32	NA	**0.004**	1.00
OSW – RI	0.09	0.10	**0.00001**	NA	**0.002**
MOSW – WPM	0.31	0.75	NA	0.21	0.32
OPC – WPM	1.00	0.31	0.27	0.43	0.96
OPM – WPM	0.33	0.63	NA	0.77	0.12
OSW - WPM	0.18	0.34	0.06	NA	0.46
RI - WPM	0.98	0.76	0.05	**0.004**	0.10
MOWS – YPC	0.85	**0.02**	NA	NA	0.93
OPC – YPC	0.56	**0.0007**	NA	NA	0.16
OPM – YPC	0.83	**0.002**	NA	NA	1.00
OSW – YPC	0.56	**0.0002**	NA	NA	**0.03**
RI – YPC	0.52	**0.01**	NA	NA	0.99
WPM - YPC	0.58	**0.01**	NA	NA	0.18

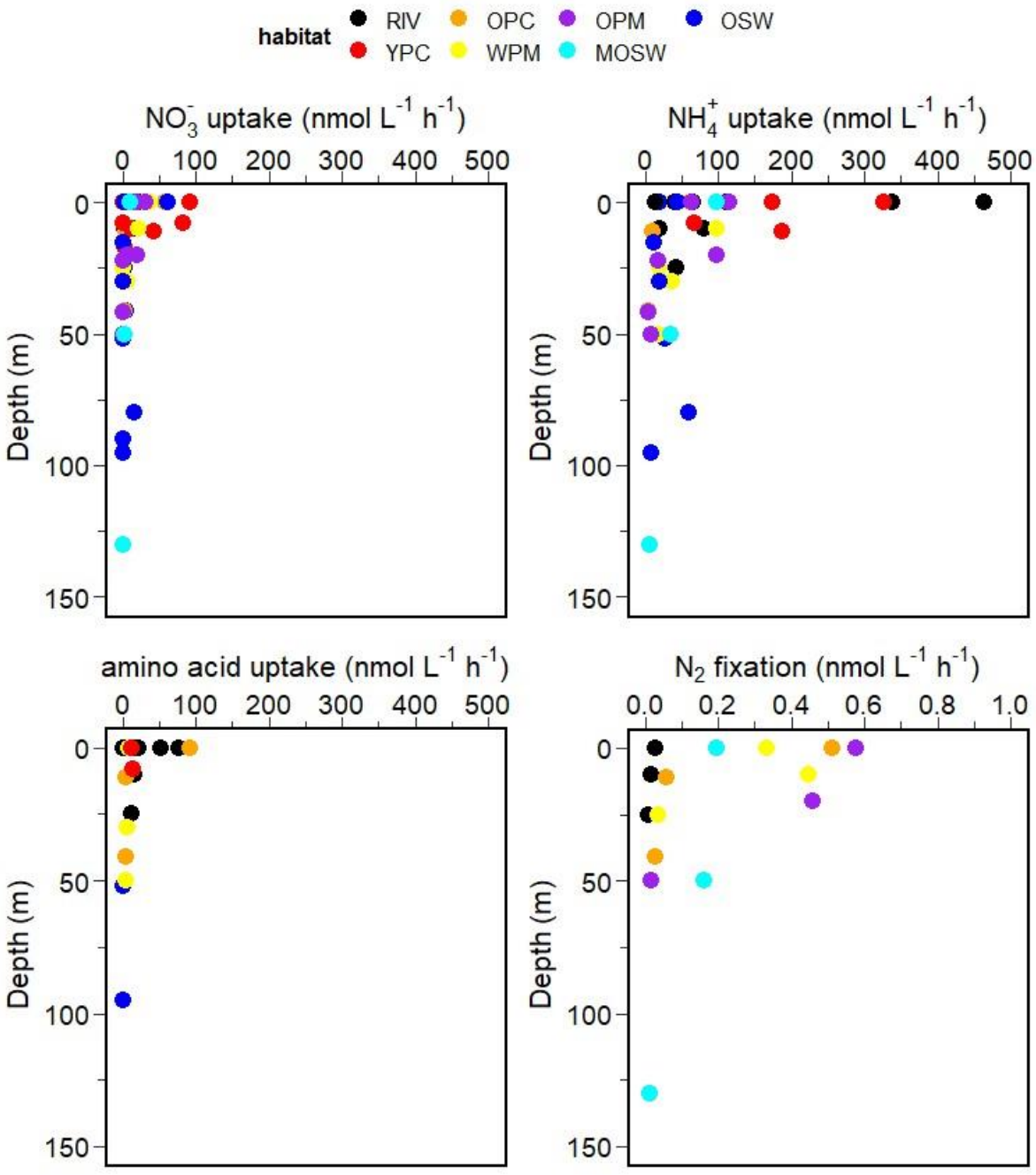

Figure A-3: *Depth profiles of N uptake rates and N2 fixation rates determined in the Amazon river plume. The colours indicate the different habitats.*

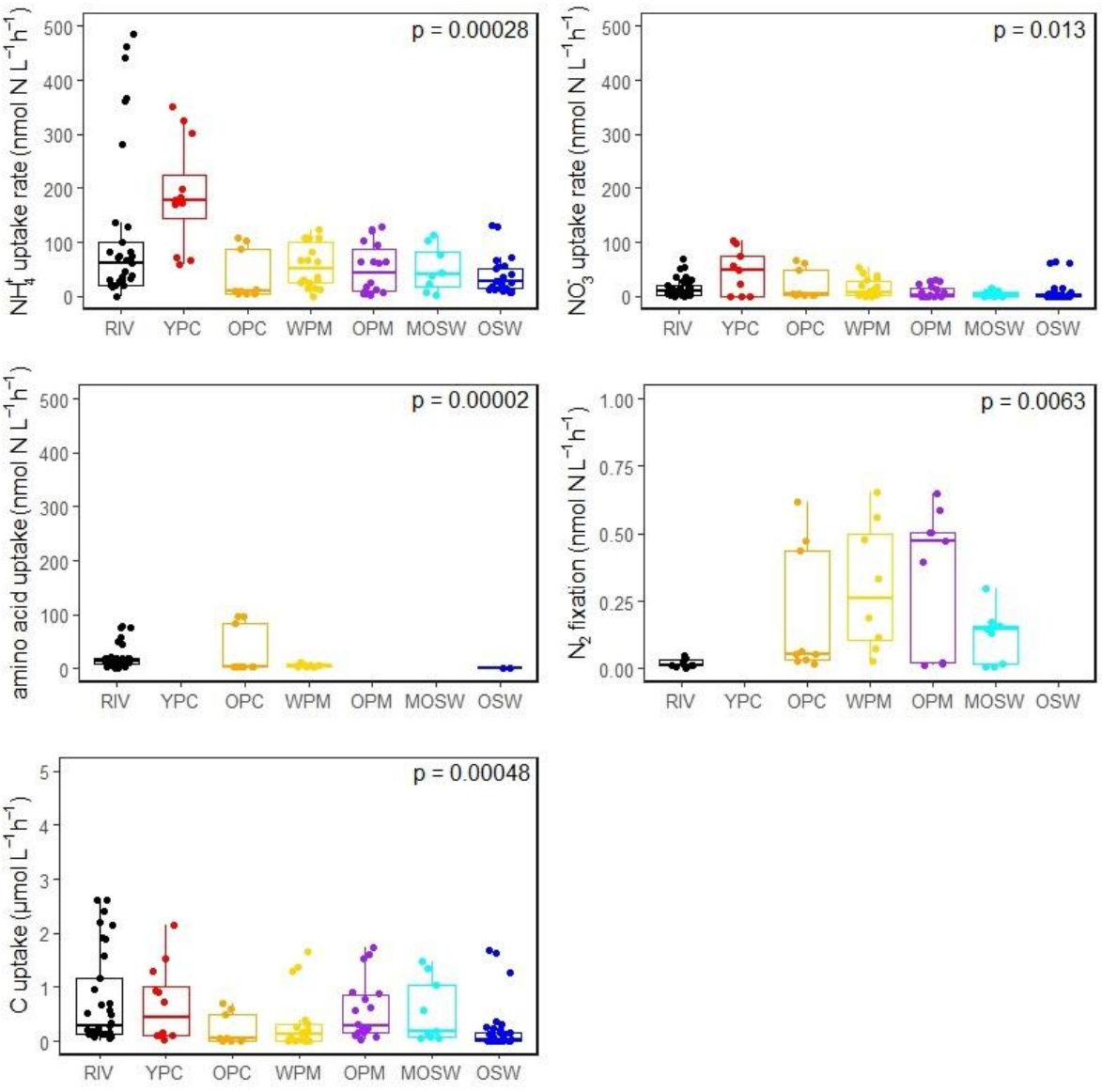

Figure A-4: *Boxplots with results from the Kruskal-Wallis tests for N uptake, N_2 fixation and primary production rates from the different habitats.*

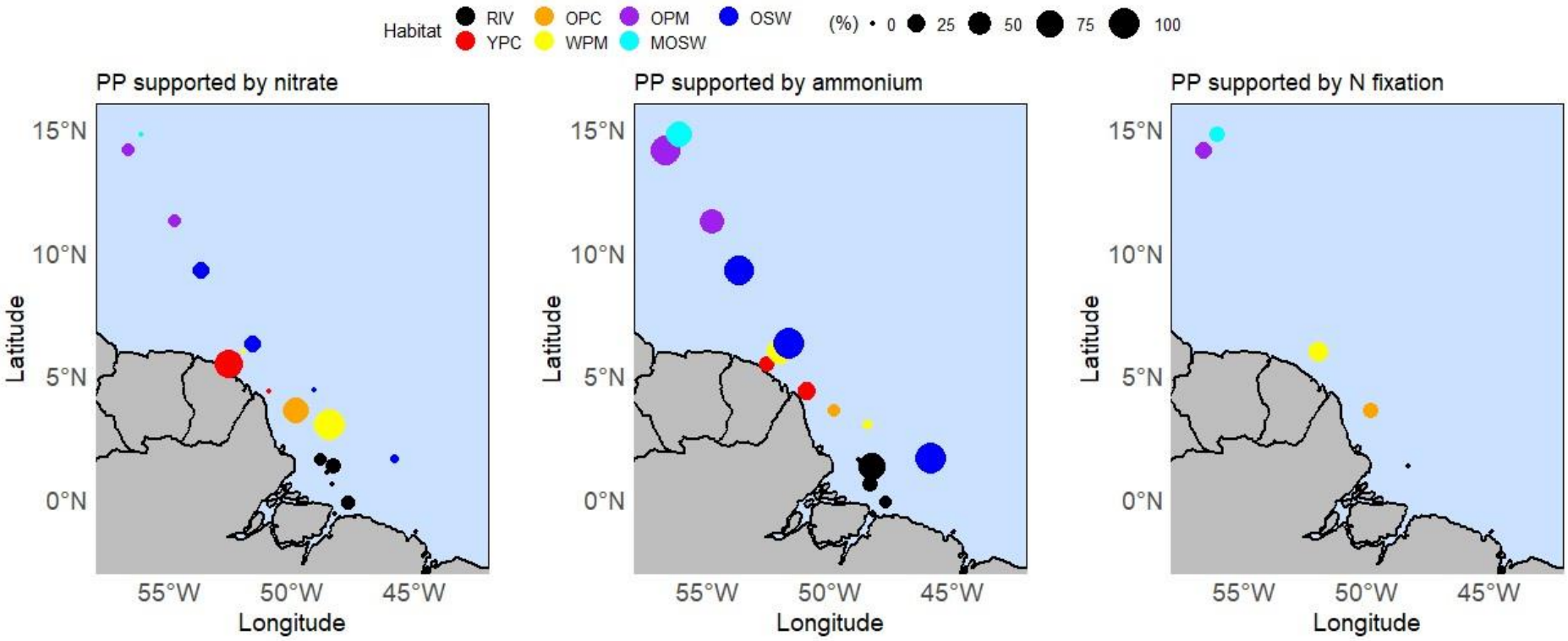

Figure A-5: *Map showing the percentage of primary production ("PP") supported by NO_3^- (left), NH_4^+ (middle) and N_2 fixation (right) in the Amazon River plume. The size of symbols indicates the percentage and the colour refers to the different habitats.*

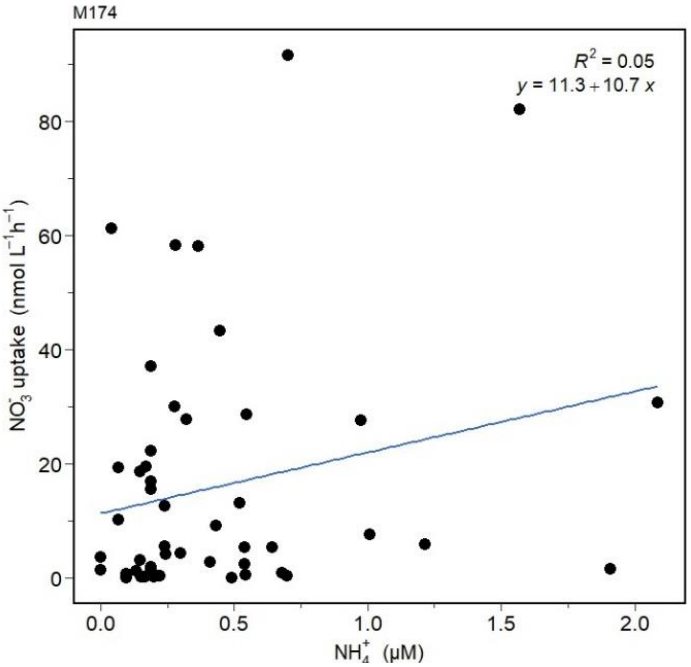

Figure A-6: *NO_3^- uptake rates versus NH_4^+ concentrations at all sampling stations in the Amazon River plume. The blue line is the linear regression line. The equation for the regression analysis is given in the upper right corner.*

Eidesstattliche Versicherung

Die Gelegenheit zum vorliegenden Promotionsvorhaben ist mir nicht kommerziell vermittelt worden. Insbesondere habe ich keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen/Betreuer für die Anfertigung von Dissertationen sucht oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

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Ort, Datum Unterschrift

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Publikationen und Vorträge auf Tagungen

- Umbricht, J., Dippner, J.W., Fry, B., Kröncke, I., Nehmer, P., Thoms, F., Voss, M. (2018). Correction of the isotopic composition (d13C and d15N) of preserved Baltic and North Sea macrozoobenthos and their isotopic interactions. Marine Ecology Progress Series 595: 1-13. https://doi.org/10.3354/meps12543

- Umbricht J., M. Benavides, Choisnard N., Montoya J. P., Subramaniam A., Voss, M. (2023) Ammonium fuels a large proportion of the primary production in the Amazon River plume. ASLO Conference, June 4-9 2023, Palma de Mallorca, Spain

- Umbricht, J., Burmeister, C., Choisnard, N., Liskow, I., Montoya, J., Subramaniam, A., Voss, M. (2022) An evaluation of the nitrogen sources supporting phytoplankton production in the Amazon River plume. Ocean Sciences Conference, February 24-March 4 2022, virtual meeting

- Umbricht J., Dippner, Joachim W., Montoya, Joseph P., Subramaniam, A., Voss, M. (2021) Nitrogen fixation and nitrate uptake in the Amazon River plume. ASLO Conference, June 22-27 2021, virtual meeting

Rostock, 17.11.2023 Jacqueline Umbricht