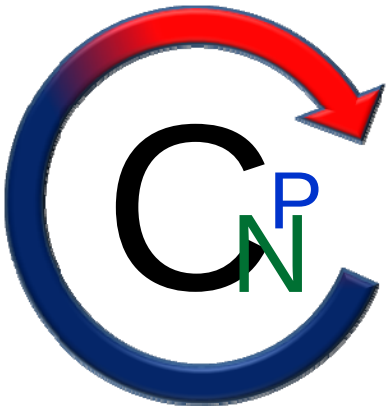
Effects of sea surface warming on elemental cycling in a pelagic system



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I.

SUMMARY

I. SUMMARY

Human activities, such as the burning of fossil fuels, changes in land-use practices and deforestation, are changing Earth's climate at an unprecedented rate in its history. While the ocean mitigates the progress of climate change by taking up and storing atmospheric carbon dioxide (CO_2) and heat, thus acting as a natural climate buffer, these anthropogenic perturbations are also affecting the ocean itself in its chemical and physical properties, e.g. through increased warming and acidification of the upper ocean layers. Future climate scenarios project an increase in global surface temperature by up to 6°C until the year 2100, whereas ocean pH will further drop by 0.14 to 0.35 units. While these abiotic deviations from contemporary surface ocean conditions are predictable with relatively high certainty, present knowledge concerning their effects on marine organisms and any potentially emerging biotic feedbacks to the climate system is still in its infancy. The biological fixation of dissolved inorganic carbon (DIC) into organic biomass via algal photosynthesis in the euphotic zone and its subsequent cycling through a complex network of interconnected food web processes determines global ocean productivity and is one of the driving forces for the flux of CO_2 from the atmosphere into the ocean. As temperature is an important factor that directly controls the rate of biological processes, anthropogenic surface ocean warming is likely to affect the delicate balance between the various food web processes.

The aim of this thesis is to elucidate such effects of rising sea surface temperature on the biogeochemical cycling of the key elements carbon, nitrogen, and phosphorus during the spring bloom of planktonic communities in a pelagic system, with particular focus on the temporal dynamics in their inorganic and organic reservoirs as well as the partitioning of organic matter between dissolved and particulate pools.

To approach these research topics, a novel indoor mesocosm set-up was used to expose a natural spring plankton community from the western Baltic Sea, encompassing all trophic levels from bacteria to mesozooplankton, to a range of four temperatures, which corresponded to the seasonal *in situ* surface temperature at the study site and future temperature scenarios of *in situ* +2°C, +4°C, and +6°C as projected for the end of the 21st century. The build-up and decline of distinct phytoplankton spring blooms in the respective treatments was closely monitored by following temporal changes in dissolved and particulate, as well as organic and inorganic pools of carbon, nitrogen, and phosphorus. These analyses were further supplemented by activity measurements of key autotrophic and heterotrophic processes, e.g. primary production, bacterial secondary production, and community respiration, as well as by a detailed characterisation of specific organic matter pools, i.e. carbohydrates.

Increasing the temperature by up to 6°C significantly affected the interplay of the various food web components, and hence also organic matter flow, due to individually different temperature-sensitivities. While warming exerted only a fairly weak effect on phytoplankton growth and primary production, it clearly stimulated bacterial secondary production, indicating an enhanced organic matter flux through the microbial loop. In addition, also the metabolic rates of presumably both autotrophic and heterotrophic food web members responded positively to rising temperature, as suggested by increased community respiration rates. These alterations in the activities of individual food web components further translated into distinct changes in the elemental cycling between inorganic and organic, dissolved and particulate pools. Thus, the quantitatively enhanced and temporally accelerated respiratory consumption of organic matter through heterotrophic processes relative to its autotrophic production in response to warming drastically reduced the net community uptake of DIC by up to 31%. Moreover, rising temperature caused an increased partitioning of accumulating organic carbon into the dissolved relative to the particulate pool. While the chemical characterisation of the carbohydrate fraction of bulk dissolved organic carbon (DOC) indicated an algal origin of the background increase in DOC, critical examination led to the conclusion that the temperature-dependent rate and magnitude of DOC accumulation was mainly caused by an enhanced bacterial release of presumably degradation-resistant DOC compounds.

In concert, these changes in food web functioning and the associated cycling of organic matter in response to sea surface warming reduced the transfer of matter and energy to higher trophic levels and lowered the availability of particulate organic matter for export processes. This has a strong potential to weaken the efficiency of biological carbon sequestration from surface to depth via the biological pump and to reduce ocean productivity, hence potentially providing a positive feedback mechanism to climate change.

In coming decades, the rise in oceanic surface temperature will, however, further interact with other environmental perturbations caused by anthropogenic climate change, e.g. increased ocean acidification or changes in the allochthonous nutrient loading of marine sites. To test for such combined effects of ocean warming and altered nutrient availability on biogeochemical cycling, a comprehensive microcosm experiment was performed in which a mixed algal-bacterial community was exposed to three temperatures and either nitrogen (N)- or phosphorus (P)-deficient growth conditions in a factorial combination. Interactively, these environmental stressors yielded unexpected shifts in the activity of the investigated autotrophic and heterotrophic components, thereby also mediating strong changes in the build-up and degradation of organic matter

in this model system. While the response of the P-deficient community to increasing temperature displayed patterns very similar to those of the natural plankton community investigated in the previous mesocosm experiment with i) a weak response of autotrophic production processes, ii) a stimulation of bacterial activities, i.e. here bacterial secondary production and extracellular enzyme activity, and iii) a pronounced shift in the partitioning of organic carbon between its particulate and dissolved pool towards the latter, that of the N-deficient community differed clearly. Here, in particular the heterotrophic utilisation of the accumulating organic matter was markedly reduced and displayed an either dampened, as in the case of bacterial secondary production, or even no response to rising temperature at all, as observed for extracellular enzyme activities. Moreover, also the partitioning of organic matter between the particulate and dissolved pools was clearly dominated by accumulation of particulate matter and did not respond notably to warming. Ultimately, these findings suggest a pronounced effect of nutrient availability on the sensitivity of pelagic food webs to surface ocean warming, thereby also mediating significant changes in the cycling and fate of organic matter. This study clearly underscores the need for more experimental investigations focussing on the interactive synergistic or antagonistic effects of various environmental stressors on pelagic ecosystem functioning and biogeochemical cycles.

II.

ZUSAMMENFASSUNG

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Die Aktivitäten des Menschen, wie die Verbrennung fossiler Energieträger, Änderungen in der Landnutzung sowie Entwaldung (Brandrodung von Wäldern), verändern das Klima der Erde in einer in der jüngeren Erdgeschichte beispiellosen Geschwindigkeit. Während der Ozean das Voranschreiten des Klimawandels durch die Aufnahme und Lagerung von Kohlendioxid und Wärme abmildert und damit als natürlicher Klimapuffer fungiert, ändern diese anthropogen bedingten Störungen gleichzeitig auch die chemische und physikalische Beschaffenheit des Ozeans selbst, z.B. durch zunehmende Erwärmung und Versauerung der oberen Ozeanschichten. Klimaszenarien der Zukunft prognostizieren einen Anstieg der globalen Oberflächentemperatur um bis zu 6°C für den Zeitraum bis zum Jahr 2100, wogegen der pH-Wert des Ozeans um weitere 0.14 bis zu 0.35 Einheiten abfallen wird. Während diese abiotischen Abweichungen von derzeitigen Ozeanbedingungen mit recht hoher Sicherheit prognostiziert werden können, ist unser Wissen um deren Auswirkungen auf marine Organismen und daraus erwachsende potentielle Rückkopplungen auf das Klimasystem der Erde noch sehr begrenzt. Die biologische Fixierung von gelöstem anorganischem Kohlenstoff (DIC) in organische Biomasse durch Photosynthese treibende Algen in der euphotischen Zone und deren anschließende Zirkulation durch ein komplexes Netzwerk von miteinander gekoppelten Nahrungsnetzprozessen entscheidet über die globale Ozeanproduktivität und ist eine der Triebkräfte für den Kohlendioxidfluss von der Atmosphäre in den Ozean. Da Temperatur ein wichtiger Umweltfaktor ist, der sich direkt auf die Geschwindigkeit biologischer Prozesse auswirkt, wird die anthropogen bedingte Ozeanerwärmung sehr wahrscheinlich auch die empfindliche Balance zwischen den verschiedenen Nahrungsnetzprozessen beeinflussen.

Das Ziel dieser Arbeit ist es, derartige Auswirkungen von ansteigender Temperatur des Oberflächenozeans auf den biogeochemischen Kreislauf der Schlüsselelemente Kohlenstoff, Stickstoff und Phosphor während der Frühjahrsblüte des Planktons in einem pelagischen System näher zu beleuchten, mit einem besonderen Schwerpunkt auf der zeitlichen Dynamik der anorganischen und organischen Reservoire, sowie auf der Partitionierung zwischen den gelösten und partikulären Pools von organischem Material.

Zur Umsetzung dieses Forschungsvorhabens wurde ein neuartiges Indoor-Mesokosmossystem eingesetzt, welches es ermöglicht, eine natürliche Frühjahrsblütengemeinschaft der westlichen Ostsee, die alle trophischen Ebenen von Bakterien bis hin zum Mesozooplankton umfasst, vier verschiedenen Temperaturszenarien auszusetzen, welche der *in situ* Oberflächentemperatur des untersuchten Standortes sowie drei für das Ende des 21. Jahrhunderts prognostizierten

erhöhten Temperaturregimes von *in situ* +2°C, +4°C und +6°C entsprechen. Der Auf- und Abbau von sich entwickelnden Phytoplankton-Frühjahrsblüten wurde über Veränderungen in den gelösten und partikulären sowie organischen und anorganischen Pools von Kohlenstoff, Stickstoff und Phosphor in hoher zeitlicher Auflösung verfolgt. Des Weiteren wurden diese Analysen ergänzt durch Aktivitätsmessungen auto- und heterotropher Schlüsselprozesse, wie z.B. Primärproduktion, bakterielle Sekundärproduktion und Respiration der Planktongemeinschaft, sowie durch eine detaillierte Charakterisierung spezifischer Pools organischen Materials, so z.B. von Kohlenhydraten.

Eine Temperaturerhöhung um bis zu 6°C beeinflusste deutlich das Zusammenspiel verschiedener Nahrungsnetzkomponenten aufgrund unterschiedlicher Empfindlichkeiten gegenüber Temperaturänderungen, und dadurch auch den Fluss organischen Materials. Während Erwärmung nur einen schwachen Einfluss auf Wachstum und Primärproduktion des Phytoplanktons ausübte, wurde die bakterielle Sekundärproduktion deutlich stimuliert, was auf einen erhöhten Fluss von organischem Material über die sogenannte „Mikrobielle Schleife“ schließen lässt. Weiterhin reagierten die Stoffwechselraten wahrscheinlich sowohl autotropher als auch heterotropher Nahrungsnetzkomponenten positiv auf steigende Temperatur, was durch zunehmende Respirationsraten der Planktongemeinschaft nahe gelegt wird. Diese Aktivitätsänderungen spiegelten sich auch direkt wider in deutlichen Änderungen der Element-Kreisläufe zwischen anorganischen und organischen sowie gelösten und partikulären Pools. Der durch Erwärmung bedingte quantitativ höhere und zeitlich beschleunigte respiratorische Abbau organischen Materials durch heterotrophe Prozesse, in Relation zu dessen autotropher Produktion, verringerte somit drastisch die Netto-Aufnahme von DIC durch die Planktongemeinschaft um bis zu 31%. Ferner verursachte die ansteigende Temperatur eine zunehmende Verschiebung in der Partitionierung von akkumulierendem organischem Kohlenstoff hin zu gelöstem relativ zu partikulärem Material. Während die nähere chemische Charakterisierung des Kohlenhydratanteils des Gesamtpool an gelöstem organischem Kohlenstoff (DOC) die Algen als Quelle des Hintergrundanstiegs von DOC vermuten ließ, führte die weitere kritische Untersuchung zu der Schlussfolgerung, dass die temperaturabhängige Geschwindigkeit und das Ausmaß der DOC-Akkumulation hauptsächlich durch eine verstärkte bakterielle Ausscheidung von wahrscheinlich abbauresistenten DOC-Komponenten verursacht wurde.

Im Zusammenspiel reduzierten diese durch Erwärmung des Oberflächenozeans bedingten Veränderungen von Nahrungsnetzfunktion und damit verbundener Zirkulation organischen Materials den Transfer von Materie und Energie zu höheren trophischen

Ebenen und verringerten die Menge an für Exportprozesse zur Verfügung stehender partikulärer organischer Substanz.

Dies besitzt ein hohes Potenzial, die Effizienz biologischer Kohlenstoff-Sequestrierung von der Oberfläche in die Tiefe mittels der biologischen Kohlenstoffpumpe wie auch die Ozeanproduktivität zu reduzieren und könnte somit möglicherweise einen positiven Rückkopplungsmechanismus zum Klimawandel darstellen.

In den kommenden Jahrzehnten wird der Anstieg in der Oberflächentemperatur der Meere mit anderen durch anthropogenen Klimawandel verursachten Umweltveränderungen, so z.B. zunehmende Ozeanversauerung und Änderungen im allochthonen Nährstoffeintrag in marine Systeme, zusammenwirken. Um derartige kombinierte Effekte von Ozeanerwärmung und veränderter Nährstoffverfügbarkeit auf biogeochemische Stoffkreisläufe zu untersuchen, wurde ein umfangreiches Mikrokosmen-Experiment durchgeführt, in dem, in faktorieller Kombination, gemischte Planktongemeinschaften aus Algen und Bakterien drei unterschiedlichen Temperaturen und zusätzlich entweder Stickstoff- (N) oder Phosphor (P)- verarmten Wachstumsbedingungen ausgesetzt wurden. Zusammenwirkend ergaben diese veränderten Umweltbedingungen unerwartete Verschiebungen in der Aktivität der untersuchten auto- und heterotrophen Komponenten, und dadurch bedingt auch deutliche Änderungen bei Aufbau und Abbau von organischem Material in diesem Modellsystem.

Während die Reaktion der P-verarmten Gemeinschaft auf steigende Temperaturen ähnliche Muster aufwies wie diejenige der natürlichen Planktongemeinschaft im vorherigen Mesokosmen-Experiment mit i) einer schwachen Reaktion autotropher Prozesse, ii) einer Stimulation bakterieller Aktivität, hier vor allem bakterieller Sekundärproduktion und extrazellulärer Enzymaktivität, und iii) einer deutlichen Verschiebung in der Partitionierung organischen Kohlenstoffs zwischen der partikulären und gelösten Phase hin zur letzteren, zeigte die N-verarmte Gemeinschaft klar erkennbare Unterschiede in ihrer Reaktion. Hier war insbesondere die heterotrophe Nutzung des akkumulierenden organischen Materials eindeutig reduziert und zeigte entweder eine abgeschwächte, wie im Fall bakterieller Sekundärproduktion, oder gar keine Reaktion auf ansteigende Temperaturen, wie z.B. im Hinblick auf extrazelluläre Enzymaktivitäten. Außerdem war hier auch die Aufteilung des organischen Kohlenstoffs zwischen partikulärer und gelöster Phase deutlich dominiert von einer Akkumulation partikulären Materials und reagierte nicht merklich auf Erwärmung.

Schließlich legen diese Ergebnisse einen deutlichen Effekt von Nährstoffverfügbarkeit auf die Empfindlichkeit pelagischer Nahrungsnetze gegenüber einer Erwärmung des

Oberflächenozeans nahe, was sich letztendlich auch auf den Kreislauf und Verbleib von organischem Material auswirken wird. Diese Studie unterstreicht deutlich den Bedarf an weiteren experimentellen Untersuchungen zu interaktiven synergistischen und antagonistischen Effekten verschiedener Umweltfaktoren auf die Funktionen und biogeochemischen Kreisläufe in pelagischen Ökosystemen.

III.
General Introduction

III. GENERAL INTRODUCTION

1. Carbon, nitrogen, and phosphorus – key elements in the marine environment

Life on Earth is inextricably linked to the availability of the three elements carbon (C), nitrogen (N), and phosphorus (P). These are the basic building blocks of most biochemical compounds constituting cells and organisms and possess important cellular functions, e.g. by providing structure, storing energy, and carrying genetic information (Sterner and Elser 2002). N and P belong to the so-called macronutrients. While N is generally seen as the major nutrient limiting primary productivity throughout most of the ocean (Falkowski 1997; Tyrrell 1999), evidence for a at least seasonally occurring P limitation in certain oceanic areas (e.g. the subtropical gyres in the North Atlantic and Pacific, and the Mediterranean Sea) increases (Ammerman *et al.* 2003; Karl *et al.* 2001; Thingstad *et al.* 2005). C, N, and P are all present in the marine environment in various dissolved and particulate, organic as well as inorganic forms. In the following, a short description of the different pools of C, N, and P, the respective reservoir sizes, as well as their main sources and sinks will be given.

Carbon. With a size of approx. 39000 Gt C the pool of dissolved inorganic carbon (DIC) represents the largest carbon reservoir in the ocean, and makes the ocean itself the second-largest pool of C on Earth. Carbon mainly enters the ocean via the exchange of gaseous carbon dioxide (CO_2) between the atmosphere and the surface ocean, as well as to a minor degree through river influx (Siegenthaler and Sarmiento 1993). A strong gradient from low to high DIC concentrations exists between the surface ocean and the deep sea. This is caused by the working of two carbon pumps: the physical pump and the biological carbon pump (Volk and Hoffert 1985). The physical pump is driven by the increased solubility of CO_2 in colder waters, which together with the formation of deep water masses through thermohaline circulation at high latitudes leads to a massive transport of carbon-enriched waters to depth. The biological carbon pump describes the photosynthetic incorporation of dissolved inorganic carbon into organic biomass in the euphotic surface layer of the ocean, which is then transported to the ocean interior via aggregation and sedimentation of particulate organic matter. This pump is responsible for approximately three quarters of the surface-to-deep gradient in DIC, and thus contributes significantly to the net air-sea flux of CO_2. Although these photosynthetic organisms, mediating the uptake of DIC in the surface ocean, only have a biomass of ~ 1 Gt C, being equivalent to 0.2% of total photosynthetically active biomass on Earth, they contribute about half of global primary production (Falkowski *et al.* 1998; Field *et al.* 1998). Besides this living biomass, particulate organic carbon (POC) also exists as dead

matter in the ocean, resulting in a total POC reservoir of ~ 3 Gt C (Siegenthaler and Sarmiento 1993). Several biological processes (e.g. algal exudation, grazing, bacterial decomposition and viral lysis) also lead to the release of dissolved organic carbon (DOC). With a size of ~ 685 Gt C (Hansell and Carlson 1998a) the DOC pool is the second largest carbon reservoir in the ocean and of comparable size to the amount of atmospheric CO_2 (~ 750 Gt C). Surface ocean DOC displays a large spatial and temporal variability in its concentration due to a variety of biological and photochemical processes that produce or consume DOC and consists of a mixture of freshly produced as well as older material. In contrast, the deep-sea concentration of DOC remains fairly constant at an average value of ~43 µmol C L^{-1} (see Hansell 2002), and is on average ~ 4000 years old (Bauer *et al.* 1992). These differences in concentration and age are also reflected in the size distribution and bioreactivity of surface and deep DOC. Thus, the latter consists mainly of refractory low-molecular-weight (LMW; < 1 kDa) compounds, whereas biological activities in the upper mixed layer of the ocean lead to a release and seasonal accumulation of labile and semi-labile compounds. Moreover, also the share of high-molecular-weight (HMW; > 1 kDa) components to total DOC is increased in the surface, which have been shown to be more bioreactive than the LMW fraction (Amon and Benner 1994; Amon and Benner 1996).

Nitrogen. The marine nitrogen cycle is one of the most complex biogeochemical cycles in the ocean. Various chemical forms of N exist in seawater, which differ in their oxidation state and their bioavailability: nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), nitrous oxide (N_2O), and dinitrogen (N_2). While N_2 constitutes with approx. 1 x 10^4 Gt N (Gruber 2008) the most abundant form in the ocean, it is not readily utilisable as a nutrient for growth to all microorganisms. Only a very specialised group of microorganisms, the diazotrophs, possess the enzymatic repertoire to fix N_2 into biologically available N. In contrast, NO_3^-, NO_2^-, and NH_4^+, constituting the so-called fixed N pool, are the main N sources for both autotrophic and heterotrophic growth in the surface ocean. Ammonium is generally preferred over nitrate and nitrite when available, because its incorporation into particulate organic biomass does not require any redox transformations and is hence less energy-demanding (Zehr and Ward 2002). Growing evidence suggests that also dissolved organic nitrogenous compounds (DON), which are like DOC a by-product of several biological processes in the surface ocean, can be used by microorganisms as a nutrient source for growth (Berman and Bronk 2003; Bronk *et al.* 2006; Glibert *et al.* 2004).

The main pathways of N into the ocean include riverine influx, atmospheric deposition of dust, as well as the biological N_2-fixation (Gruber 2008 and references therein). These processes are counteracted by a loss of N from the water column

through denitrification and anammox, both of which require low oxygen concentrations and therefore occur mainly in the oxygen minimum zones (OMZ) of the ocean and in sediments. The projected expansion of OMZ due to anthropogenic climate change (Matear and Hirst 2003) may hence shift the balance between the sources and sinks of N with severe effects on the marine N-cycle and the availability of nitrogen for autotrophic growth. To a minor degree N may also be lost from the water column through the sinking and burial of particulate organic nitrogen (N) in deep-sea sediments (Gruber 2008 and references therein).

Phosphorus. The cycling of phosphorus in the marine realm is much simpler than that of nitrogen as P exists only as dissolved inorganic (DIP), particulate organic (POP) and dissolved organic phosphorus (DOP), and does not include a gaseous phase or any redox reactions. The main inputs of P into the ocean occur via river water carrying P components from the chemical weathering of rocks, aeolian dust deposition, and to a lesser and spatially restricted degree from volcanic sources (Hutchins and Fu 2008). Although the atmospheric input of P through dust is only small, it may provide an important P source in the oligotrophic areas of the open ocean, as it has been shown that 21-50% of dust-borne P is bioavailable (Duce *et al.* 1991; Graham and Duce 1982). DIP is mainly present as orthophosphate. Almost all DIP in the surface ocean is utilised by the photosynthetic primary producers in the euphotic zone and incorporated into organic biomass, which is partly transported to the deep ocean via the biological pump. Approximately 95% of this organically fixed P are remineralised within the water column (Föllmi 1996), whereas the remaining 5% are buried in sediments (Benitez-Nelson 2000). New evidence suggests that also scavenging of P onto particles, such as $CaCO_3$ shells, may play a role in transporting P from surface to depth (Diaz *et al.* 2008).

2. The biogeochemical cycling of organic matter in the surface ocean – its sources, sinks, and composition

The biogeochemical cycling of organic matter in the pelagic realm is largely driven by a complex network of food web processes that contribute to its production and/or consumption (Fig. 1). The food web structure is critical for the fate of the primary produced matter in the surface ocean, thereby also controlling the sequestration of climatically active elements to the ocean interior and the contribution of biological processes to the ocean's capacity to take up atmospheric CO_2.

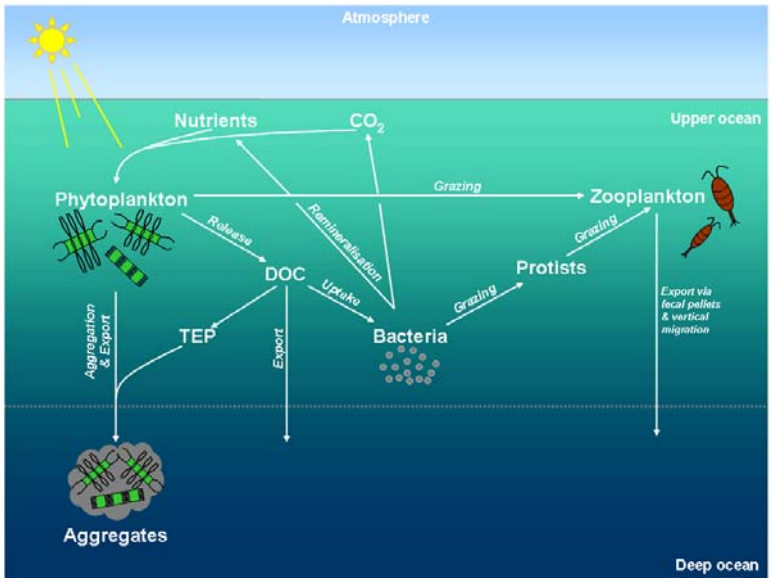

Figure 1.
Simplified scheme of pelagic food web processes driving organic matter cycling in the surface ocean. Details on displayed processes are given in the text.

Organic matter sources

The photosynthetic incorporation of DIC and nutrients into particulate biomass by phytoplankton cells in the euphotic layers of the ocean provides the base of the pelagic food web, serving as an energy and matter source for the metabolism and growth of higher trophic levels. Although phytoplankton biomass accounts only for approx. 0.2% of total photosynthetically active carbon on Earth, these microscopically small organisms contribute about half of global net primary production (Falkowski *et al.* 1998; Field *et al.* 1998). This illustrates the immensely rapid turnover of biomass in the pelagic realm, which is on the order of one week (Falkowski *et al.* 1998).

A significant fraction of primary production is also directly channelled into the pool of dissolved organic matter (DOM). Several studies have shown that this extracellular release (ER) is a normal process occurring in healthy and actively growing phytoplankton cells (Baines and Pace 1991; Fogg 1983; Mague *et al.* 1980). While measured rates of extracellular release relative to total dissolved and particulate primary production can differ greatly, depending on factors such as community structure, light intensity, nutrient availability, and temperature, they were found to be on average 13% (Baines and Pace 1991). Two mechanisms have been proposed, the overflow model (Fogg 1966) and the passive diffusion model (Bjørnsen 1988; Fogg 1966), for both of which experimental evidence exists (see also Carlson 2002). According to the overflow model, the light-dependent production of photosynthate may at times be faster than its incorporation into biomass, which also depends on the availability of nutrients, hence resulting in an active release of excess photosynthates. In line with this, the algal release and accumulation of dissolved organic compounds has often been observed at

the end of phytoplankton blooms, when inorganic nutrients were used up (Ittekkot *et al.* 1981; Lignell 1990; Smith *et al.* 1998; Wood and Van Valen 1990). The passive diffusion model is based on the concentration gradient of LMW photosynthates, e.g. dissolved free carbohydrates and amino acids, between the algal cytoplasm and the outer medium, thereby driving a passive leakage of these compounds out of the cell.

In addition to the algal release of DOM, this pool is also fuelled by several heterotrophic processes. Thus, sloppy feeding by zooplankton, the egestion of protistan food vacuoles, leaking from zooplankton fecal pellets, as well as the lysis of living cells via autocatalytic cell death, bacterial activity or viral infection have been shown to release dissolved and colloidal organic compounds into the surrounding medium (Nagata 2000 and references therein).

Organic matter sinks

Several biotic and abiotic processes lead to the removal of organic matter from the surface ocean, i.e. grazing by phagotrophic organisms, microbial degradation, and the aggregation and subsequent sinking of organic matter to deeper water layers. Their interplay critically controls the fate of the primary produced matter, resulting either in a rapid recycling back into the inorganic constituents or in the storage of photosynthetically-fixed matter in the deep sea.

In temperate and high latitude ocean areas, which are in general characterised by the annual occurrence of high algal biomass events, so-called phytoplankton blooms, and belong to the most productive marine systems, both grazing and sedimentation of organic aggregates often provide the major loss terms of surface ocean primary production. Grazing by zooplankton, e.g. copepods, provides a direct link between the base of the pelagic food web and higher trophic levels. This classical algae-zooplankton-fish food chain has for a long time been regarded as the dominant pathway of matter and energy through the pelagic food web.

The pronounced accumulation of organic biomass in surface waters during bloom situations is generally attributed to a temporal decoupling of autotrophic primary producers, which depend largely on light and nutrient availability, and their heterotrophic consumers, i.e. zooplankton, whose development naturally displays a certain temporal time lag to their prey due to a complex reproduction cycle via larval stages. This often leads to a massive formation of organic aggregates at the end of phytoplankton blooms (Alldredge *et al.* 1995; Fowler and Knauer 1986), which rapidly sink out of the upper mixed layer, hence providing an important vertical transport mechanism for photosynthetically bound carbon and nutrients to the ocean interior via the biological pump. In addition to this, also grazing activities may promote an accelerated transport of

organic matter to depth by repackaging ingested material into dense fecal pellets, by the discarding of larvacean houses and pteropod feedings webs, or by the release of food, ingested at the surface, at greater water depths by vertically migrating zooplankton species (Alldredge and Gotschalk 1990; Bochdansky and Herndl 1992; Pomeroy and Deibel 1980).

Although the surface-to-deep flux of organic matter is quantitatively dominated by the particulate fraction, also dissolved organic compounds accumulating at the surface during the productive season contribute significantly to the global oceanic export of carbon and other climatically active elements. Thus, Hansell and Carlson (1998b) have estimated an annual transport of DOC from surface to depth of 1.2 Gt C yr^{-1} via deep-water formation at high latitudes and the downwelling of surface water masses by Ekman pumping in low latitude subtropical gyres (Hansell 2002). Moreover, it has been shown that the HMW fraction of DOM can spontaneously assemble to colloidal polymer gels (Chin *et al.* 1998; Kerner *et al.* 2003) or even form microparticles, e.g. transparent exopolymer particles (TEP; Engel *et al.* 2004; Passow 2000) by abiotic coagulation and aggregation processes, thereby moving up the size spectrum from the dissolved to the particulate phase. TEP were found to play an important role in the aggregation and sedimentation of phytoplankton blooms in the ocean. Due to their surface-active nature and high stickiness, TEP promote the aggregation on non-sticking solid particles, thereby facilitating the formation of larger aggregates (Passow 2002). Thus, the abiotic formation of TEP from dissolved organic precursors may provide an important mechanism affecting the export of organic matter to the ocean interior.

The efficiency of organic matter loss by grazing and sedimentation is counteracted and controlled by the decomposition and recycling of organic matter through heterotrophic bacteria in the water column. The acknowledging of the importance of microbial processes in pelagic food webs and their potential role in marine biogeochemical cycles only came about 30 years ago with the discovery of the vast abundance of microbial organisms in seawater (Pomeroy 1974). Shortly after, this was followed by the formulation of the “microbial loop” concept (Azam *et al.* 1983; Ducklow 1983), describing the reintroduction of dissolved primary production back into the classical food web by incorporation into bacterial biomass, which is then transferred to higher trophic levels via a chain of protist and mesozooplankton grazing. Today, it is widely acknowledged that a large fraction of photosynthetic primary production of on average 50% is channelled via DOM through the microbial loop (Williams 2000). Most of the energy and matter is, however, lost from the food web through respiration, as the growth efficiency of marine bacteria is typically 30% or less (Del Giorgio and Cole 1998). Hence, this pathway often rather represents a trophic sink of organic matter instead of a

trophic link to higher-level food web members. Although being in general an important food web component in the pelagic realm, the microbial loop dominates organic matter fluxes in particular in the low-latitude oligotrophic areas of the ocean (e.g. Gasol *et al.* 1997). Here, a very efficient recycling of carbon and nutrients by microbial organisms leads to a high retention of these elements in the surface ocean and reduces the loss of organic matter to depth via the biological pump.

However, bacteria are not only the major consumers of DOM in seawater (Azam and Hodson 1977; Williams 1981), but are also key mediators of particle solubilisation and decomposition (Cho and Azam 1988; Smith *et al.* 1992), hence controlling the efficiency of organic matter export via the biological pump. Organic particles and aggregates sinking through the water column are in general rapidly colonised by bacteria and provide hot spots of organic matter degradation in the pelagic realm (Simon *et al.* 2002). Through the synthesis and release of a diverse set of ecto- and extracellular hydrolytic enzymes (e.g. carbohydrases, phosphatases, peptidases) particle-attached bacteria cleave polymeric organic matter into utilisable oligo- and monomeric units, thus promoting the disaggregation of particles. Smith *et al.* (1992) showed, however, that a large proportion of the solubilised material is not directly utilised by the attached bacterial fraction, but diffuses in dissolved and colloidal form into the surrounding seawater, where it is rapidly assimilated by free-living microbes (Cho and Azam 1988).

Organic matter composition

The photosynthetic incorporation of carbon, nitrogen, phosphorus and other elements into particulate biomass by autotrophic phytoplankton provides the stoichiometric basis of the pelagic food web and controls the productivity of higher trophic levels, which strongly depend on the quality of ingested prey or substrate. In general, the stoichiometric ratio of C, N, and P in particulate organic matter (POM) appears to be fairly well constrained in the ocean, which seems logical considering the important functions of these elements in cells and organisms, i.e. serving as structural components, energy storage, carriers of genetic information, etc. (see also Sterner and Elser 2002 for more information on this topic). In line with this, Alfred Redfield and co-workers (Redfield 1934; Redfield *et al.* 1963) reported an astonishingly uniform atomic ratio of C:N:P = 106:16:1 contained in marine particulate organic matter, the Redfield ratio. Moreover, the particulate N:P ratio of 16:1 strongly resembled the relative concentrations of dissolved NO_3^- and PO_4^{3-} in deeper waters, which led Redfield to conclude that the oceanic biota themselves determine the availability of nutrients according to their own needs. He was, however, aware that this ratio represents an

average of present particulate matter and allows for a certain variability of the individual components. Following Redfield, many laboratory studies have investigated the effects of varying nutrient availability on the cellular stoichiometry of autotrophic and heterotrophic organisms (see Sterner and Elser 2002). One emerging pattern of these studies is that in autotrophs, despite being also constrained by the basic elemental needs of the underlying cell machinery, the acquisition of carbon and inorganic nutrients is not perfectly coupled, thereby causing large variations in autotrophic stoichiometry as a consequence of changes in the nutrient content of the surrounding medium. One example for this is, for instance, the luxury consumption of inorganic nutrients by diatoms, which posses specialised storage vacuoles (Falkowski *et al.* 2004), at times of high nutrient availability, resulting in deviations from Redfieldian behaviour. In contrast, the stoichiometric range seems to be less variable in heterotrophic organisms (Sterner and Elser 2002), whose reproductive success often depends on an appropriate quantity and quality of their food.

In contrast to POM, the stoichiometric relations between C, N, and P are much less constrained in dissolved organic matter (DOM), as they are rather determined by active release of elements that are available in excess of cellular needs (e.g. extracellular release of C-rich photosynthates, when the availability of N and P restricts biomass synthesis), the passive leakage of LMW compounds across cell membranes, or the unintentional loss of organic matter by grazing (i.e. sloppy feeding). In addition to this, the pool of DOC, being the largest organic carbon reservoir in the ocean, consists of a vast amount of old and refractory carbon compounds, which can have markedly higher turnover times (i.e. on the time-scales of centuries to millennia; Bauer *et al.* 1992) than the average ocean circulation time of ~ 1000 years. In line with this, C:N:P ratios of DOM in surface and deep waters deviate substantially from the Redfield ratio with average values of 300:22:1 and 444:25:1, respectively (Benner 2002). The refractory and diagenetically altered nature of the DOM pool is also illustrated by the fact that on average only 4-11% of surface ocean DOC can be chemically identified as amino acids, carbohydrates, or other biochemical compounds. This proportion further decreases with increasing depth, yielding only 1-3% in the deep sea (Benner 2002). Among the identified components of marine DOM, carbohydrates represent the most abundant one and typically account for 10-25% of surface ocean DOC (Bhosle *et al.* 1998; Børsheim *et al.* 1999; Pakulski and Benner 1994). Concomitant to the decrease in identifyable DOM fractions, also the size spectrum changes from surface waters to the ocean interior with a decline in the proportion of HMW to LMW matter, which is in accordance with the notion that the surface ocean is the major site of fresh DOM production and that the accumulating material becomes increasingly smaller in size and less biodegradable

through microbial and photochemical processes on its journey through the ocean (Amon and Benner 1996; Opsahl and Benner 1998; Smith *et al.* 1992).

3. The ocean in a state of change

Reconstructions of past climate conditions on Earth obtained from the Vostok ice core have revealed that the atmospheric concentrations of CO_2 and methane (CH_4), two of the most powerful greenhouse gases, have oscillated regularly between low and high values throughout Earth's geological history, the so-called glacial/interglacial cycles. Here, glacial times were characterised by low CO_2 and CH_4 concentrations of 180 ppmv and 320-350 ppbv, which increased to maximum values of 280-300 ppmv and 650-750 ppbv, respectively, with the transition to interglacial conditions (Petit *et al.* 1999). Moreover, these oscillations were closely followed by pronounced changes in the isotopic temperature proxy deuterium (δD_{ice}), hence indicating a close coupling between atmospheric CO_2 and CH_4 concentrations and Antarctic air temperature (Petit *et al.* 1999).

Since the beginning of the industrial era, human activities have led to a substantial increase in the atmospheric content of CO_2 and other greenhouse gases, i.e. CH_4 and nitrous oxide (N_2O), through burning of fossil fuels, changes in land-use practices and deforestation (Solomon *et al.* 2007). With a present CO_2 concentration of 380 ppmv in the atmosphere, we have, however, not only reached levels far above those observed within at least the last 420,000 years (Petit *et al.* 1999), but this change within a geologically minute period of roughly 200 years has also taken place at an alarmingly fast, and still speeding up (Joos and Spahni 2008; Raupach *et al.* 2007) rate compared to the glacial/interglacial cycles, which occurred on time scales of approximately 100,000 years (Petit *et al.* 1999). Greenhouse gases are a natural component of the atmosphere and play a decisive role in Earth's climate system, because they affect the absorption, scattering and emission of radiation within the atmosphere and at the Earth's surface, thereby controlling the overall heat balance of the planet. However, the recent anthropogenic increase in their concentration far beyond what the Earth system has experienced within the last hundreds of thousands of years has caused a substantial deviation from natural conditions with an increase in global surface temperature of 0.8°C within the last century (Hansen *et al.* 2006).

Throughout Earth's history, the ocean has played an important role in the regulation of its climate system, and recently also in the mitigation of anthropogenic climate change. Thus, it has taken up approximately 48% of the emissions caused by fossil fuel burning over the period of 1800-1994 (Sabine *et al.* 2004). In addition, the

ocean also acts as a temperature buffer and has absorbed about 84% of the increase in Earth's heat budget within the last 50 years (Levitus *et al.* 2005). In turn, these processes lead themselves to significant changes in the ocean's chemical and physical properties. Thus, the increased penetration of atmospheric CO_2 into the upper ocean has already led to a decrease in ocean pH by 0.1 units (Bindoff *et al.* 2007), as inferred from both direct observations at several ocean time series (Hawaii Ocean Time series (HOT), Bermuda Atlantic Time Series (BATS), European Station for Time-Series in the Ocean (ESTOC); see Bindoff *et al.* 2007) and calculations of estimated anthropogenic carbon uptake between 1750 and 1994 (Raven *et al.* 2005; Sabine *et al.* 2004). Moreover, a warming signal of anthropogenic origin has already intruded into all ocean basins (Barnett *et al.* 2005), with a globally averaged increase in the temperature of the upper 3000 m of the water column of 0.037°C (Levitus *et al.* 2005).

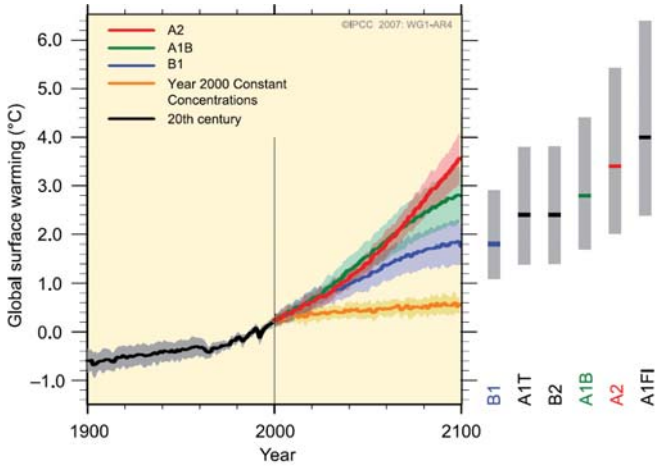

Figure 2: Climate model projections of future global surface warming. Solid lines represent multi-model global averages of surface warming (relative to the period 1980-1999) for the scenarios A2, A1B, and B1, shown as continuations of the 20th century simulations. Shading denotes the plus/minus one standard deviation range of individual model annual averages. The orange line depicts a simulation, where greenhouse gas concentrations were held constant at year 2000 values. The gray bars at right indicate the best estimate (solid line within each bar) and the likely range assessed for the six SRES marker scenarios. Source: IPCC 4th assessment report (2007).

For the projection of future climate on Earth, the International Panel on Climate Change (IPCC) has published several emission scenarios in its 4th assessment report, which vary in the development pathways regarding future emissions of greenhouse gases. Basically all of these scenarios project a further rise in atmospheric CO_2 concentration, and hence also in global surface temperature, yielding a likely range of future warming between 1.1 (B1 scenario) and 6.4°C (A1FI scenario) until the end of the 21st century (Fig. 2) (Meehl *et al.* 2007). Regionally, these estimates may even be higher as, for

instance, warming in Northern Europe is expected to be strongest during the winter season with an increase in surface temperature of 4-10°C (Giorgi *et al.* 2001). Additionally, a further drop in ocean pH by 0.14 to 0.35 units has been projected until the year 2100 (Solomon *et al.* 2007).

While these abiotic changes of the marine environment are predictable with a fairly high certainty, our knowledge of their effects on the marine biota and the associated biological processes driving the biogeochemical cycling of climatically active elements in the ocean are largely unknown. Planktonic microorganisms constitute the base of marine pelagic food webs. Primary production by phytoplankton does not only contribute about half of global primary production on Earth (Falkowski *et al.* 1998; Field *et al.* 1998), thereby providing matter and energy for higher trophic levels and sustaining global fisheries, but is also responsible for the incorporation of inorganic carbon into organic biomass in the ocean's euphotic zone. Driven by a complex network of food web processes in the surface ocean, a significant fraction of this biomass-bound carbon is transported to the ocean interior via the biological pump, hence removing it from contact with the atmosphere and contributing to the net flux of CO_2 from the atmosphere into the ocean. Ultimately, temperature-driven changes in the interplay of these processes involved in organic matter cycling and export may hence significantly affect ocean productivity as well as the ocean's role in the climate system.

Temperature is a major environmental factor that directly controls the rate of virtually all biological processes due to the temperature-dependence of the various biochemical reactions underlying all cellular functions (e.g. enzyme-mediated reactions). This is of great importance for the physiological and ecological fitness of organisms, as it affects important functional traits such as growth, metabolism, developmental times, foraging rates, etc. (Gillooly *et al.* 2001; Woods *et al.* 2003). However, the response magnitude, or temperature-sensitivity, of individual biological processes can differ greatly. This sensitivity is generally expressed as the Q_{10} factor, which describes the factorial increase of the biological process rate for a given increase in temperature of 10°C. Also the various processes driving biogeochemical cycling of organic matter in the surface ocean have been shown to differ in their response to changing ambient temperature. Thus, phytoplankton growth and photosynthesis are generally only weakly affected by temperature with a Q_{10} of 1-2 (Eppley 1972) or, under light-limited conditions, even temperature-independent, as suggested by Tilzer *et al.* (1986). Here, other environmental factors, such as light intensity or nutrient availability, likely exert a stronger control. In contrast, heterotrophic processes often display a considerably higher dependency on ambient temperature. Thus, Q_{10} values of bacterial processes have been reported to range between 2 and 3 (Pomeroy and Wiebe 2001). Moreover, Rivkin

and Legendre (2001) found in a literature-based analysis of reported bacterial growth efficiencies (BGE) that these decrease with rising temperature, thus yielding a higher respiratory loss of incorporated carbon at elevated temperature. In contrast to this, Vázquez-Dominguez and co-workers (2007) reported from on-deck incubations of a Mediterranean coast bacterial community that a warming by 2.5°C above ambient values did not affect BGE, but led to an increase in total bacterial carbon demand. As the BGE critically determines how much of the organic carbon consumed by heterotrophic microbes is incorporated into biomass, thus being available for transfer to higher trophic levels, and how much is directly respired back into CO_2, it may impose a particularly critical control on the cycling and fate of biologically-fixed carbon in the surface ocean. For other important members of planktonic food webs, i.e. micro- and mesozooplankton, Q_{10} values of, for instance, 2.5 for microflagellate growth (Caron *et al.* 1986) and 1.8-2.1 for zooplankton respiration (Ikeda *et al.* 2001) have been reported in the literature. In contrast, only little information on the temperature-dependence of aggregation processes, driving much of the vertical flux of organic matter from surface to depth, is available to date. However, a stimulating effect on organic matter aggregation has been observed in a laboratory study using a diatom monoculture (Thornton and Thake 1998).

Considering these widely differing sensitivities of pelagic key processes driving the biogeochemical cycling of organic matter in the surface ocean, a climate-change induced warming of the surface ocean is likely to shift the delicate balance between the autotrophic production and heterotrophic consumption processes, with yet unknown effects on ocean productivity and the biological sequestration of climatically active elements to depth.

In addition to these direct effects of temperature on marine biota, sea surface warming will also indirectly affect life in the marine realm. Thus, the projected increase in vertical stratification of the upper water column (Sarmiento *et al.* 1998) will lead to an increase in light availability, but concomitantly decrease the input of nutrients due to a reduced upwelling of nutrient-rich deep waters. This may lead to differential responses of marine communities at low and high latitudes. In high-latitude areas, primary production is frequently limited by light due to a deep vertical mixing of the water column, resulting in a very short period of net growth. Here, a shoaling of the upper mixed layer may indeed increase productivity by prolonging the growing season. In contrast, the oligotrophic ocean areas at low latitudes, being already characterised by a permanent stratification of the water column and a low availability of nutrients, may exhibit an additional decrease in productivity in response to sea surface warming. However, the consequences for global ocean productivity are not understood at present.

Further complicating the picture, the direct and indirect effects of surface ocean warming will also interact with other anthropogenic perturbations of the marine environment, i.e. ocean acidification (Solomon *et al.* 2007), as well as increasing allochthonous inputs of nitrogen and phosphorus (Jickells 1998). The decrease in oceanic pH associated with ocean acidification has already been shown to have a detrimental impact on a variety of marine calcifying organisms (Orr *et al.* 2005; Riebesell *et al.* 2000). Human activities have also severely disturbed the nutrient balance of the oceans by increasing allochthonous inputs via river runoff and atmospheric deposition (Falkowski *et al.* 2000; Jickells 1998). This may even further increase in future decades due to changes in global precipitation patterns, as projected for this century (Trenberth *et al.* 2007). Here, in particular, the macronutrients nitrogen and phosphorus may play a major role, as they frequently limit primary productivity in the marine environment.

In concert, these perturbations of the marine environment will likely have a profound effect on marine organisms and the associated biogeochemical cycling of key elements, with yet unknown consequences for ocean productivity and the ocean's role in Earth's climate system.

4. Thesis outline

The experimental studies described in this thesis have been conducted at the IFM-GEOMAR (Kiel) within the framework of the national priority program AQUASHIFT, which is funded by the Deutsche Forschungsgemeinschaft (DFG). The overarching aim of AQUASHIFT is to investigate the effects of progressing climate change, in particular of global warming, on aquatic systems, bringing together experimentally working and modelling scientists from both limnic and marine research areas. Within AQUASHIFT, the "Kiel Plankton Mesocosm Cluster" focuses on the effects of winter and spring warming on the succession of different trophic levels within a natural spring plankton community and the associated biogeochemical cycling of major elements. Here, Kiel Bight serves as a model system for moderately deep water bodies, where the onset of the phytoplankton spring bloom is not dependent on the establishment of a thermal stratification of the water column. The experiments were conducted in joint collaboration with colleagues from the IFM-GEOMAR Leibniz Institute of Marine Sciences/Kiel, the Alfred Wegener Institute for Polar and Marine Research (AWI)/Bremerhaven, and the Leibniz Institute for Baltic Sea Research (IOW)/Warnemünde.

The aim of this thesis is to elucidate the effects of sea surface warming, as projected for the end of the 21st century, on the biogeochemical cycling of carbon, nitrogen, and phosphorus during spring plankton blooms in a pelagic system. Here, the experimental studies focussed in particular on temporal changes in the inorganic and organic reservoirs of these key elements, as well as on the partitioning of organic matter between its particulate and dissolved pools.

In **Chapters 1** and **2**, I present results from a mesocosm experiment in which a natural spring plankton community was exposed to a range of four different temperatures, corresponding to the present-day seasonal *in situ* temperature of the study site and to three elevated temperature scenarios, *in situ* +2°C, *in situ* + 4°C, and *in situ* + 6°C, as projected for the year 2100. Here, a novel indoor-mesocosm set-up was applied with eight mesocosms, which were deployed in duplicate in four temperature-controlled climate chambers. Computer-controlled light benches above each mesocosm, which were equipped with full-spectrum light tubes covering the whole range of photosynthetically active radiation (PAR), allowed a very close simulation of natural light conditions.

Chapter 1 describes the build-up, elemental composition and stoichiometry, as well as the aggregation potential of organic matter in response to rising sea surface temperature. A comprehensive set of analytical methods was applied to monitor changes in the dissolved and particulate, as well as organic and inorganic pools of carbon, nitrogen, and phosphorus. These measurements were further supplemented

with state-of-the-art analyses of specific organic matter pools (i.e. carbohydrates), particle dynamics (i.e. transparent exopolymer particles), and rate measurements of autotrophic primary production.

Chapter 2 focuses specifically on temperature-driven changes in the flow of carbon through the planktonic food web. Here, biogeochemical analyses of the various carbon pools (i.e. dissolved inorganic carbon, as well as particulate and dissolved organic carbon) were combined with different activity measurements characterising the heterotrophic utilisation of organic carbon in the plankton community, i.e. bacterial secondary production and community respiration. These data sets were further used for the calculation of a carbon budget, depicting the balance between the autotrophic build-up and heterotrophic consumption of organic carbon.

Chapter 3 presents results from a microcosm study, which targets the question of interactive effects of sea surface warming and altered nutrient availability on the build-up, composition, and degradability of organic matter in a model algal-bacterial community. Natural seawater containing a bacterial community was inoculated with an axenic culture of *Skeletonema costatum*, a dominant algal species during the spring bloom in Kiel Bight. The mixed algal-bacterial assemblage was exposed to a range of three different temperatures, simulating weak to strong warming as projected for the end of this century, as well as either nitrogen- or phosphorus-deficient growth conditions in a factorial combination. Biogeochemical analyses of the various pools of carbon, nitrogen, and phosphorus were combined with rate measurements characterising the degradability of the accumulating organic material by heterotrophic microbes.

This will be followed by a comprehensive **Synthesis**, discussing the results and embedding them into current knowledge on climate change, as well as by a personal outlook on important future research directions emerging from this thesis.

IV.
CHAPTERS

CHAPTER 1

EFFECTS OF RISING SEA SURFACE TEMPERATURE ON THE BIOGEOCHEMICAL CYCLING OF ORGANIC MATTER DURING A SPRING PLANKTON BLOOM: AN INDOOR-MESOCOSM STUDY

Effects of rising sea surface temperature on the biogeochemical cycling of organic matter during a spring plankton bloom: an indoor-mesocosm study

ABSTRACT

Rising concentrations of atmospheric greenhouse gases and the associated heating of the Earth system due to human burning of fossil fuels and land-use change are causing an increase in oceanic surface temperatures. To investigate how warming may affect the biogeochemical cycling of organic matter in the upper ocean, an indoor-mesocosm experiment was conducted, using a natural plankton community from the western Baltic Sea. The community was exposed to four different temperatures with the lowest temperature being equivalent to present-day conditions, and three elevated temperatures corresponding to the warming scenarios projected by the International Panel on Climate Change (IPCC) until the year 2100. The build-up and decline of the developing phytoplankton blooms was monitored over a period of 30 days. In this study, we focussed in particular on the magnitude, stoichiometric composition, and aggregation potential of organic matter accumulating in the water column. For this purpose, the dissolved and particulate pools of inorganic and organic carbon, nitrogen, and phosphorus were analysed on a daily basis. These data were supplemented by analyses of the carbohydrate fraction of dissolved organic matter, of transparent exopolymer particles, as well as by rates of autotrophic primary production. Increasing the temperature by 2 to 6°C clearly affected several important aspects of organic matter cycling. While autotrophic processes responded only weakly to warming, a pronounced acceleration of heterotrophic activities was observed, resulting in a faster remineralisation and recycling of suspended particulate organic matter. Moreover, rising temperature caused a distinct shift in the partitioning of carbon between particulate and dissolved organic matter towards an enhanced accumulation of carbon-rich dissolved compounds. Thus, the rate of accumulation of bulk dissolved organic carbon as well as of polysaccharides displayed a more than two-fold increase when raising the temperature by up to 6°C. A contribution of bacterially-derived refractory DOC to this temperature-dependent accretion is suggested. A pronounced increase in the concentration of transparent exopolymer particles (TEP) in the two warmest treatments also indicated a stimulation of aggregation processes by rising temperature. In concert, the observed alterations of organic matter cycling due to surface ocean warming have the potential to profoundly affect the major biogeochemical cycles in the ocean with likely consequences for the functioning of the pelagic ecosystem.

INTRODUCTION

The interaction of biological processes in the surface ocean plays an integral role in regulating the biogeochemical cycling of organic matter, total ocean productivity and the ocean's capacity to mitigate climate change. The transport of photosynthetically fixed carbon from the euphotic zone into the ocean interior, i.e., the biological carbon pump, is one of the key processes driving the net air-sea flux of CO_2. In addition, the ocean also acts as a temperature buffer in the Earth's climate system by absorbing excess heat, largely originating from the anthropogenic emission of greenhouse gases. This has, in turn, already led to a detectable warming of the global ocean in the last 40 years (Levitus *et al.* 2005). Recent climate projections predict a further rise in global surface temperature by 1.1 to 6.4 °C until the end of the 21st century (Meehl *et al.* 2007).

Temperature is a major environmental factor, directly controlling the rate of biological processes. The sensitivity of individual biotic processes, generally expressed as the Q_{10} factor (i.e. the factorial increase in a process rate for a 10°C increase in temperature), can differ considerably, especially between autotrophic and heterotrophic processes. While phytoplankton growth and photosynthesis, for instance, generally respond only weakly to an increase in temperature with a Q_{10} of 1-2 and are rather controlled by the supply of light and inorganic nutrients (Eppley 1972), heterotrophic activities often show a stronger dependency on temperature with Q_{10} values generally exceeding 2 (e.g. bacterial degradation of organic matter: 2-3 (Pomeroy and Wiebe 2001); microflagellate growth: 2.5 (Caron *et al.* 1986); zooplankton respiration: 1.8-2.1 (Ikeda *et al.* 2001). In contrast, information on the sensitivity of aggregation processes to rising temperature is still scarce. A response to ocean warming seems, however, likely, because the underlying mechanisms, i.e., Brownian motion and the conformation of molecules, determining the availability of binding sites, are known to be temperature-dependent. Concordantly, results from a laboratory study using a diatom monoculture indicated a stimulating effect of rising temperature on aggregate formation (Thornton and Thake 1998).

Moreover, temperature may also affect the flux of organic matter through changes in the chemical structure and/or composition of the accumulating material. The high-molecular-weight fraction of dissolved organic matter (e.g. complex carbohydrates), being a major constituent of freshly produced phytoplankton exudates, is frequently involved in the abiotic formation of so-called transparent exopolymer particles (TEP). These particles have been shown, both in laboratory (Passow and Alldredge 1994) and field studies (Engel 2004), to play an important role in the aggregation and sedimentation of phytoplankton blooms owing to their surface-active nature. Conversely, also the heterotrophic break-down of specific compounds or elements alters the

composition of the organic matter pools. For instance, the selective microbial utilisation of dissolved carbohydrates has been suggested to affect the hydrophobic behaviour of DOM by increasing the relative abundance of deoxy sugars, thereby potentially promoting the formation of TEP particles (Giroldo and Vieira 2003). Similarly, Shaffer et al. (Shaffer *et al.* 1999) reported a preferential remineralisation of phosphorus and nitrogen over carbon in sedimenting particulate matter on its way through the water column, causing a release of nutrients at shallow depth and a concomitant enrichment of the sinking matter in carbon.

Ultimately, such changes in the balance between autotrophic production and heterotrophic consumption of organic matter or in its composition due to surface ocean warming may have a strong impact on the flow of energy and matter through the pelagic food web and the sequestration of carbon from the surface ocean to depth.

However, present atmosphere-ocean general circulation models (AOGCM) still lack a good representation of biological processes and of biotic feedbacks to climate change. In this study, we aim at elucidating the multiple effects of rising sea surface temperature on organic matter cycling, with a focus on the timing and magnitude of organic matter build-up and decline, its composition and stoichiometry, as well on its aggregation and export potential. To investigate these, we conducted an indoor-mesocosm experiment using a natural winter/spring plankton community from Kiel Fjord (Western Baltic Sea) and exposing the community to a range of four temperatures: the *in situ* temperature (T+0), and the elevated temperatures *in situ* + 2°C (T+2), *in situ* + 4°C (T+4), and *in situ* + 6°C (T+6). The build-up and decline of the phytoplankton blooms was closely monitored over a period of 30 days.

MATERIAL AND METHODS

Experimental set-up.

The indoor-mesocosm study was conducted between 6th January and 5th February 2006 at the IFM-GEOMAR, Leibniz Institute of Marine Sciences (at Kiel University), Germany. Eight mesocosms with a volume of 1400 L were set up in four temperature-controlled climate chambers and filled with unfiltered seawater from Kiel Fjord (Western Baltic Sea) containing a natural winter/spring plankton community. Mesozooplankton was added from net catches in natural over-wintering densities of approx. 10 individuals L^{-1}. The temperature of the four climate chambers was adjusted to 2.5, 4.5, 6.5, and 8.5°C, respectively (Fig. 1A).

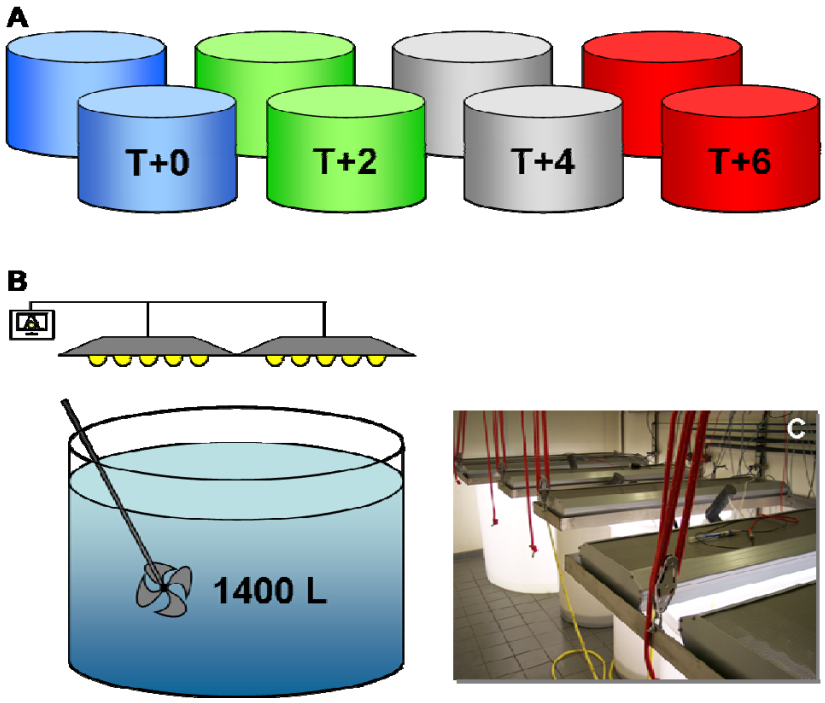

Figure 1. Experimental set-up. (A) Four temperature-controlled climate chambers were equipped with two replicate mesocosms each. The chambers were adjusted to the following temperatures: 2.5 (*in situ* temperature T+0; blue), 4.5 (T+2; green), 6.5 (T+4; grey), and 8.5 °C (T+6; red). (B) Scheme of a mesocosm. Displayed are the water tank with a volume of 1400 L, the agitator mixing the water column, as well as the controlled illumination system supplying full-spectrum light. (C) A picture showing the replicate mesocosms as well as the light benches above in one of the climate chambers (also displayed are two smaller benthic chambers in the middle, which were not used in this experiment).

The lowest temperature of 2.5°C (*in situ* treatment T+0) was derived from a 10-year (1993-2002) meteorological database for Kiel Bight. The elevated temperature regimes, *in situ* +2°C (T+2), *in situ* +4°C (T+4), and *in situ* +6°C (T+6), were chosen according to the projected increase in surface temperature of 4-10°C during winter in the Baltic Sea region (based on AOGCM climate model simulations as summarised in (Giorgi *et al.* 2001). Light was supplied by a computer-controlled illumination system, generating a diurnal triangular light curve with maximum light intensities at midday and an overall 12:12 hours light:dark-cycle. The illumination system contained full-spectrum light tubes (10x JBL T5 Solar Tropic [4,000 K], 2x JBL T5 Solar Natur [9,000 K]), which covered the full range of photosynthetically active radiation (PAR; 400-700 nm) (Tab. 1).

Mesocosm #	T+0_1	T+0_2	T+2_1	T+2_2	T+4_1	T+4_2	T+6_1	T+6_2
Mean water temperature (°C)	2.1	2.4	4.1	4.8	5.9	6.5	7.0	8.0
Light:Dark cycle (h:h)				12:12				
Max. light intensity (µmol photons m^{-2} s^{-1})	177	201	193	204	187	171	127	176
Initial nutrient concentration (µmol L^{-1})								
NO_3^-				21.1 ± 0.20				
NH_4^+				5.6 ± 0.30				
PO_4^{3-}				0.9 ± 0.01				
$Si(OH)_4$				20.4 ± 0.50				

Table 1. Mean water temperature, light:dark-cycle, maximum light intensity (measured at 12:00 in approx. 10 cm water depth), and initial nutrient concentrations.

Based on the light measurements at midday, a daily photon flux of 1.93 mol photons m^{-2} d^{-1} was calculated for the mesocosms (see Calculations section within Material & Methods for details). This value compares well with latitudinal-averaged community compensation irradiances of 0.9 to 1.75 mol photons m^{-2} d^{-1} estimated to generate the initiation of the North Atlantic spring bloom (Siegel *et al.* 2002). Initial concentrations of dissolved inorganic nutrients were 0.9 µM phosphate (DIP), 8 µM nitrate, 5.6 µM ammonium, µM nitrite and 20.4 µM silicate (DSi), respectively. Due to the unusually low nitrate concentration in this year, another 13 µM of NO_3^- were added to ensure bloom development, yielding a total dissolved inorganic nitrogen (DIN = sum of inorganic nitrogen species nitrate, nitrite, and ammonium) concentration of 27 µM. The water body was gently mixed with a propeller attached to the side of the mesocosm (Fig. 1B). With this, cells and smaller particles were kept in suspension, whereas larger particles and aggregates forming during the bloom sank out of the water column. Water samples were taken daily with a silicon hose from intermediate depth. After addition of nutrients and mesozooplankton, we followed the build-up and decline of the phytoplankton bloom over 30 days.

Measurements.

Temperature, salinity and pH were measured 3x per week with a WTW conductivity/pH probe. The maximum light intensity at midday was measured 5x per week with a submersible 4π-PAR sensor (LiCOR Inc., USA) in a water depth of approx. 10 cm. Samples for dissolved inorganic nitrate, nitrite, phosphate, and silicate were pre-filtered through 5 µm cellulose acetate filters and measured with an autoanalyser (AA II) (Hansen and Koroleff 1999). Ammonium was determined from unfiltered water samples

(Holmes *et al.* 1999). Samples for the analysis of chlorophyll a were filtered onto glass fibre filters (GF/F, Whatman) and stored at -20°C. Pigments were extracted in 90% acetone and measured on a 10-AU Turner fluorometer (Welschmeyer 1994). Primary production (PP) was determined through measurements of ^{14}C-bicarbonate ($H^{14}CO_3^-$) uptake (Gargas 1975; Steemann Nielsen 1952). For this purpose, $H^{14}CO_3^-$ was added to 30 ml-samples at a final activity of 4 µCi per bottle. The samples were then incubated *in situ* for 4 – 5 h at intermediate water depth inside the respective mesocosms. All incubations were carried out in duplicate with one dark bottle serving as a blank. After incubation, sample aliquots were filtered onto 0.2 µm cellulose acetate and 3.0 µm polycarbonate filters, respectively. The filters were fumed with concentrated hydrochloric acid (HCl) for 10 min in order to remove excess $H^{14}CO_3^-$. Finally, samples were radio-assayed in 4 ml of scintillation cocktail (Lumagel Plus) on a Packard TriCarb scintillation counter. Daily PP (µmol C L^{-1} d^{-1}) was calculated by multiplying the obtained production rates with a light factor (total amount of light throughout the day divided by the amount of light received during the incubation).

For the determination of particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP), samples were filtered onto pre-combusted (5 h, 450°C) GF/F-filters and stored at -20°C until analysis. POC and PON filters were dried for 6 h at 60°C and analysed on a Eurovector EuroEA-3000 elemental analyser (Sharp 1974). POP was determined colorimetrically after oxidation with potassium peroxodisulphate (Hansen and Koroleff 1999). Samples for dissolved organic carbon (DOC), dissolved organic phosphorus (DOP), and dissolved carbohydrates were filtered through pre-combusted GF/F-filters. For the determination of DOC and dissolved carbohydrates, the filtrate was collected in pre-combusted (12 h, 450°C) glass vials and stored at -20°C. DOC analysis was carried out on a Shimadzu TOC_{VCN} using the HTCO method (Qian and Mopper 1996). For dissolved carbohydrates, the 2, 4, 6-tripyridyl-s-triazine (TPTZ) spectrophotometric method was applied (Myklestad *et al.* 1997). DOP samples were stored in acid-cleaned polyethylene (PE) vials at -20°C and analysed following the protocol of Hansen and Koroleff (1999). The molecular composition of the polysaccharide fraction of DOC was detected by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) on a Dionex IC 3000. Samples were pre-filtered through Acrodisc® syringe filters (0.45 µm, GHP-membrane, PALL, USA) and stored at -20°C in combusted (12h, 450°C) glass vials. Prior to analysis, samples were desalinated through membrane dialysis (MWCO: 1kDa), hydrolysed with 0.8 mol L^{-1} HCl and neutralised by evaporation with N_2 (Engel and Händel *subm.*). The concentration of transparent exopolymer particles (TEP) was determined colorimetrically (Passow and Alldredge 1995). Samples were gently (< 150

mbar) filtered through 0.4 µm polycarbonate filters (Whatman), stained with Alcian Blue and stored at -20°C until analysis. The dye was redissolved in 80% H_2SO_4 for 3 hours and the supernatant analysed on a Hitachi U-2000 spectrophotometer. All filters were prepared in duplicate. The acidic polysaccharide Gum Xanthan was used as a standard. The carbon content of TEP (µmol C L^{-1}) was calculated using a conversion factor (f' = 0.63) (Engel 2004).

Calculations.

Daily light flux: Based on the average maximum light intensity of 179.6 µmol photons m^{-2} s^{-1} at the surface of the mesocosms at midday and following the equation $I_{mix} = I_0(1 - e^{-kz})(kz)^{-1}$ (Riley 1957) with I_0 being the incident light intensity at the surface, a typical winter water light attenuation coefficient k = 0.25 m^{-1} (Sommer and Lengfellner 2008), and a water depth of z = 1 m, a mean daily photon flux of 1.93 mol photons m^{-2} d^{-1} was calculated.

Cardinal points of the bloom: To determine the day of bloom onset the natural logarithm (ln) of Chl a and POC data was calculated and plotted against time. During the exponential growth of algal cells, the logarithmic values generally scale linearly with time. Thus, the first data point fulfilling this linearity criterion was taken as the day of bloom onset.

The end of the bloom period was calculated in a similar manner. Logarithmic Chl a and POC data also showed a linear decline after the peak. The end of the bloom period was hence defined as the first data point deviating from this linear behaviour.

POM build-up: The maximum net build-up of POC (∆POC), PON (∆PON), and POP (∆POP) (in units of µmol C, N, or P L^{-1}) was determined by calculating the difference between the start (average of first 2 days of the experiment) and the maximum concentration. Each mesocosm was treated individually.

POM decline: To determine the rate of decline in POC (v_{decr}(POC)), PON (v_{decr}(PON)), and POP (v_{decr}(POP)) concentration (in units of µmol C, N, or P L^{-1} day^{-1}), the difference between the maximally accumulating amount of the respective variable and the subsequent minimum concentration was calculated. This value was then divided by the number of days between these two sampling points. In the two warmest treatments, T+4 and T+6, a respiratory signal was observed in the dissolved inorganic carbon data sets from days 23/24 (T+4) and 20/24 (T+6) onward (see also Chapter 2), which may affect the observed decline in POM. Since it cannot be ruled out that degradation of sedimented organic matter accumulating at the bottom of the mesocosm contributed to this signal, only data points before the occurrence of the respiratory signal were included in the calculations.

DOM build-up: Time means of DOC (ø DOC) and dissolved polysaccharide (ø PCHO) concentration were calculated for the period from nutrient exhaustion (i.e. DIP exhaustion) to the end of the experiment using all available data points. Each mesocosm was treated individually. For the same period of time, the coefficient of variation (CV_{DOC}) was calculated for DOC by dividing the respective standard deviation by the time mean. The rate of increase in DOC (v_{incr}(DOC)) and PCHO (v_{incr}(PCHO)) was determined by applying a linear regression model to the individual data sets for the time period from DIP exhaustion to the end of the experiment. As DOC and PCHO concentrations stagnated in both replicates of the T+6 treatment after day 26 and day 23, respectively, later obtained data points were not included in the analyses. In order to assess the effect of temperature on v_{incr}(DOC) and v_{incr}(PCHO), the regression slopes were plotted against the respective temperature. The contribution of the polysaccharide fraction to total DOC concentration (PCHO/DOC; in %) was assessed for each mesocosm individually at the start of the experiment (day 0) and six days after the respective bloom peak (average of day 5, 6, and 7 after the peak).

Statistical analysis. The effect of temperature on the various variables and parameters was assessed by using linear regression analysis with a statistical significance level of $p < 0.05$.

RESULTS

Autotrophic growth and organic matter build-up

In all mesocosms the development of distinct phytoplankton blooms was observed. The onset of algal growth was marked by a rapid decline in dissolved inorganic nutrients (Fig. 2) and a concomitant increase in Chl a concentration and primary production rates (Fig. 3). While all inorganic nutrients were scavenged until the detection limit, the initial ratio of DIN to DIP of 30.3:1 indicates a prevailing deficiency of phosphorus compared to the Redfield ratio of 16:1 (Redfield *et al.* 1963). The developing bloom was dominated by chain-forming diatoms (i.e. *Skeletonema costatum*, *Chaetoceros minimum*, *Chaetoceros curvisetus*, and *Thalassiosira nordenskioldii*), constituting more than 87% of total phytoplankton abundance at the time of maximum Chl a concentration (U. Sommer, *unpubl. data*). This predominance of large phytoplankton taxa is also reflected in size-fractionated measurements of primary productivity (PP), showing that the bulk of total primary production (> 0.2 µm; Fig. 3B) occurred in the size fraction >3 µm (Fig. 3C).

Temperature clearly affected the timing of bloom development with an accelerated onset of autotrophic growth (e.g. start of exponential increase in Chl a: 0.7 days/°C), a faster utilisation of inorganic nutrients (i.e. earlier onset of nutrient depletion with DIN: 1.0 days/°C; DIP: 0.7 days/°C; DSi: 1.4 days/°C) and an earlier bloom peak

(e.g. peak of total primary production rates: 1.1 days/°C) at elevated temperatures (Tab. 2). In contrast, the magnitude and total duration of the bloom did not respond notably to rising temperature. Maximum Chl a values, for instance, ranged between a minimum of 44.8 ± 5.0 at T+2 and a maximum of 65.5 ± 2.2 µg L^{-1} at T+0. The average duration of the bloom was 22 ± 2 days (see *Material and Methods* for details on calculations).

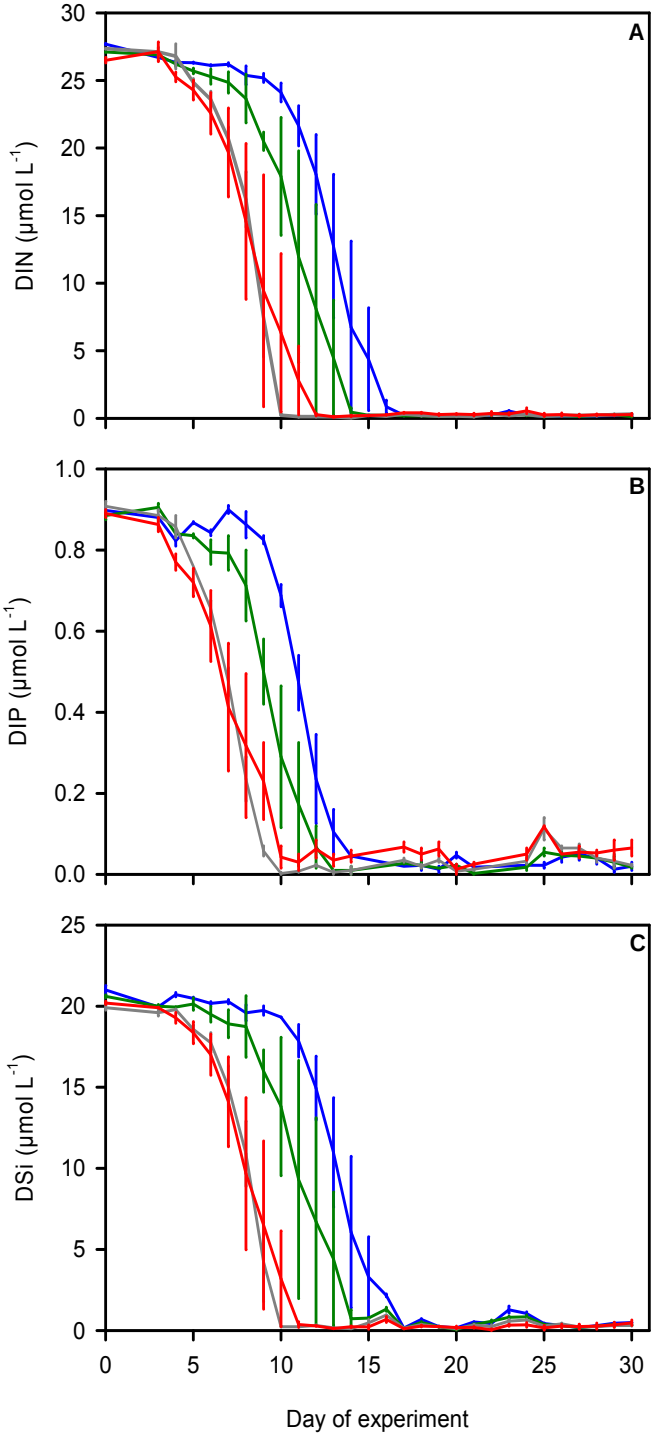

Figure 2. Temporal development of dissolved inorganic nutrient concentrations. (A) dissolved inorganic nitrogen (DIN), (B) dissolved inorganic phosphorus (DIP), (C) dissolved inorganic silicate ($Si(OH)_4$). The different colours represent the four temperature regimes: the *in situ* temperature T+0 (blue) and the elevated temperature regimes T+2 (green), T+4 (grey), and T+6 (red). Solid lines denote the average of two replicate mesocosms, error bars indicate the range of the replicates.

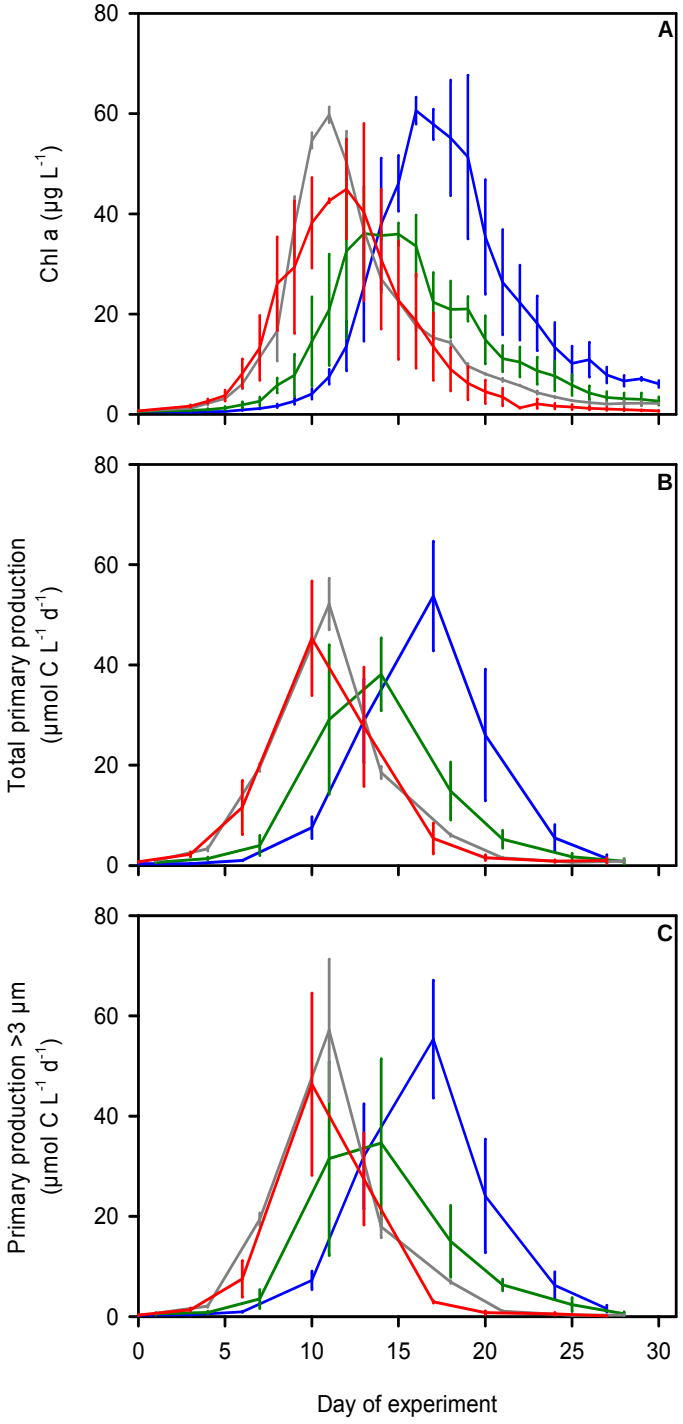

Figure 3. Temporal development of autotrophic growth. (A) chlorophyll a (Chl a); Rates of (B) total primary production (> 0.2 µm), and (C) primary production > 3 µm. The data on primary production rates were kindly provided by P. Breithaupt. Colour and symbol code as in Fig. 2.

	T+0	T+2	T+4	T+6	acceleration	R^2	p
bloom onset:							
Chl a	6.5 ± 1.5	4.5 ± 0.5	3.0 ± 0	3.0 ± 0	-0.71	0.75	0.005*
POC	8.5 ± 0.5	6.5 ± 1.5	5.0 ± 0	4.0 ± 1.0	-0.89	0.85	0.001*
nutrient utilisation:							
DIN	15.5 ± 1.5	13.0 ± 1.0	10.0 ± 0	11.0 ± 1.0	-1.02	0.76	0.005*
DIP	13.5 ± 0.5	12.0 ± 1.0	9.5 ± 0.5	10.5 ± 0.5	-0.69	0.71	0.009*
DSi	17.0 ± 0	14.5 ± 2.5	10.0 ± 0	10.5 ± 0.5	-1.42	0.81	0.002*
bloom peak:							
Chl a	17.5 ± 1.5	14.0 ± 2.0	11.0 ± 0	11.5 ± 1.5	-1.31	0.79	0.003*
Primary production	17.0 ± 0	12.5 ± 1.5	11.0 ± 0	11.5 ± 1.6	-1.13	0.76	0.005*
POC	18.5 ± 0.5	17.0 ± 1.0	11.5 ± 0.5	12.5 ± 1.5	-1.40	0.82	0.002*

Table 2. Timing of the algal bloom development. Given are the days of bloom onset, nutrient depletion, and bloom peak, calculated for each temperature treatment as the average of two replicate mesocosms ± their range. The acceleration of bloom onset, nutrient utilisation, and bloom peak in units of days $°C^{-1}$ was determined by linear regression analysis.

	T+0	T+2	T+4	T+6	slope	R^2	p
build-up:							
Δ POC	326.2 ± 3.9	314.7 ± 7.4	352.0 ± 28.7	245.2 ± 37.9	-8.3	0.13	0.38
Δ PON	23.1 ± 0.2	21.2 ± 0.6	30.1 ± 4.3	20.9 ± 1.1	0.35	0.03	0.7
Δ POP	0.67 ± 0.02	0.62 ± 0.01	0.64 ± 0.03	0.59 ± 0.02	-0.01	0.52	0.04*
decline:							
v_{decr} (POC)	-21.2 ± 1.7	-19.1 ± 5.8	-23.6 ± 1.5	-27.1 ± 5.7	1.1	0.35	0.13
v_{decr} (PON)	-1.2 ± 0.08	-1.1 ± 0.07	-1.81 ± 0.17	-1.84 ± 0.12	0.14	0.67	0.01*
v_{decr} (POP)	-0.037 ± 0.003	-0.033 ± 0.001	-0.038 ± 0.003	-0.049 ± 0.003	0.002	0.39	0.10

Table 3. Build-up and decline of particulate organic matter (POM). Given are the values for maximum net build-up of POC (Δ POC), PON (Δ PON), and POP (Δ POP), as well as the rate of decline in POC, PON, and POP concentration after the peak (in units of µmol L^{-1} and µmol L^{-1} day^{-1}, respectively). Values represent the average of two replicate mesocosms ± their range. The effect of temperature on the different variables and parameters was assessed using linear regression analysis with the slope describing the direction and magnitude of change with increasing temperature.

Similarly, also the build-up of particulate organic matter (POM) showed a temperature-dependent development with an acceleration of 1.0 (PON) to 1.2 days/°C (POC). While the maximum net accumulation of particulate organic carbon (ΔPOC) and nitrogen (ΔPON) was not affected by temperature with average values of 309.5 ± 49.5 µmol C L^{-1} (Fig. 4A) and 23.8 ± 4.6 µmol N L^{-1} (Fig. 4B), respectively, the maximum net amount of particulate organic phosphorus (ΔPOP) accumulating in the water column showed a significant decrease by more than 10% from 0.67 ± 0.02 µmol L^{-1} at *in situ*

temperature to 0.59 ± 0.02 at T+6 ($p = 0.04$; Fig. 4C and Tab. 3). Moreover, temperature also affected the loss of POM from the water column, as indicated by the rate of decline in POC, PON, and POP concentration after the bloom peak. PON concentrations decreased significantly faster at elevated temperatures with a maximum rate of -1.84 µmol N L^{-1} day^{-1} in the warmest treatment T+6 compared to -1.2 µmol N L^{-1} day^{-1} at T+0 (v_{decr}(PON): $p = 0.01$; Tab. 3). POC and POP decline rates also showed a clear positive correlation with rising temperature, but in contrast to PON these temperature trends were marginally insignificant (v_{decr}(POC): $p = 0.13$; v_{decr}(POP): $p = 0.10$; Tab. 3).

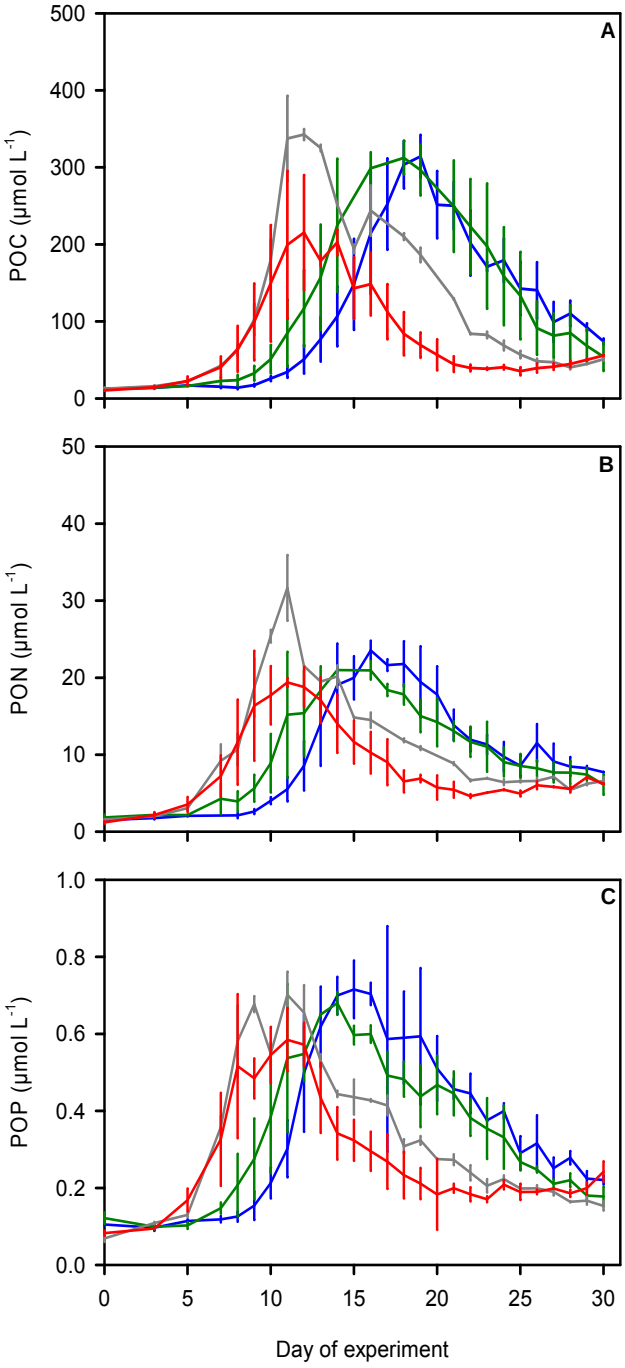

Figure 4.
Temporal development of particulate organic matter concentrations. (A) particulate organic carbon (POC), (B) particulate organic nitrogen (PON), particulate organic phosphorus (POP). Colour and symbol code as in Fig. 2.

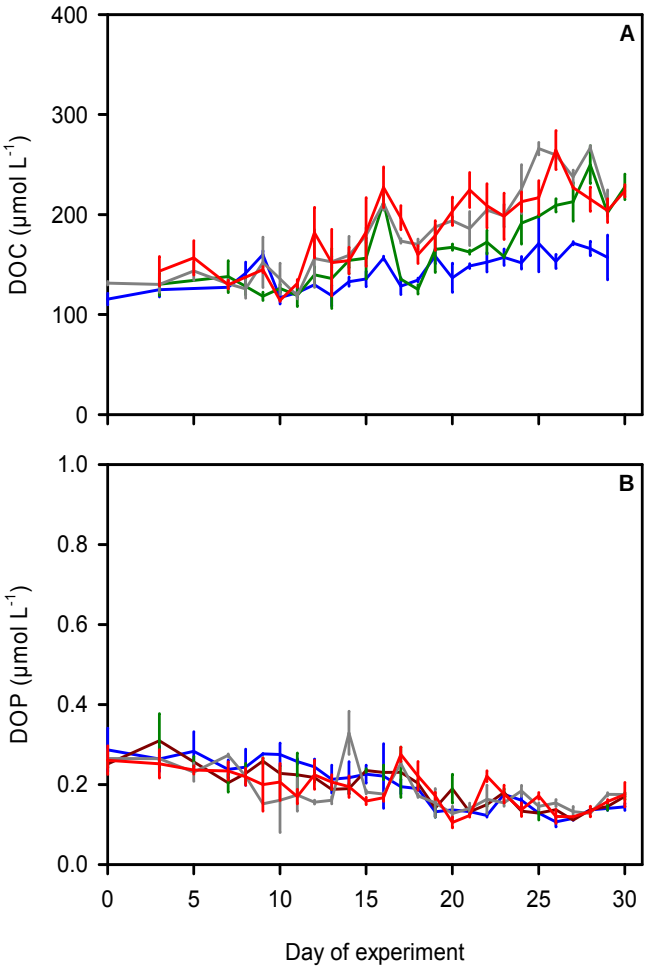

Figure 5.
Temporal development of dissolved organic matter concentrations. (A) dissolved organic carbon (DOC), (B) dissolved organic phosphorus (DOP). Colour and symbol code as in Fig. 2.

With the onset of nutrient limitation also the concentration of dissolved organic carbon (DOC) started to rise (Fig. 5A). The rate of accumulation, as indicated by the slope of linear regressions fitted individually to each mesocosm data set (see also *Material and Methods* for details on calculations), showed a significant increase with rising temperature (v_{incr}(DOC): p = 0.02; Tab. 4). This is also reflected in the average DOC concentration (ø DOC), calculated for the period of beginning nutrient depletion until the end of the experiment, which rose significantly from 151 ± 4.3 at *in situ* temperature to 192.4 ± 8.5 µmol C L^{-1} at T+6 (p = 0.001; Tab. 4). In addition, also the coefficient of variation (CV_{DOC}), calculated for the same period, showed a positive response to elevated temperatures with an increase from 14.0 ± 1.3 % at T+0 to 26.2 ± 0.5% at T+4 (Tab. 4). In contrast to these observations, the concentration of dissolved organic phosphorus (DOP) continuously decreased in the course of the experiment in all treatments from initially 0.27 ± 0.04 to 0.17 ± 0.02 (Fig. 5B), without displaying a temperature-related response.

	T+0	T+2	T+4	T+6	slope	R^2	p
ø DOC	151.1 ± 4.3	173.0 ± 8.5	196.4 ± 1.7	192.4 ± 8.5	8.89	0.86	0.001*
CV_{DOC}	14.0 ± 1.3	21.8 ± 0.5	26.2 ± 0.5	21.5 ± 0.8	1.34	0.45	0.067
v_{incr} (DOC)	2.4 ± 0.6	5.1 ± 0.7	6.4 ± 0.0	5.3 ± 0.4	0.61	0.61	0.02*
ø PCHO	35.4 ± 2.6	48.7 ± 1.4	65.0 ± 10.4	50.6 ± 6.1	4.2	0.48	0.058
v_{incr} (PCHO)	2.8 ± 0.2	4.8 ± 0.6	5.7 ± 0.2	6.1 ± 0.1	0.61	0.83	0.002*
PCHO/DOC	25.3 ± 1.6	31.5 ± 7.4	33.2 ± 2.0	44.6 ± 3.3	2.96	0.52	0.04*

Table 4. Build-up and composition of dissolved organic matter (DOM). Given are the values for the average DOC concentration (DOC_{avg}; in µmol C L^{-1}), the coefficient of variance of DOC data (CV_{DOC}), and the average concentration of polysaccharides ($PCHO_{avg}$; in µmol C L^{-1}), calculated for the period from the onset of nutrient depletion onward, as well as the rate of increase in DOC ($DOC_{increase}$; in µmol C L^{-1}) and PCHO concentration ($PCHO_{increase}$; in µmol C L^{-1}) and the contribution of polysaccharides to the total DOC pool (PCHO/DOC; in %), calculated on day 6 after the respective bloom peak. Values represent the average of two replicate mesocosms ± their range. The effect of temperature on the different variables and parameters was assessed using linear regression analysis with the slope describing the direction and magnitude of change with increasing temperature.

	T+0	T+2	T+4	T+6	slope	R^2	p
ø $N:P_{part}$	32.2 ± 0.2	33.3 ± 0.9	35.7 ± 0.5	31.6 ± 0.5	0.1	0.01	0.78
ø $C:P_{part}$	378.8 ± 22.3	405.3 ± 46.4	384.5 ± 13.9	290.6 ± 22.9	-13.8	0.27	0.19
max. $C:P_{part}$	647.4 ± 65.0	755.7 ± 19.3	682.8 ± 25.6	613.3 ± 66.5	-5.2	0.02	0.73
v_{decr} ($C:P_{part}$)	-29.4 ± 6.0	-51.5 ± 16.3	-66.3 ± 3.7	-52.7 ± 6.6	5.7	0.49	0.054
ø $C:P_{diss}$	981.8 ± 13.6	1057.0 ± 22.6	1175.2 ± 60.4	1163.0 ± 44.9	40.5	0.84	0.002*

Table 5. Stoichiometry of particulate and dissolved organic matter. Given are data on the maximum C:P ratio of particulate organic matter ($C:P_{part}$), the rate of decrease in $C:P_{part}$ after the peak, and the average C:P ratio of dissolved organic matter ($C:P_{diss}$), calculated for the time period of nutrient limitation to the end of the experiment. Values represent the average of two replicate mesocosms ± their range.

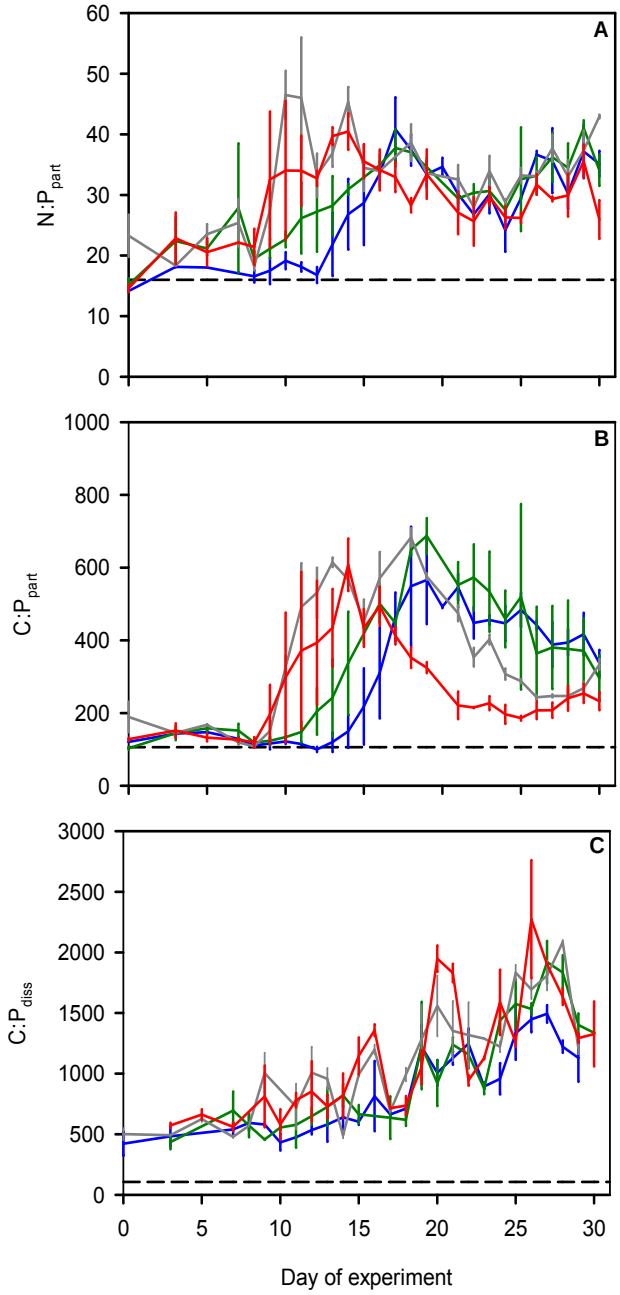

Figure 6.
Stoichiometry of particulate and dissolved organic matter in the course of the experiment.
(A) particulate carbon:nitrogen ratio ($C:N_{part}$), (B) particulate carbon:phosphorus ratio ($C:P_{part}$), (C) dissolved carbon:phosphorus ratio ($C:P_{diss}$). Colour and symbol code as in Fig. 2.

Stoichiometry of particulate and dissolved organic matter

At the start of the experiment, the initial ratio of PON to POP ($N:P_{part}$; Fig. 6A) was close to the Redfield ratio (N:P = 16:1), averaging 16.8 ± 4.5. As inorganic nutrients became depleted, the ratio increased and remained well above Redfield until the end of the experiment with an average value of 33.2 ± 1.8, hence clearly indicating a phosphorus deficiency. A temperature-related difference in $N:P_{part}$ between treatments was not observed (Tab. 5). In contrast, the ratio of POC to POP ($C:P_{part}$; Fig. 6B) was already

higher than Redfield (C:P = 106:1) at the start of the experiment with an average value of 134.9 ± 41.4. With the onset of phosphate depletion it then further increased to maximum values of 546.7 to 775.0. While these maxima did not show a response to warming, a notably faster decline in $C:P_{part}$ after the peak was observed with rising temperature (p = 0.054; Tab. 5).

Compared to POM, the pool of dissolved organic matter (DOM) showed an enhanced enrichment in carbon relative to phosphorus throughout the experiment (Fig. 6C). The initial C:P ratio of DOM ($C:P_{diss}$), for instance, was already approximately four times higher than the Redfield ratio and $C:P_{part}$ with an average value of 462.5 ± 82.6. $C:P_{diss}$ further increased in the course of the experiment in all treatments. However, the ratio tended to be higher at elevated temperatures, as reflected by average $C:P_{diss}$ values (calculated for the time period from nutrient depletion to the end of the experiment; see also *Material and Methods* for details on calculations). These showed a significant increase with rising temperature from 981.8 ± 19.3 at *in situ* temperature to a maximum of 1175.2 ± 85.4 at T+4 (p = 0.002; Tab. 5).

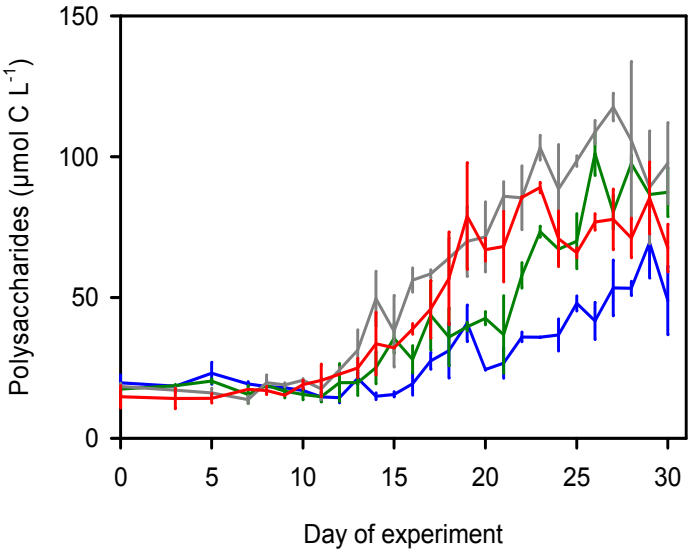

Figure 7.
Temporal development of dissolved polysaccharides (PCHO). Colour and symbol code as in Fig. 2.

Composition of dissolved organic matter and formation of transparent exopolymer particles (TEP)

Concomitant to the increase in DOC also a pronounced accumulation of dissolved carbohydrates, particularly of polysaccharides (PCHO; Fig. 7), was observed. Linear regression analysis revealed a significant effect of temperature on the rate of PCHO increase with rates rising from 2.8 ± 0.2 at T+0 to 6.1 ± 0.1 µmol C L^{-1} d^{-1} at T+6 (v_{incr}(PCHO): p = 0.002; Tab. 4). This is also reflected in observed maximum concentrations of PCHO with values of 101.7 ± 7.1, 123.3 ± 10.4, and 89.0 ± 1.8 µmol C L^{-1} at T+2, T+4 and T+6, respectively, compared to 69.5 ± 12.6 µmol C L^{-1} at T+0. The

lower maximum PCHO value in the warmest treatment was associated with a levelling-off of PCHO concentrations from day 23 onward. At the start of the experiment, the PCHO fraction accounted on average for 14.2 ± 3.8% of the total DOC pool. As the bloom proceeded and PCHO started to accumulate, this percentage increased in all treatments. Thus, six days after the respective bloom peaks, PCHO already constituted between 25.3 ± 1.5 at *in situ* temperature and 44.6 ± 3.3 % of total DOC at T+6, respectively (Tab. 4). Linear regression analysis confirmed that the increase in the contribution of PCHO to total DOC at this time point with rising temperature was statistically significant ($p = 0.04$; Tab. 4), hence clearly indicating a temperature-driven shift in DOM composition.

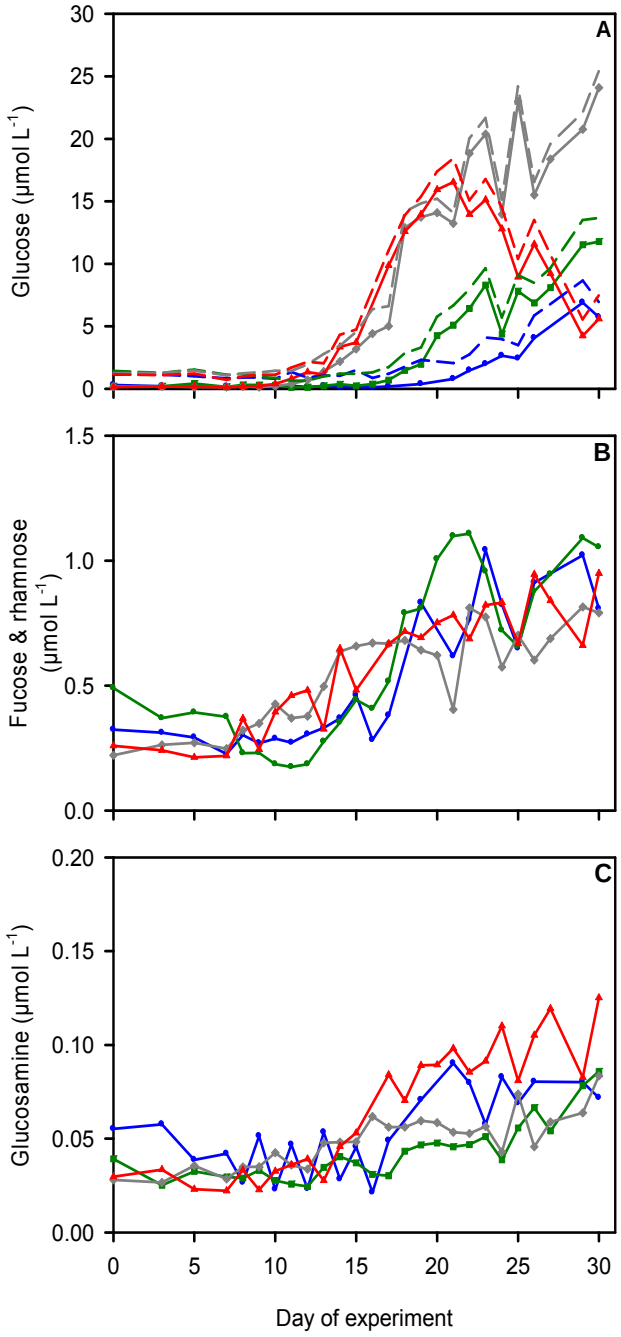

Figure 8.
Composition of dissolved combined carbohydrates (dcCHO).
Temporal development of (A) glucose (solid line) and total dcCHO (dashed line), (B) deoxy sugars (fucose and rhamnose), and (C) glucosamine concentration. Solid lines represent data from one of the replicates of each temperature treatment. Colour code as in Fig. 2. Data from Engel *et al.* (*to be submitted*).

A detailed analysis of the monomeric composition of the PCHO pool revealed that glucose was the most abundant component and largely responsible for the bulk increase, constituting on average (after the onset of nutrient limitation) 44.2, 62.8, 76.4, and 78.3% of total PCHO concentration at T+0, T+2, T+4, and T+6, respectively (Fig. 8A). Albeit other components of the PCHO pool were detected at markedly lower concentrations, they also displayed temporal changes. The concentration of the deoxy sugars fucose and rhamnose, for instance, increased from initially 0.32 ± 0.12 to a maximum of 0.98 ± 0.13 µmol L^{-1} (Fig. 8B). Glucosamine, an amino sugar and a major component of bacterial cell walls, also increased throughout the experiment (Fig. 8C). Highest glucosamine concentrations were observed in the most elevated temperature treatment T+6 with an average of 77 nmol L^{-1} (calculated for the period from the onset of nutrient limitation onward). Average glucosamine values of the other treatments T+0, T+2, and T+4 were markedly lower with 64, 49, and 54 nmol L^{-1}, but did not show a clear trend with rising temperature. In accordance with the bulk measurements of PCHO, also the chromatographic analysis revealed a temperature-dependent increase in the total concentration of dissolved polysaccharides with highest concentrations at T+4, as well as a decrease in PCHO concentration in the warmest treatment T+6 from day 21 onwards.

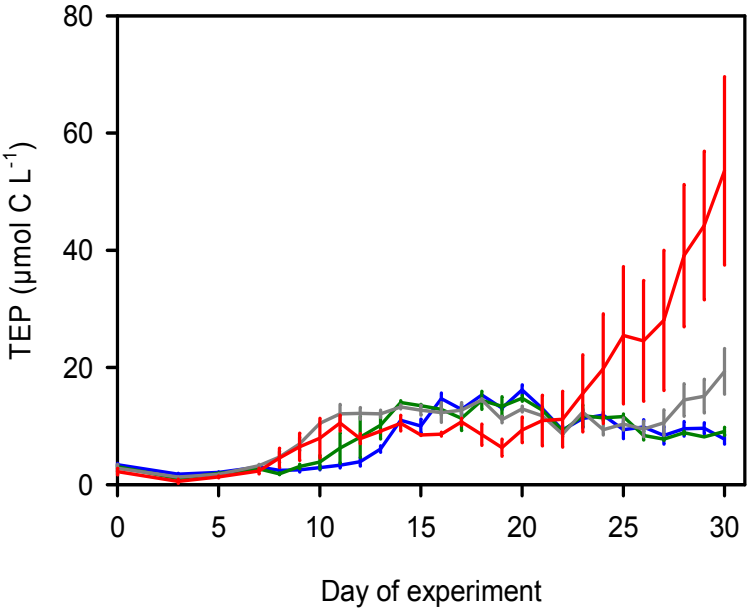

Figure 9.
Temporal development of transparent exopolymer particles (TEP). Colour and symbol code as in Fig. 2.

Concurrent to the onset of bloom development, the concentration of transparent exopolymer particles (TEP) started to increase from initially 2.9 ± 0.7 µmol C L^{-1} on day 0 to maximum values of 11.8 to 17.0 µmol C L^{-1}, with highest amounts of TEP at *in situ*

temperature (Fig. 9). While TEP concentrations declined thereafter in the colder treatments T+0 and T+2, a second increase, exceeding the first peak by several-fold, was observed at T+6 and to a lesser extent also at T+4 from days 20 and 27 onwards, reaching final concentrations of 53.5 ± 16.1 and 19.3 ± 3.9 µmol C L^{-1}, respectively. In the warmest treatment, this increase coincided temporally with a drop-off in PCHO concentrations.

DISCUSSION

Production versus loss of organic matter in a warming ocean

In line with theoretical expectations, the development of autotrophic organisms showed only a weak sensitivity to rising temperature. Thus, different parameters characterising autotrophic growth and biomass production (e.g. nutrient utilisation, Chl a accumulation, primary production) were accelerated by approx. 1 day/°C, shifting the onset of the bloom forward by up to one week at elevated temperatures relative to the *in situ* treatment. These results agree with those reported by Sommer and Lengfellner (2008) from three other indoor-mesocosm experiments. Here, albeit based on different methods and data sets (i.e. phytoplankton cell counts and a cell volume-based calculation of algal carbon content), also a weak advancement of bloom development by 1-1.4 days/°C was observed. As the onset of the spring bloom in the ocean naturally varies by more than one month between years, the observed acceleration due to warming is likely negligible from an ecological point of view. Similarly, neither a direct nor indirect effect (e.g. through changes in grazing pressure) of global warming on the beginning of the spring bloom could be detected to date in two long-term data sets from the central North Sea (Edwards and Richardson 2004) and Helgoland Roads (Wiltshire *et al.* 2008), despite a substantial warming of the North Sea in the past 40 years (Wiltshire and Manly 2004).

While also the magnitude of primary production rates and the build-up of organic biomass (i.e., POC) did not respond markedly to elevated temperature, further indicating a general insensitivity of autotrophic production processes to experimental warming, the maximum accumulation of POP (∆POP) significantly decreased with rising temperature. Previous chemostat experiments investigating phosphorus competition between algae and heterotrophic bacteria have suggested a superior ability of the latter to acquire inorganic phosphorus (Currie and Kalff 1984). Hence, a reduced accumulation of POP at elevated temperature may indicate a shift in the competitive performance of bacteria. Alternatively, the observed temperature effect on ∆POP could also have been caused by an acceleration of heterotrophic degradation processes. A comparison of bacterial secondary production rates at the time of maximum biomass accumulation indeed revealed a pronounced stimulating effect of rising sea surface temperature, clearly

indicating an enhanced degradation activity of bacterial cells at these elevated temperatures already during the phase of bloom build-up (Wohlers *et al.* 2009), see also Chapter 2, Fig. 3A). Further support of the latter hypothesis is also given by an accelerated decrease of PON and POP concentrations with rising temperature during the post-bloom phase. A preferential remineralisation of phosphorus has been reported for both particulate and dissolved organic matter (Clark *et al.* 1998; Shaffer *et al.* 1999). The recycling of organic phosphorus is, however, often difficult to detect in the bulk particulate and dissolved pools as organisms generally reabsorb the released inorganic phosphate as fast as it is produced (Downing 1997). This may, in fact, have reduced the rate of POP decline in our study by continuously re-fuelling the bulk pool of POP, thereby obscuring the response to elevated temperatures. A temperature-related shift in the remineralisation and rapid re-utilisation of phosphoric compounds relative to carbon is also indicated by the stoichiometric ratio of POC to POP ($C:P_{part}$), displaying a faster decline in $C:P_{part}$ after the peak of the bloom with rising temperature.

Partitioning of organic matter between particulate and dissolved pools

Our study revealed a pronounced effect of sea surface warming on DOC dynamics and on the partitioning of carbon between particulate and dissolved organic matter. While primary production rates and the maximum net build-up of POC did not differ notably between treatments, rising temperature stimulated both the rate and the magnitude of DOC accumulation. Timing of the increase (i.e., at the onset of nutrient limitation) and chemical composition suggest that the dissolved material largely originated from phytoplankton exudation. Thus, a concomitant increase in polysaccharides, which often comprise the major fraction of fresh and labile DOM (Benner *et al.* 1992; Biddanda and Benner 1997; Biersmith and Benner 1998), was observed. Moreover, an analysis of the monomeric composition of the PCHO pool revealed that glucose, being the basic module of the major diatom storage polysaccharide chrysolaminaran (i.e., ß-1, 3-glucan) (Granum *et al.* 2002), accounted for the bulk of this increase.

Although rising temperature markedly stimulated microbial activities, they were apparently not able to counteract the accumulation of DOC compounds. Similarly, a seasonal accumulation of DOC in the surface ocean, in particular during and after phytoplankton blooms, has also been reported from different ocean provinces and has been attributed to a temporal decoupling of production and consumption processes (Carlson *et al.* 1998; Carlson *et al.* 1994; Copin and Avril 1993). While the release of dissolved organic compounds by phytoplankton cells generally increases with the onset of nutrient limitation, a phenomenon that is ascribed to a cellular overflow of carbon-rich dissolved organic compounds due to ongoing photosynthetic carbon acquisition while

nutrient deficiency limits further cell growth and division (Wood and Van Valen 1990), its consumption by heterotrophic microorganisms may be constrained by several, not mutually exclusive mechanisms. Fry *et al.* (1996) reported, for instance, a partly high biochemical resistance of freshly produced DOC to microbial degradation. In contrast, other studies suggested that heterotrophic bacteria may not be able to utilise DOC compounds efficiently due to a limitation by inorganic nutrients (e.g. DIP) rather than carbon (Rivkin and Anderson 1997; Thingstad *et al.* 1997).

As the production processes of organic matter (e.g., primary production rates) did not show a marked response to rising temperature in our study, the observed alterations of the DOM pool most likely result from changes in the utilisation and diagenetic processing of dissolved carbon compounds by heterotrophic processes. A severe deficiency of microbial organisms in inorganic nutrients, for instance, is indicated by the ratios of DIN:DIP and PON:POP, both greatly exceeding the Redfield ratio by a factor of 2, and may have hindered an efficient uptake and utilisation of dissolved organic compounds by bacterial cells. This is also reflected by a shift in the stoichiometric composition of DOM towards higher C:P ratios at elevated temperatures, indicating a poorer nutritious quality of the accumulating matter. However, nutrient deficiency alone may only explain a temperature-dependent shift in the timing of DOC accumulation, caused by the earlier onset of nutrient limitation at elevated temperatures. In contrast, a significant increase in the rate of DOC and PCHO accumulation by a factor of 2 was observed when raising the temperature by 2-6°C above *in situ*. This strongly suggests that another, temperature-dependent process contributed to the build-up of the DOC and PCHO pools. According to recent studies, bacteria are not only the major consumers of dissolved organic compounds, but may themselves produce copious amounts of DOC, being highly resistant to rapid microbial degradation and thus contributing to the refractory background pool of DOC in the ocean (Azam 1998; Ogawa *et al.* 2001; Stoderegger and Herndl 1998). Two potential mechanisms have been proposed so far: a partial hydrolysis of complex dissolved organic compounds (e.g., polysaccharides) by bacterial hydrolytic enzymes (Azam 1998), and the continuous release of capsular material from the cell surface of metabolically active bacteria (Stoderegger and Herndl 1998). This material surrounding bacterial cells consists mainly of high-molecular-weight polysaccharides (Sutherland 1977) and has been suggested to exhibit a high resistance to microbial ectoenzymes, because a large fraction of these may actually be embedded in the capsule itself (Stoderegger and Herndl 1998). In both cases, higher bacterial activities would result in an enhanced accumulation of slowly degradable dissolved material. In our study, a positive effect of rising temperature on bacterial activities is clearly indicated by an increase in bacterial secondary production.

Moreover, the concentration of glucosamine, being a major constituent of the bacterial cell wall, increased in the course of the experiment in all treatments, but reached highest values in the warmest treatment T+6. These observations strongly suggest that the proposed mechanisms of bacterial DOC release may have contributed to the observed temperature-dependent increase in DOC and polysaccharide accumulation.

Temperature-sensitivity of aggregation processes

A distinct effect of temperature was also observed concerning the aggregation of organic matter. The concentration of TEP began to increase in all treatments with the onset of nutrient limitation. However, in the T+6 and to a lesser extent also in the T+4 treatments a second and much more pronounced increase in TEP concentration occurred in the post-bloom phase, but was absent in the T+2 and T+0 treatments. Concurrent to the increase in TEP at elevated temperatures, we observed a decrease in PCHO concentration, indicating that TEP was formed by aggregation of the polysaccharide fraction of DOC. Lower concentrations of PCHO in the T+2 and T+0 treatments may have limited TEP formation, which may explain the observed decrease in TEP concentrations during bloom decline. Transformation of dissolved into particulate organic matter via abiotic aggregation to TEP was shown both in laboratory (Chin *et al.* 1998; Kerner *et al.* 2003) and mesocosm studies (Engel *et al.* 2004). The timing of the pronounced increase in TEP at T+6 and T+4 suggests that also the stimulation of bacterial activities by rising temperature may have additionally promoted enhanced TEP-formation through, for instance, an enhanced bacterial release of capsular fibrils (Stoderegger and Herndl 1999) or a selective microbial degradation of polysaccharide components, causing shifts in the hydrophobic behaviour of the accumulating matter. Due to their surface-reactive nature, TEP promote the aggregation and sinking of particulate matter (Engel 2004; Passow and Alldredge 1994). To what extent enhanced TEP formation affected particle sinking in our study is, however, difficult to assess as TEP concentrations in the elevated temperature treatments showed the strongest increase at a time when particulate matter concentrations had nearly decreased to pre-bloom levels, hence limiting the potential for TEP-mediated particle export.

Conclusions

The balance between the build-up of organic matter and its consumption in the surface layer of the ocean is an important aspect of marine biogeochemical cycling, as it controls the flux of energy and matter through the food web and the sequestration of organic matter to depth. Our study provides evidence that rising sea surface temperature due to climate change may markedly affect this balance. While the

production processes of organic matter were only weakly affected by rising temperature, heterotrophic activities displayed a markedly stronger sensitivity to experimental warming, thereby mediating shifts in the remineralisation and recycling of organic matter, in the partitioning of carbon between the particulate and dissolved pools, and also in the aggregation potential of the organic material accumulating in the water column. Projecting these findings to a larger scale, such alterations of organic matter fluxes may profoundly affect the major biogeochemical cycles in a warming ocean.

CHAPTER 2

CHANGES IN BIOGENIC CARBON FLOW IN RESPONSE TO SEA SURFACE WARMING

Changes in biogenic carbon flow in response to sea surface warming

ABSTRACT

The pelagic ocean harbors one of the largest ecosystems on Earth. It is responsible for approximately half of global primary production, sustains world-wide fisheries, and plays an important role in the global carbon cycle. Ocean warming caused by anthropogenic climate change is already starting to impact the marine biota, with possible consequences for ocean productivity and ecosystem services. Because temperature-sensitivities of marine autotrophic and heterotrophic processes differ greatly, ocean warming is expected to cause major shifts in the flow of carbon and energy through the pelagic system. Attempts to integrate such biological responses into marine ecosystem and biogeochemical models suffer from a lack of empirical data. Here, we show, using an indoor-mesocosm approach, that rising temperature accelerates respiratory consumption of organic carbon relative to autotrophic production in a natural plankton community. Increasing temperature by 2 to 6°C hence decreased the biological drawdown of dissolved inorganic carbon in the surface layer by up to 31%. Moreover, warming shifted the partitioning between particulate and dissolved organic carbon toward an enhanced accumulation of dissolved compounds. In line with these findings, the loss of organic carbon through sinking was significantly reduced at elevated temperatures. The observed changes in biogenic carbon flow have the potential to reduce the transfer of primary produced organic matter to higher trophic levels, weaken the ocean's biological carbon pump, and hence provide a positive feedback to rising atmospheric CO_2.

INTRODUCTION

The ocean plays a dominant role in the climate system through storage and transport of heat (Barnett *et al.* 2005) and by mitigating global warming through the uptake and sequestration of anthropogenic carbon dioxide (CO_2) (Sabine *et al.* 2004). Over the past 40 years, approximately 84% of the increase in the earth's heat budget have been absorbed by the surface oceans (Levitus *et al.* 2005), thereby increasing the average temperature of the upper 700 m by 0.1°C (Bindoff *et al.* 2007). This process is likely to accelerate in the next decades with a predicted increase in global mean surface temperature between 1.1°C (low CO_2 emission scenario B1) and 6.4°C (high CO_2 emission scenario A1FI) until the end of the 21st century (Meehl *et al.* 2007).

Sea surface warming will affect the pelagic ecosystem in two ways: directly through its effect on the rates of biological processes, and indirectly through decreased surface layer mixing, causing decreased nutrient supply and increased light availability for photosynthetic organisms suspended in the upper mixed layer. It is expected that these changes in the physical and chemical environment will have drastic effects on the marine biota. The sensitivity of biological processes to temperature is commonly described by the Q_{10} factor – the factorial increase in the process rate for a 10°C increase in temperature. While phytoplankton growth and photosynthesis show only a moderate temperature-response ($1<Q_{10}<2$), and are primarily controlled by incident light intensity and nutrient availability (Eppley 1972), bacterial heterotrophic activities typically have a Q_{10} factor between 2 and 3 (Pomeroy and Wiebe 2001). Moreover, bacterial growth efficiency is an inverse function of temperature, causing an increased fraction of the assimilated carbon to be respired with rising temperature (Rivkin and Legendre 2001). According to these findings, surface ocean warming is expected to shift the balance between autotrophic production and heterotrophic consumption of organic matter toward enhanced recycling and respiration. Ultimately, such alterations in the interplay of key processes involved in ocean carbon cycling may affect both pelagic food web structures and the functioning of the biological carbon pump, which transports surface-bound organic carbon to the deep ocean and hence contributes to the ocean's capacity to take up atmospheric CO_2.

To investigate the impact of rising sea surface temperature on the cycling and fate of organic carbon during a phytoplankton spring bloom, eight mesocosms with a volume of 1400 L each were set up in four temperature-controlled climate chambers. The mesocosms were simultaneously filled with unfiltered seawater from Kiel Bight (Baltic Sea) containing a natural winter/spring plankton community. The four climate chambers, each containing two replicate mesocosms, were adjusted to the following

temperatures: *in situ* (T+0), following the natural seasonal temperature regime observed in Kiel Bight, *in situ* +2°C (T+2), *in situ* +4°C (T+4) and *in situ* +6°C (T+6). In each of the mesocosms the build-up and decline of the spring bloom was closely monitored over 30 days.

MATERIALS AND METHODS

Experimental set-up.

The indoor-mesocosm study was conducted between 6th January and 5th February 2006 at the IFM-GEOMAR, Leibniz Institute of Marine Sciences (at Kiel University), Germany. A detailed description of the experimental set-up is given in *Chapter 1*. In brief, eight mesocosms with a volume of 1400 L were placed in four temperature-controlled climate chambers. Unfiltered seawater from Kiel Fjord (western Baltic Sea), containing a natural winter/spring plankton community, was filled into the mesocosms and exposed to four different temperatures. The lowest temperature of approximately 2.5°C (*in situ* treatment T+0) corresponded to a 10-year-mean (1993-2002) for Kiel Bight. The elevated temperature regimes were 2°C (T+2), 4°C (T+4), and 6°C (T+6) higher than the *in situ* temperature and corresponded to the projected increase in surface temperature of 4-10°C during winter in the Baltic Sea region until the year 2100 as reported by the IPCC (Giorgi *et al.* 2001). Mesozooplankton was added in natural over-wintering densities of approx. 10 individuals L^{-1}. Light was supplied by a computer-controlled illumination system containing full-spectrum light tubes. A diurnal triangular light curve with an overall 12h:12h light-dark cycle was applied. Dissolved inorganic nutrients were initially available in concentrations of 0.9 µmol L^{-1} phosphate (PO_4^{3-}), 21 µmol L^{-1} nitrate (NO_3^-), 5.6 µmol L^{-1} ammonium (NH_4^+), and 20.4 µmol L^{-1} silicate ($Si(OH)_4$), respectively. Throughout the study, the water body was gently mixed by means of a propeller attached to the side of the mesocosm. Samples were taken daily from intermediate depth with a silicon hose. The development of the phytoplankton blooms was monitored over a period of 30 days.

Measurements.

Temperature, salinity, pH, and maximum light intensity at midday were monitored 3- 5x per week using a WTW conductivity/pH probe and a submersible 4π-PAR sensor (LiCOR Inc., USA), respectively. Samples for dissolved inorganic nitrate, nitrite, phosphate, and silicate were pre-filtered through 5 µm cellulose acetate filters and measured with an autoanalyzer (AA II) (Hansen and Koroleff 1999). Ammonium was determined from unfiltered water samples (Holmes *et al.* 1999). Dissolved inorganic carbon was measured on sterile-filtered samples via coulometric titration (Johnson *et al.*

1987). Total alkalinity was determined from $HgCl_2$-poisoned samples using the Gran electrotitration method (Gran 1952). Samples for the analysis of chlorophyll a were filtered onto glass fibre filters (GF/F, Whatman) and stored at -20°C. Pigments were extracted in 90% acetone and measured on a 10-AU Turner fluorometer (Welschmeyer 1994). For the determination of particulate organic carbon and nitrogen, samples were filtered onto pre-combusted (5 h, 450°C) GF/F-filters and stored at -20°C until analysis. Filters were dried for 6 h at 60°C and analyzed on a Eurovector EuroEA-3000 elemental analyzer (Sharp 1974). Samples for dissolved organic carbon were filtered through pre-combusted GF/F-filters. The filtrate was collected in pre-combusted (12 h, 450°C) glass vials and stored at -20°C. The analysis was carried out on a Shimadzu TOC_{VCN} using the HTCO method (Qian and Mopper 1996). Bacterial secondary production (BSP) was assessed by measuring the incorporation of ^{3}H-leucine (^{3}H-leu; indicator for protein synthesis) and ^{3}H-methyl-thymidine (^{3}H-thy; indicator for DNA synthesis) (Fuhrman and Azam 1982; Simon and Azam 1989). ^{3}H-leu (specific activity: 160 µCi nmol^{-1}) and ^{3}H-thy (specific activity: 63 µCi nmol^{-1}) were added to the samples at final concentrations of 103 nmol L^{-1} and 8 nmol L^{-1}, respectively. The samples were dark-incubated at *in situ* temperature (i.e. in the respective climate chambers) for 1.5 - 3 h. Incubation was terminated by adding formaldehyde (1% v/v). In order to distinguish between total and particle-associated carbon production, 5 ml of sample were filtered onto 0.2 µm and 3 µm polycarbonate membrane filters (Poretics®), respectively. The filters were subsequently rinsed with ice cold 5% TCA solution before being radio-assayed in 4 ml of scintillation cocktail (Lumagel Plus) on a Packard TriCarb scintillation counter. All incubations were carried out in triplicate. A formalin-killed sample was used to correct for background absorption of radioactivity. To convert the incorporation of ^{3}H-leu and ^{3}H-thy into carbon production (µg C L^{-1} h^{-1}), a theoretical conversion factor of 3.1 kg C mol^{-1} leucine (Simon and Azam 1989) and an empirically determined conversion factor of 30.87 kg C mol^{-1} thymidine (P. Breithaupt, *pers. comm.*), respectively, were used. The daily carbon production rates (µmol C L^{-1} d^{-1}) were then calculated, assuming that bacterial production rates were constant throughout the day. Community respiration (CR) was determined using the Winkler titration method with automated photometrical end-point detection. In order to distinguish between total community respiration and bacterial respiration (CR < 3 µm), one half of the samples was gently filtered (< 200 mbar) through 3 µm polycarbonate filters prior to the incubation. Samples were dark-incubated at *in situ* temperature for 48 h. All incubations were carried out in triplicate. To calculate respiratory C-utilisation (µg C L^{-1} h^{-1}), respiration in terms of O_2 uptake (mg L^{-1} h^{-1}) was multiplied with a factor of 0.32 as determined for marine substrates (Gocke and

Hoppe 1977). Daily respiration rates (µmol C L^{-1} d^{-1}) were then calculated, assuming that respiration rates remained constant throughout the day.

Calculations.

Air-sea exchange of CO_2: DIC concentrations were corrected for CO_2 gas exchange between mesocosm water and atmosphere following the approach described in Delille *et al.* (2005), including a chemical enhancement factor (Kuss and Schneider 2004).

Net consumption of DIC (∆DIC), and net build-up of POC (∆POC) and DOC (∆DOC): For each mesocosm, the first two days of sampling were set as the baseline for all calculations, since they were not significantly affected by any biological activities. For the determination of ΔDIC, each data point was subtracted from this baseline value. In the case of ΔPOC and ΔDOC, the baseline value was subtracted from the respective data point to yield the net organic carbon production.

Maximum net consumption of DIC (ΔDIC_{max}): ΔDIC_{max} was calculated from the difference between the initial (DIC_i) and the minimum DIC concentration (DIC_{min}): $\Delta DIC_{max} = DIC_i - DIC_{min}$. DIC_{min} was calculated as the mean of DIC concentrations measured after the period of exponential bloom development. For this, the natural logarithm (ln) of DIC measurements was plotted against time. During the period of exponential phytoplankton growth ln [DIC] scaled linearly with time. The first data point deviating from the linear regression was the first to be included in the calculation of DIC_{min}. In the T+0 and T+2 treatments [DIC] stabilised thereafter. Hence in these cases, all DIC measurements after the period of exponential growth were included in the calculation of DIC_{min}. In the T+4 and T+6 treatments [DIC] started to increase again due to respiratory processes after a short stagnant period. Here, DIC_{min} was calculated from measurements obtained after exponential growth and before the subsequent increase in [DIC]. Data points used for calculating DIC_{min} are marked by a solid black line in Figs. 2A, B.

Net organic carbon loss (ΔC_{loss}): For reasons of comparability, ΔC_{loss} was calculated for each mesocosm at the time of maximum biomass. It is defined as the difference between ΔDIC_{max} and the maximum net build-up of total organic carbon (ΔTOC_{max}) with $\Delta TOC = \Delta POC + \Delta DOC$. ΔTOC_{max} was calculated as the average of three consecutive days (the day with the highest biomass concentration as well as one day before and after).

Rates of BSP and CR at the time of maximum biomass represent mean values of measurements obtained right before and after the biomass peak (i.e. 1-3 days).

Daily photon flux: Based on the average maximum light intensity of 179.6 µmol photons m^{-2} s^{-1} at the surface of the mesocosms at midday and following the equation $I_{mix} = I_0(1 -$

$e^{-kz})(kz)^{-1}$ (Riley 1957) with I_0 being the incident light intensity at the surface, a typical winter water light attenuation coefficient k = 0.25 m^{-1} (Sommer and Lengfellner 2008), and a water depth of z = 1 m, a mean daily photon flux of 1.93 mol photons m^{-2} d^{-1} was calculated.

RESULTS AND DISCUSSION

The development of a diatom-dominated (in particular *Skeletonema costatum*) bloom was marked by an increase in chlorophyll a (Chl a) concentration (Fig. 1A), a rapid decline in dissolved inorganic nutrients (as shown for phosphate concentration, Fig. 1B), and the drawdown of dissolved inorganic carbon (DIC) caused by photosynthetic production of organic matter (Fig. 1C).

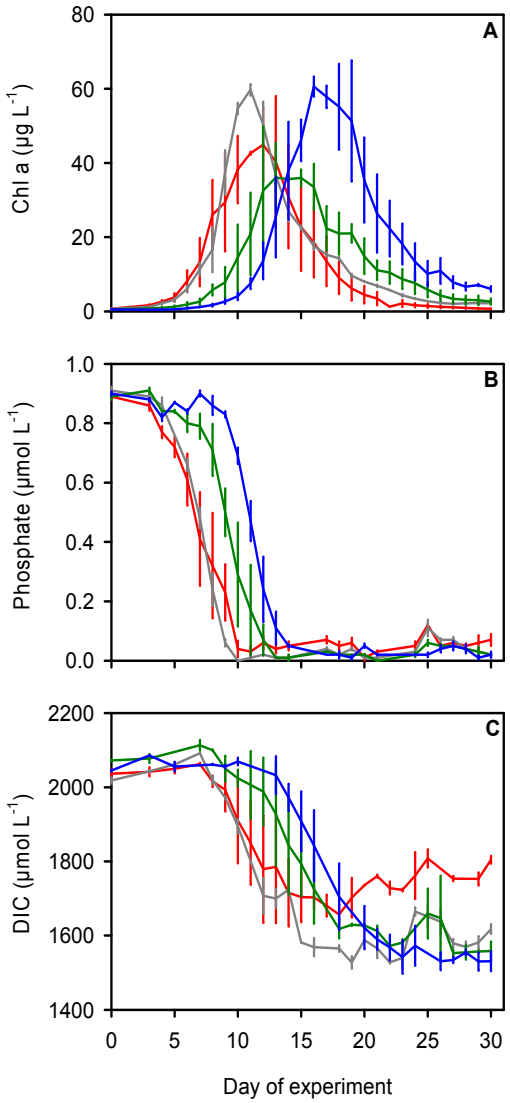

Figure 1. Temporal bloom development. Concentrations of **(A)** Chlorophyll a (Chl a), **(B)** dissolved inorganic phosphate (PO_4^{3-}) and **(C)** dissolved inorganic carbon (DIC). The different colors represent the four temperature regimes: *in situ* temperature T+0 (blue) and elevated temperatures T+2 (green), T+4 (grey), and T+6 (red). Solid lines denote the average of two replicate mesocosms, error bars indicate the range of the replicates.

All parameters showed an earlier onset of the bloom at elevated temperatures with an acceleration of approximately 1 day/°C. While maximum Chl a concentration and nutrient consumption did not differ significantly between treatments, a distinct effect of temperature on the uptake of DIC was observed (Fig. 2). Here, elevated temperatures led to a significant decline in the maximum net consumption of DIC (ΔDIC_{max}) (Fig. 2C, p = 0.014) (see *Materials and Methods* for details on calculations), yielding an average difference of 153 µmol C L^{-1} between the *in situ* treatment (T+0) and the warmest treatment (T+6).

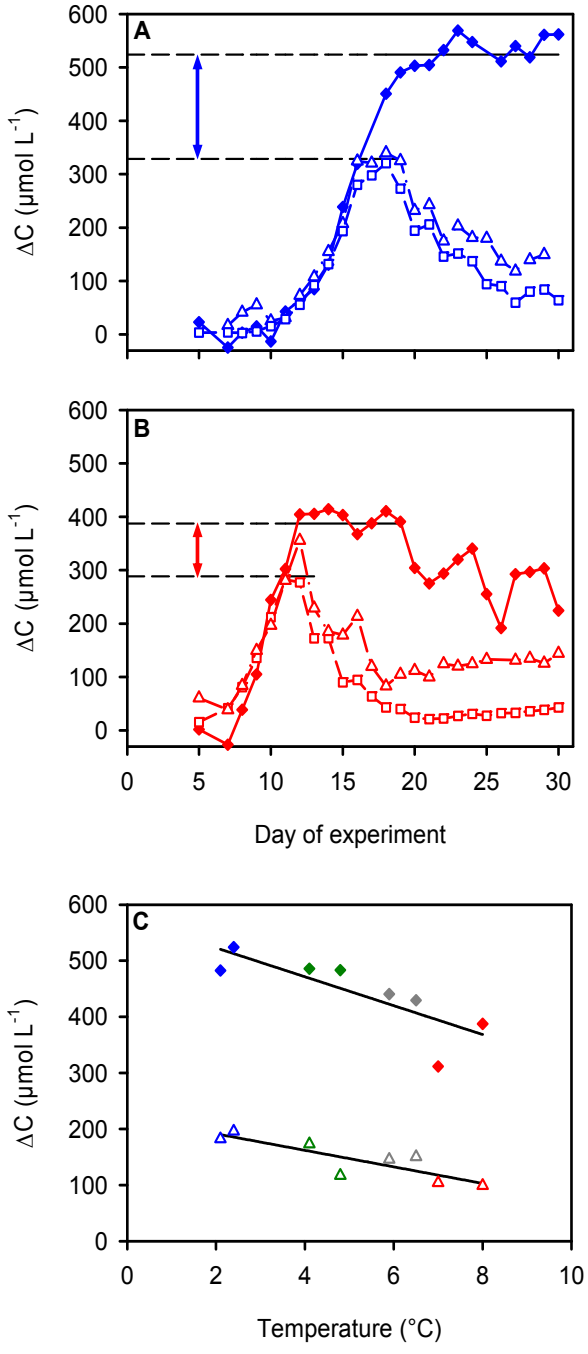

Figure 2. Temperature-sensitivity of inorganic carbon consumption, organic carbon build-up and loss. **(A, B)** Temporal development of the net drawdown of dissolved inorganic carbon (ΔDIC, full diamonds/solid line), the net build-up of particulate organic carbon (ΔPOC, open squares/dashed line), and the net build-up of total organic carbon (ΔTOC = ΔPOC + ΔDOC, open triangles/dashed-dotted line) at *in situ* temperature T+0 (**A**, blue) and at elevated temperature T+6 (**B**, red) (data from one replicate mesocosm of each treatment). The two horizontal lines in each panel indicate the magnitude of the maximum net consumption of DIC (ΔDIC_{max}, upper line) and the maximum net build-up of total organic carbon (ΔTOC_{max}, lower line), the arrow indicates the magnitude of the net loss of organic carbon from the water column (ΔC_{loss}) at the time of maximum organic carbon accumulation. **(C)** ΔDIC_{max} (full diamonds) and ΔC_{loss} (open triangles) as a function of temperature. Color code as in Fig. 1. Solid lines denote linear regressions (n = 8; ΔDIC_{max}: R^2 = 0.66, p = 0.014; ΔC_{loss}: R^2 = 0.74, p = 0.006).

The photosynthetic uptake of DIC was accompanied by a rapid build-up of particulate organic carbon (POC) and a gradual accumulation of dissolved organic carbon (DOC) (see Chapter 1, Fig. 3A, 4A). In contrast to the observed temperature effect on DIC drawdown, the maximum net build-up of both POC (ΔPOC; Fig. 2A, B) and suspended total organic carbon (ΔTOC = ΔPOC + ΔDOC; Fig. 2A, B) was not markedly affected by changes in temperature. Yet, immediately after the biomass peak an enhanced accumulation of DOC occurred at elevated temperatures, indicating a pronounced shift in the partitioning of organic matter between the dissolved and particulate pool.

In all treatments, the photosynthetic drawdown of DIC exceeded the build-up of suspended organic carbon, revealing the presence of an additional carbon sink. This loss of organic carbon (ΔC_{loss}) from the water column, calculated as the difference between ΔDIC_{max} and the maximum net build-up of total organic carbon ($\Delta C_{loss} = \Delta DIC_{max} - \Delta TOC_{max}$), showed a significant decline with increasing temperature (Fig. 2C, $p = 0.006$), resulting in an up to 46% lower loss at T+6 compared to the *in situ* temperature. In principle, three processes may account for the loss of carbon from the water column: (i) CO_2 outgassing, (ii) organic carbon fixation through algal growth on mesocosm walls, and (iii) sinking of organic matter to the bottom of the mesocosms. As photosynthetic carbon uptake caused a decline in mesocosm pCO_2 below the atmospheric level of 380 ppm, resulting in a net flux of CO_2 into the mesocosm water, outgassing can be excluded as a loss term. Moreover, DIC concentrations were corrected for the air-water exchange of CO_2 (see also *Materials and Methods* for details on calculations). Based on repeated inspections of the mesocosm walls, carbon loss through fouling was also found to be negligible. In contrast, accumulation of particulate matter at the bottom of the enclosures was prominent in all treatments. Although attempts to obtain quantitative measurements of the amount of particulate matter accumulating at the bottom of the enclosures were impeded by its highly patchy distribution, a crude extrapolation of POC measured in individual patches confirmed that bottom accumulation represented the predominant loss term for carbon from the water column. A similar temperature effect on the sinking of particulate matter was also observed in other mesocosm studies, where the amount of organic carbon lost via sedimentation decreased with increasing temperature (Keller *et al.* 1999; Müren *et al.* 2005).

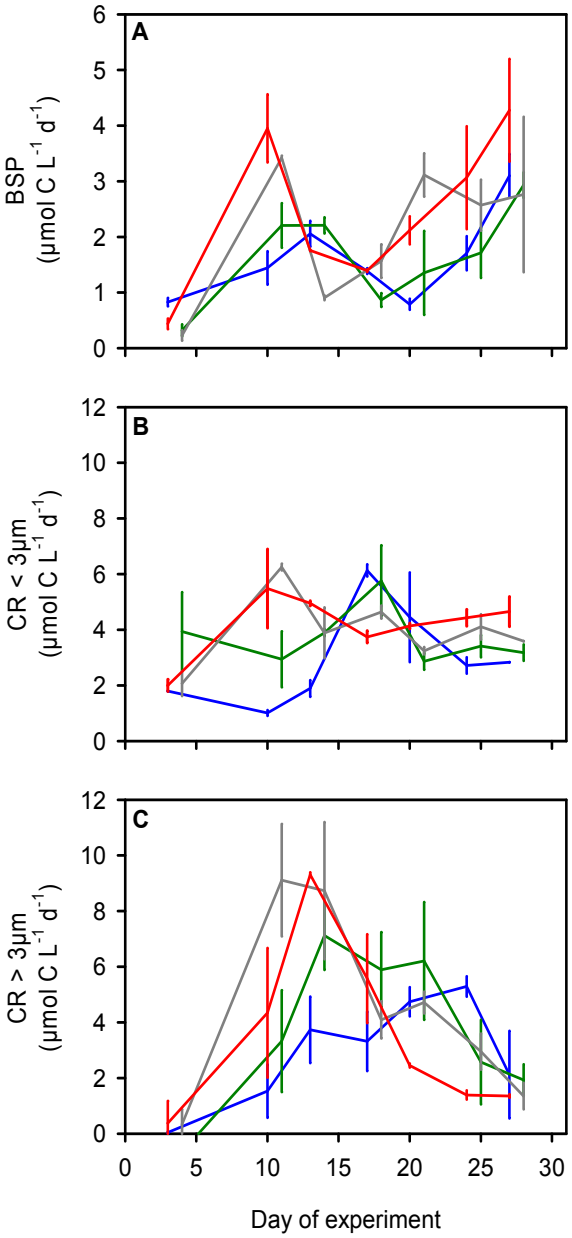

Figure 3. Temporal development of bacterial secondary production and community respiration. (A) bacterial secondary production (BSP) rates of free-living bacteria (<3 µm) as calculated from ^{3}H-leucine uptake; **(B, C)** community respiration rates in the bacterial size fraction (CR <3 µm) and in the non-bacterial size fraction (CR >3 µm). Color and symbol code as in Fig. S1. Data kindly provided by P. Breithaupt.

The combination of the observed responses to rising temperature, namely the decrease in both net inorganic carbon consumption (ΔDIC_{max}) and net loss of organic carbon from the water column (ΔC_{loss}), poses questions regarding the underlying mechanism(s). Although the decline in ΔDIC_{max} with rising temperature could have resulted from a reduction in photosynthetic carbon fixation, this is neither supported by ^{14}C primary production measurements (see also Chapter 1, Fig. 2B, C) nor by data on phytoplankton biomass, both in terms of cell counts (data not shown) and Chl a concentrations (Fig. 1A). Alternatively, this inverse relationship of ΔDIC_{max} with temperature may have been caused by enhanced heterotrophic recycling and respiration of primary produced organic matter, thereby replenishing the DIC pool and reducing the availability of organic

carbon for export processes. This notion, in fact, is supported by data on bacterial secondary production (BSP), showing an earlier increase in and higher maxima of production rates at elevated temperatures (Fig. 3A). Thus, at the time of maximum organic carbon accumulation BSP rates of free-living bacteria significantly increased by a factor of 2 to 3 between the *in situ* temperature and T+6, both in terms of protein production (^{3}H-leucine incorporation) and cell production (^{3}H-thymidine incorporation) (Fig. 4A, BSP_{leu}: p = 0.012; BSP_{thy}: p = 0.003). This pattern was driven by an increase in the abundance of bacterial cells as well as in cell-specific BSP rates with, for instance, BSP_{thy} values rising from 0.4 ± 0.01 pmol C cell^{-1} d^{-1} at *in situ* temperature to 0.99 ± 0.19 pmol C cell^{-1} d^{-1} in the T+6 treatments. Compared to the activities of free-living bacteria at the time of maximum biomass accumulation (e.g. BSP_{thy}: 0.4-2.5 µmol C L^{-1} d^{-1}, see Fig. 4A), particle-associated bacteria were only of minor importance for carbon turnover as their BSP rates were markedly lower (e.g. BSP_{thy}: 0.08-0.17 µmol C L^{-1} d^{-1}).

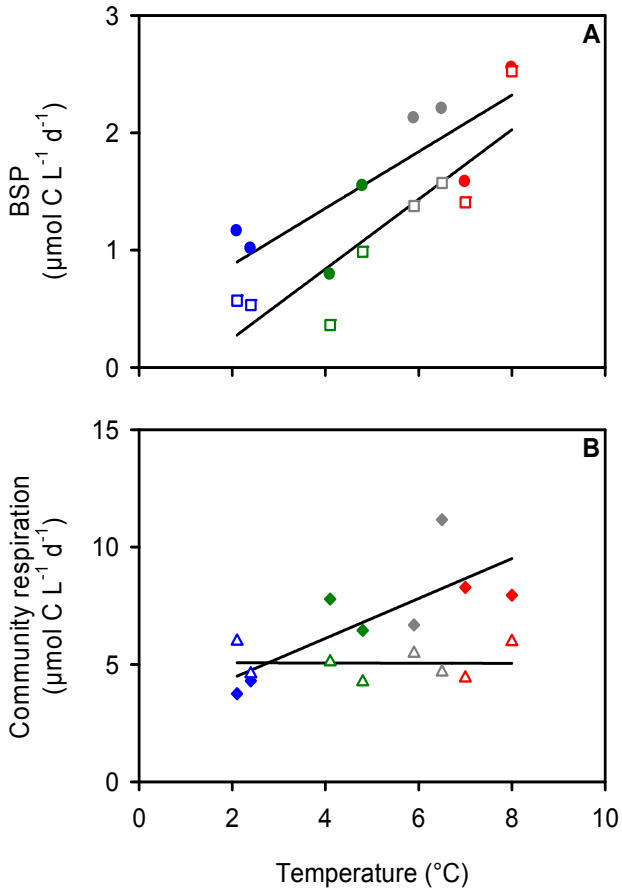

Figure 4. Temperature-dependency of bacterial secondary production (BSP) and community respiration (CR) rates at the time of maximum organic carbon accumulation. (A) BSP of free-living bacteria (< 3 µm), as measured by ^{3}H-leucine (BSP_{leu}, full circles) and ^{3}H-thymidine incorporation (BSP_{thy}, open squares); **(B)** CR in the bacterial (CR < 3 µm, open triangles) and non-bacterial (CR > 3 µm, full diamonds) size fractions. Color code as in Fig. 1. Solid lines denote linear regressions (n = 8; BSP_{leu}: R^2 = 0.68, p = 0.012; BSP_{thy}: R^2 = 0.79, p = 0.003; CR < 3µm: R^2 = 0.0002, p = 0.98; CR > 3µm: R^2 = 0.60, p = 0.025). Data kindly provided by P. Breithaupt.

Size-fractionated respiration measurements indicate that the bacterially-consumed organic carbon was not immediately respired, because community respiration in the <3µm fraction remained low and apparently unaffected by temperature (Figs. 3B, 4B, $p = 0.98$). Instead, it is the respiration in the fraction >3µm that responded positively to warming (Fig. 3C). A comparison of respiration rates in this larger size fraction at the time of maximum organic carbon accumulation revealed a significant, two-fold increase in daily organic carbon consumption with rising temperature (Fig. 4B, $p = 0.025$), ranging from 4.0 ± 0.3 µmol C L^{-1} d^{-1} at *in situ* temperature to 8.1 ± 0.2 µmol C L^{-1} d^{-1} at T+6. Most likely, this signal results both from enhanced transfer of bacterial carbon to higher trophic levels (e.g. through protist grazing) and increased algal respiration at elevated temperatures.

The pronounced response of respiratory processes to rising temperature also became apparent in the post-bloom phase. Here, DIC concentrations started to increase again in the T+6 treatments by on average 115 ± 31 µmol C L^{-1} (Fig. 2B), whereas they remained unaffected at *in situ* temperature until the end of the experiment (Fig. 2A). Because it cannot be ruled out that degradation of organic matter accumulating at the bottom of the mesocosms during the post-bloom phase contributed to the respiratory signal in the water column, this phase was not considered in any of the calculations given above.

Another process that showed a response to elevated temperatures was the aggregation of organic matter. The concentration of transparent exopolymer particles (TEP) increased considerably in the warmest treatment T+6 and to a lesser extent also in the T+4 treatment during the post-bloom phase of the experiment, whereas it remained low at T+2 and T+0 (see also *Chapter 1, Fig. 9*). Elevated temperature also stimulated TEP production (Claquin *et al.* 2008) and aggregation of organic matter (Thornton and Thake 1998) in incubations of monoalgal cultures. The potential of TEP to promote aggregation and sinking of particulate matter has been shown in both mesocosm (Passow and Alldredge 1994) and field studies (Engel 2004). The extent to which enhanced TEP formation could affect particle sinking in a warming ocean critically depends on the timing of TEP production and on the interplay with other biological processes, e.g. microbial degradation and grazing. In our experiment, particulate matter concentrations had decreased to nearly pre-bloom levels when TEP concentrations increased, hence limiting the potential for TEP-mediated particle export.

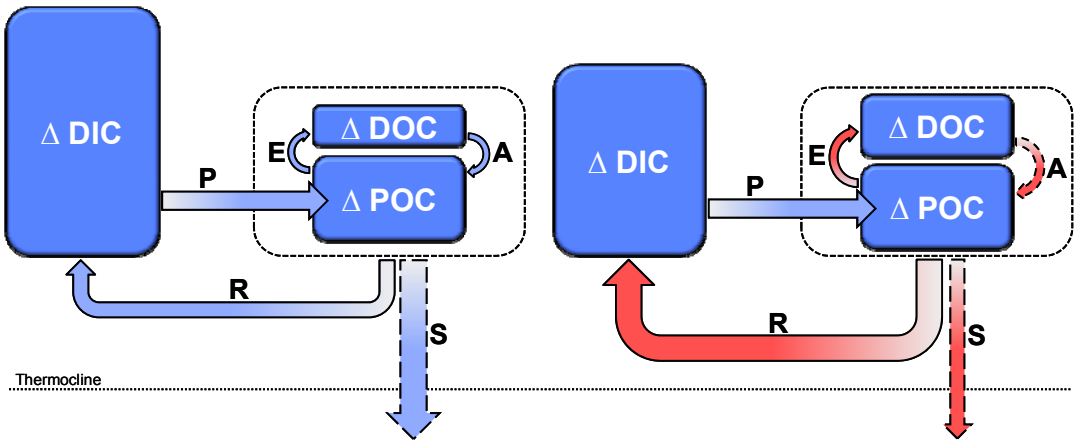

Figure 5. A schematic illustrating the surface layer carbon flow during an algal spring bloom under present (left) and elevated (right) sea surface temperature. Boxes (ΔDIC, ΔPOC, ΔDOC) represent the change in the respective reservoir over time, arrows depict processes (P = primary production, E = exudation, R = respiration, S = sinking, A = aggregation). Processes found to be sensitive to warming in this study are marked in red in the right panel. According to our findings, rising sea surface temperature leads to enhanced respiratory consumption of organic carbon relative to autotrophic production, occurring already during the build-up of the spring bloom. This results in a decline in net DIC consumption (ΔDIC) and a lowered availability of organic carbon for export processes. Additionally, the partitioning of organic carbon between the dissolved and particulate pools is affected, with enhanced accumulation of dissolved organic matter. Together, these changes in biogenic carbon flow have the potential to alter the efficiency of the biological carbon pump.

Combining the observed temperature-sensitivities in a chart of processes driving surface layer carbon flow illustrates one piece of the complex puzzle of biological responses to ocean warming (Fig. 5). In line with theoretical considerations, our study provides evidence for a temperature-induced shift in the balance between autotrophic production and heterotrophic consumption of organic matter. At elevated temperatures enhanced respiratory loss and recycling of organic carbon become effective already during the build-up of the phytoplankton bloom. As a result, for the same amount of nutrient drawdown, the net DIC consumption decreases significantly with rising temperature. In addition, the proportion of primary produced organic matter channelled into DOC, most notably after nutrient exhaustion, strongly increases with rising temperature. Apparently, the enhanced bacterial activity at elevated temperatures could not prevent this increase in DOC accumulation, suggesting that DOM degradation may have been limited by nutrient availability (Thingstad *et al.* 1997). On the other hand, it has been reported that

the bacterial break-down of organic matter through the action of exoenzymes can lead to the production and release of more refractory dissolved organic compounds (Azam 1998). Thus, a stronger bacterial activity may, in fact, even contribute to the accumulation of DOC.

In concert, the observed temperature effects, in particular the enhanced rate of POC degradation and alterations in the partitioning of organic carbon, reflect a shift in food web structure increasing the flow of organic matter through the microbial loop (Fig. 5). This shift has the potential to reduce both the transfer of organic matter and energy to higher trophic levels and the efficiency of the biological carbon pump in sequestering carbon from the surface ocean to depth - with far-reaching implications for the sustainability of global fisheries and the ocean's mitigating effect on anthropogenic climate change.

In a changing future ocean, direct effects of rising sea surface temperature, as observed here, will interact with indirect effects via increased surface layer stratification, leading to higher light availability and reduced nutrient supply. The interplay of these effects is likely to exert the strongest impact in temperate and high latitude regions. These are characterized by a seasonally high availability of nutrients due to deep winter-mixing and include some of the most productive ocean areas. Spring blooms, as investigated in this study, are a dominant feature in the seasonal cycle of these systems, caused by a temporal decoupling of autotrophic and heterotrophic processes due to a stronger temperature limitation of the latter. The fate of the organic matter accumulating during these events is largely controlled by zooplankton grazing and export below the winter-mixed layer. In a warming ocean, the interplay of reduced nutrient supply with shifts in food web structure and a more rapid cycling of organic matter and nutrients through the microbial loop, as reminiscent of the oligotrophic ocean provinces at lower latitudes, will likely reduce both the transfer to higher trophic levels and the export potential in these areas. Our findings are consistent with model results (Laws *et al.* 2000) suggesting that especially the productive parts of the ocean may be sensitive to sea surface warming in terms of organic matter export. The model shows that in areas of moderate to high productivity the ratio of export production to total primary production (i.e. the ef-ratio) is mainly controlled by and negatively correlated with temperature. At temperatures below 20°C a steep transition from high to low ef-ratios occurs as the productivity of the system declines. Thus, the interaction of direct and indirect effects of rising sea surface temperature may shift mid- to high-latitude ocean areas from high to low ef-modes and significantly reduce global carbon export.

In addition to sea surface warming, CO_2-induced ocean acidification and its effects on planktonic calcification (Bijma *et al.* 1999; Orr *et al.* 2005; Riebesell *et al.* 2000) and primary production (e.g. Riebesell *et al.* 2007) will further complicate the picture. Together, these changes in the physical and chemical environment may, aside from their impact on biogeochemical cycling, also modify pelagic ecosystem functioning, for instance through shifts in the abundance and biogeographical distribution of key species or in the phenological coupling of different trophic levels ('match-mismatch') (e.g. Beaugrand *et al.* 2003; Beaugrand *et al.* 2002; Edwards and Richardson 2004; Paerl and Huisman 2008; Perry *et al.* 2005; Pörtner and Knust 2007). Surface-ocean warming is therefore bound to be one of the most powerful drivers for future changes in ocean productivity, biogeochemical cycling, and air-sea CO_2 exchange. In order to project the impacts of these changes on marine ecosystem services and the climate system it is imperative that we gain a quantitative understanding of the underlying processes. These are presently not well constrained, partly due to the lack of data providing an integrated representation of upper-ocean, biotically-driven processes, and partly because of the lack of realistic biological simulations in Atmosphere Ocean General Circulation (AOGC) modelling. Understanding the full suite of interrelated responses and predicting their impacts on ecosystem dynamics, biogeochemical cycling, and feedbacks to the climate system requires a multidisciplinary effort of seagoing and experimental marine scientists in concert with modellers covering the range from ecosystem to AOGC modelling.

CHAPTER 3

INTERACTIVE EFFECTS OF RISING TEMPERATURE AND NUTRIENT AVAILABILITY ON ORGANIC MATTER BUILD-UP, COMPOSITION AND DEGRADABILITY

Interactive effects of rising temperature and nutrient availability on organic matter build-up, composition, and degradability

ABSTRACT

A microcosm experiment was conducted to investigate the interactive effects of rising sea surface temperature and altered nutrient stoichiometry, caused by anthropogenic climate change, on the biogeochemical cycling of organic matter in a mixed algal-bacterial community. Natural seawater, containing a bacterial community, was inoculated with an axenic culture of the diatom species *Skeletonema costatum*. A factorial design of three temperatures, simulating medium to strong warming as projected for the end of the 21st century, and either nitrogen- or phosphorus-limited growth conditions was applied. Depending on the type of nutrient limitation, the mixed algal-bacterial communities displayed pronounced differences in the build-up and microbial utilisation of organic matter in response to warming. Under N-deficient conditions the build-up of particulate organic matter dominated, and only little dissolved material accumulated. The subsequent bacterial consumption of organic matter was low, as indicated by measurements of bacterial secondary production and extracellular enzyme activities, and remained largely unaffected by rising temperature. In contrast, warming under P-deficient growth conditions caused a pronounced shift in the partitioning of organic matter between the particulate and dissolved pools towards the latter. Moreover, also the bacterial activity parameters were notably stimulated by warming, indicating an enhanced flow of organic matter through the microbial loop. In concert, these findings suggest strong shifts in the biogeochemical cycling of organic matter in the surface ocean in response to the projected changes in surface temperature and nutrient loading. This will likely affect pelagic food web structures and the biological sequestration of organic matter from surface to depth.

INTRODUCTION

Human activities, such as burning of fossil fuels and changes in land-use practices, are causing major perturbations in the ocean's chemical and physical properties through increased warming and progressive acidification of the upper water layers (Barnett *et al.* 2005; Caldeira and Wickett 2003; Levitus *et al.* 2005; Sabine *et al.* 2004; see also *General Introduction*). These changes in the marine environment are expected to further intensify in the future with, for instance, a projected increase in global surface temperature of 1 to 6°C until the end of the 21st century (Meehl *et al.* 2007).

The biogeochemical cycling and fate of organic matter in the pelagic surface ocean is largely determined by the interplay between (i) the autotrophic production of organic matter by phytoplankton cells, (ii) its consumption and respiration by heterotrophic organisms, and (iii) the export of organic matter to the ocean interior (Rivkin and Legendre 2001). In a recent mesocosm study, enhanced sea surface warming has been shown to directly affect the balance between these processes due to large discrepancies in their respective temperature-sensitivities, with likely consequences for food web structure and the efficiency of biogenic carbon sequestration to depth via the biological carbon pump (Wohlers *et al.* 2009; see also *Chapters 1* & *2*). Thus, rising sea surface temperature led to an enhanced and accelerated heterotrophic consumption of organic carbon relative to autotrophic primary production and a pronounced increase in the accumulation of dissolved carbon-rich compounds. These changes in biogenic carbon flow ultimately reduced the net community drawdown of CO_2 and lowered the availability of organic carbon for export.

In addition to sea surface warming, fossil fuel burning and the extensive use of synthetic fertilisers have caused severe anthropogenic perturbations of the major element cycles in the ocean through increased inputs of allochthonous nutrients (i.e. nitrogen (N) and phosphorus (P)) via river runoff and/or atmospheric dust deposition along the coasts (Falkowski *et al.* 2000; Jickells 1998). This is expected to be further enhanced in the future due to projected changes in global precipitation patterns (Trenberth *et al.* 2007). However, human activities do not only increase the magnitude of riverine and atmospheric inputs, but also perturb the relative abundance of the various nutrients through coastal water management practices, currently resulting in rising N:P ratios (Jickells 1998 and references therein). While primary productivity is generally considered to be limited by nitrogen throughout most of the ocean (Falkowski 1997; Tyrrell 1999), several studies have suggested a (seasonal) P-limitation in certain areas, e.g. strongly freshwater-influenced coastal zones or marginal seas like the Baltic Sea (Zweifel *et al.* 1993), the subtropical gyres in the North Pacific (Karl et al. 2001) and Atlantic Ocean (Ammerman *et al.* 2003), as well as the Mediterranean Sea (Thingstad *et*

al. 2005). Hence, the reported rise in N- compared to P-loading will likely further enhance the potential for P-limitation in such areas.

A number of studies have reported a pronounced effect of the type of nutrient limitation, i.e. phosphorus- versus nitrogen-limited and nutrient replete growth, on the magnitude, composition, and microbial utilisation of organic matter during algal blooms (Guerrini *et al.* 1998; Magaletti *et al.* 2004; Myklestad 1977; Obernosterer and Herndl 1995; Puddu *et al.* 2003; Urbani *et al.* 2005). Here, phosphorus-limited growth conditions led to a distinctly higher algal release of dissolved organic carbon (DOC), in particular of carbohydrates, compared to nitrogen-limited and/or nutrient-replete conditions. However, severe P-deficiency (N:P = 100-145, (Obernosterer and Herndl 1995; Puddu *et al.* 2003) also markedly reduced the efficiency of the associated bacterial communities to utilise the released material, hence causing a pronounced accumulation of dissolved organic carbon in the water column.

Thus, both sea surface warming and the availability of inorganic nutrients have been shown separately to significantly affect the autotrophic build-up and subsequent heterotrophic consumption of organic matter in the surface ocean. In this study, we aim to further elucidate the combined impact of such anthropogenic perturbations on the build-up, composition and degradability of organic matter in a mixed algal-bacterial spring community, and the associated implications for pelagic ecosystem functioning and the biological sequestration of organic matter to the deep sea.

MATERIAL AND METHODS

Experimental design and sampling procedure.

The microcosm experiment was conducted from 5th july to 13th august at the IFM-GEOMAR Leibniz Institute of Marine Sciences (at Kiel University), Germany. These microcosms consisted of acid-cleaned and autoclaved 25 L polycarbonate bottles (Nalgene, USA). Eighteen bottles in total were equally distributed onto three temperature-controlled climate chambers (Fig. 1). The temperature of the chambers was adjusted to 4, 8, and 12 °C, respectively, simulating weak to strong warming as projected for the winter season in the Baltic Sea region until the end of the 21st century (4-10°C; Giorgi *et al.* 2001). The decadal-average (1993-2002) sea surface temperature in the investigation area Kiel Bight during spring is approximately 2.5°C. Prior to the experiment, natural seawater was collected in late spring at the Booknis Eck station in Kiel Bight at a depth of approx. 10 m and allowed to age in the dark for several weeks. For use in the experiment, the water was pre-filtered through pre-combusted (450 °C, 5 h) GF/F- and 0.45 µm cellulose acetate filters (Sartorius) in order to retain only the natural bacterial community. The bottles were then each filled with 24 L of filtered water

and inoculated with an axenic culture of the diatom species *Skeletonema costatum* (CCMP 1332), yielding an initial algal density of approx. 1000 cells ml^{-1}. *Skeletonema costatum* is one of the dominant algal species forming the yearly phytoplankton spring bloom in Kiel Fjord, and has been the most abundant species in a previous mesocosm experiment (see *Chapter 1*).

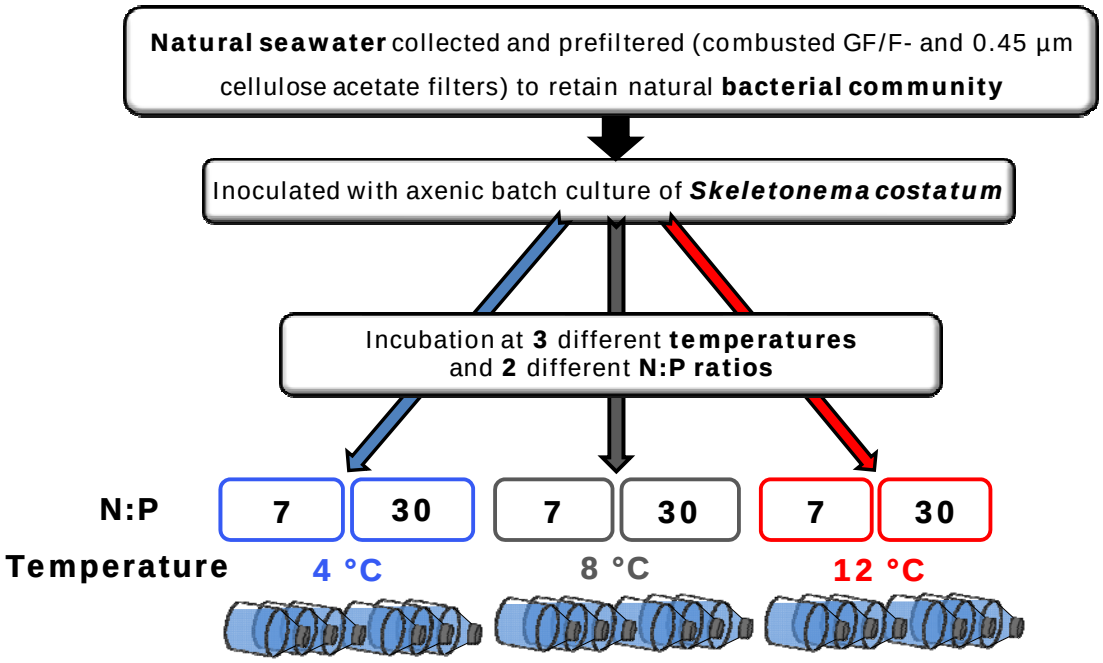

Figure 1. Schematic representation of the experimental set-up.

Initial dissolved inorganic nutrient concentrations were 5.4 µmol L^{-1} nitrate (NO_3^-), 0.4 µmol L^{-1} nitrite (NO_2^-), 1.7 µmol L^{-1} ammonium (NH_4^+), 0.3 µmol L^{-1} phosphate (PO_4^{3-}), and 5.9 µmol L^{-1} silicate ($Si(OH)_4$). In order to establish two different nutrient regimes, one half of the bottles received additional PO_4^{3-}, whereas the other half received both PO_4^{3-} and NO_3^-, yielding final concentrations of 0.87 ± 0.06 µmol L^{-1} dissolved inorganic phosphorus (DIP) and of 25.5 ± 0.6 and 7.0 ± 0.7 µmol L^{-1} dissolved inorganic nitrogen (DIN), respectively. This resulted in initial N:P ratios of approximately 29:1 ("P-limit" treatment) and 8:1 ("N-limit" treatment). Dissolved silicic acid ($(SiOH)_4$) was added to a final concentration of 44 µmol L^{-1} in order to ensure silica-replete conditions. Trace metals, vitamins, and selenium were added in f/10 amounts (Guillard and Ryther 1962). All treatments were run in triplicates. The bottles were placed horizontally below light benches. These contained full-spectrum light tubes covering the complete range of photosynthetically active radiation (PAR). The light benches were controlled by separate computer units, generating a triangular light curve with a light:dark cycle of 12:12 h.

Maximum light intensities of 377 µE m^{-2} s^{-1} ± 19 at midday were measured using a 4π-light sensor (LiCor, USA). Bottles were rotated every day in order to provide identical light conditions for all replicates. Prior to sampling, each bottle was mixed with a magnetic stirrer for approximately 1 min. A volume of 1.5 L was withdrawn at each sampling point. A comprehensive analysis of the autotrophic and heterotrophic compartments was generated through biogeochemical analyses of particulate and dissolved organic matter pools (this study), autotrophic and heterotrophic activity measurements (by P. Breithaupt, *in prep.*), and analyses of bacterial community composition (by K. Walther, *in prep.*).

Biogeochemical analyses.

Chlorophyll a. In order to closely monitor the development of the algal blooms, daily measurements of relative chlorophyll a (Chl a) fluorescence and photosynthetic efficiency (F_v/F_m) were made on a PhytoPAM equipped with Optical Unit ED-101US/MP (Walz, Germany) based on Kolbowski and Schreiber (1995). Samples were dark-adapted for 10 min prior to the measurement. Additionally, Chl a was determined fluorometrically on a Turner 10-AU fluorometer (Welschmeyer 1994). For this purpose, 50 to 100 ml of sample were filtered onto combusted GF/F-filters and stored in polypropylene tubes at -20°C. Prior to analysis, Chl a was extracted in 90% acetone.

Dissolved inorganic nutrients. The concentrations of NO_3^-, NO_2^-, PO_4^{3-}, and $Si(OH)_4$ were analysed colorimetrically from filtered (cellulose acetate filters, 5 µm pore size) water samples following the protocol of Hansen and Koroleff (1999). Ammonium was determined from unfiltered water samples (Holmes *et al.* 1999). All nutrient analyses were carried out on the day of sampling except for the last two sampling occasions, where samples were filtered and stored at -20°C until analysis.

Particulate organic matter (POM). For the determination of particulate organic carbon (POC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP) 100-200 ml of sample were filtered onto pre-combusted (450°C, 5h) GF/F-filters (Whatman) and stored at -20 °C until analysis. Prior to analysis on a Eurovector EuroEA-3000 elemental analyser (Sharp 1974), samples for POC and PON were dried at 60°C for 6h. POP concentrations were determined colorimetrically after oxidation with peroxodisulphate (Hansen and Koroleff 1999).

Dissolved organic matter (DOM). Dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved organic phosphorus (DOP), and dissolved carbohydrates were analysed from the filtrate of the particulate samples. The filtrate was collected in pre-combusted (450 °C, 12 h) 20 ml glass ampoules (DOC, carbohydrates) or acid-cleaned polypropylene vials (DON, DOP). The analysis of DOC was carried out on a

Shimadzu TOC_{VCN} using the HTCO method (Qian and Mopper 1996). DON and DOP concentrations were determined colorimetrically after oxidation with the reagent Oxisolv® following the protocol by Hansen and Koroleff (1999). The concentration of total carbohydrates (TCHO), mono- (MCHO), and polysaccharides (PCHO) was measured spectrophotometrically using the 2,-4,-6-tripyridyl-s-triazine approach (Myklestad *et al.* 1997). Additionally, the compound-specific composition of the dissolved polysaccharide pool was determined at selected time points on a Dionex IC 3000 using High Performance Anion Exchange Chromatograpyh with Pulsed Amperometric Detection (HPAEC-PAD). Prior to analysis, samples were desalinated via membrane dialysis (molecular weight cut-off 1kDa) and hydrolysed with 0.8 M hydrochloric acid (HCl) at 100°C for 20h. The hydrolysate was neutralised by evaporation with N_2 gas and subsequently redissolved in 4 ml of MilliQ water (Engel and Händel *subm*.).

Microbial activities.

Bacterial secondary production (BSP). BSP was assessed through the incorporation of ^{3}H-leucine following the protocol of Simon and Azam (1989). 10 ml of sample were spiked with 50 µl of radioactively-labelled ^{3}H-leucine (specific activity 77 µCi nmol^{-1}) and 50 µl of unlabelled leucine, yielding a final concentration of 106.49 nmol leucine L^{-1}. Duplicate samples plus an additional formalin-killed control were dark-incubated at in situ temperature (i.e. in the respective climate chamber) for 1.5-3 hours. The incubation was terminated by adding formaldehyde (1% v/v). Samples were filtered onto 0.2 µm polycarbonate filters, rinsed with ice-cold TCA solution (5%) and subsequently radio-assayed in 4ml of scintillation cocktail (Lumagel plus) on a Packard TriCarb scintillation counter. ^{3}H-leucine incorporation (pmol L^{-1} h^{-1}) was converted into BSP (µg C L^{-1} h^{-1}) by applying a theoretical conversion factor of 3.1 kg C mol^{-1} leucine (Simon and Azam 1989).

Extracellular enzyme activities. Maximum hydrolytic activities (v_{max}) of the bacterial extracellular enzymes β-D-glucosidase (β-D-gluc), leucine-aminopeptidase (Leu-amp), and BOC-peptidase, as well as of alkaline phosphatase (APA) were determined following the protocol of Hoppe (1983). Subsamples of 200 µL each were pipetted in quadruplicate into microtiter plates. Fluorogenic model substrates, i.e. 4-methylumbelliferyl-β-D-glucoglucopyranosid (MUF-β-glucoside), L-leucine-4-methyl-7-coumarinylamide (Leu-MCA), butoxycarbonyl-phenylalanine-serine-arginine-4-methyl-7-coumarinylamide (BOC-MCA), and 4-methylumbelliferyl-phosphate (all Sigma-Aldrich, Germany, except BOC-MCA: Pepta Nova GmbH, Germany), were added to the samples at a saturating concentration of 250 µmol L^{-1}. Sample fluorescence was determined immediately after addition of the substrates by using a microtiter plate fluorometer

(Fluoroskan Ascent, Ascent Software; excitation 364 nm, emission 445 nm), as well as after a dark-incubation period of approximately 3 hours at *in situ* temperature. Fluorescence signals were converted into maximum hydrolytic enzyme activities (µmol L^{-1} h^{-1}) by using empirical conversion factors, which were determined from fluorescence readings of low MUF and MCA concentrations dissolved in seawater (P. Breithaupt, *pers. comm.*).

Calculations.

Cardinal points of the bloom. For a better comparison of the various temperature and nutrient treatments, three characteristic bloom phases (i.e. "pre-bloom", "bloom", as well as "post-bloom") were identified on the basis of changes in chlorophyll a concentration [Chl a]. To determine the day of bloom onset, and thus the transition from "pre-bloom" to "bloom", the natural logarithm (ln) of [Chl a] was calculated and plotted against time. During the exponential growth of algal cells, ln [Chl a] should scale linearly with time. Thus, the first data point fulfilling this linearity criterion was defined as the day of bloom onset. The end of the "bloom" phase was defined as the day of maximum [Chl a] in the respective microcosms. The bloom peak was accelerated by 1.75 days $°C^{-1}$ in both P- and N-limit treatments. The "post-bloom" phase started with the first day after the respective Chl a peak. Because the development was markedly slowed down at low temperature, the sampling period did not fully cover the degradation phase in the 4°C treatments, hence yielding higher biomass concentrations on the last day of sampling at 4°C compared to elevated temperatures. To exclude possible temperature-artefacts when calculating, for instance, time means for the "post-bloom" phase, the last day included in the calculations was shifted forward by 1.75 days $°C^{-1}$ according to the acceleration of the bloom peak. Thus, at 4°C, the last day of sampling (day 42) was included in the calculations, whereas the bloom end was shifted forward by 7 and 14 days in the 8°C (day 35) and 12°C (day 27 (P-limit)/ 24 (N-limit)) treatments, respectively.

Algal growth rate μ_{Chla}. μ_{Chla} was calculated for the period of exponential growth based on changes in Chl a concentration according to:

μ_{Chla} = (Chl a_{t2} – Chl a_{t1})/(t_2-t_1), with t_1 being the first day of exponential growth and t_2 being the last day of exponential growth.

DIN and DIP uptake rates. These were calculated following the equation:

(X_i-X_e)/(t_e-t_i), with X = DIN or DIP, i = the first day of detectable nutrient drawdown, e = the day, at which concentrations fell below the detection limit.

POM build-up. The maximum net build-up of POC (∆POC), PON (∆PON), and POP (∆POP) (in units of µmol C, N, or P L^{-1}) was determined by calculating the difference

between the initial (day 0) and the maximum concentration. The initial concentration was averaged over all microcosms.

DOM build-up. For a comparison of DOC concentrations between the various treatments, a time mean of DOC (ø DOC) was calculated for the period from exhaustion of the limiting nutrient (i.e. DIP or DIN, respectively) to the end of the experiment. Each mesocosm was treated individually. The rate of increase in polysaccharide concentration (v_{incr} (PCHO)) was determined by applying a linear regression model to the individual data sets for the time period from DIP or DIN exhaustion, respectively, to the end of the experiment.

The net build-up of PCHO (∆PCHO) was calculated according to:

$\Delta PCHO = (PCHO_e - PCHO_i)$, with i referring to the initial PCHO concentration (day 0), and e referring to the PCHO concentration at the end of the bloom (i.e., days 42, 35, 27 (P-limit)/24 (N-limit) at 4, 8, and 12°C). To account for the differences in biomass formation between the P- and N-limit treatments, ∆PCHO was normalised to the maximum net accumulation of POC (∆POC).

The contribution of the PCHO fraction to total DOC concentration (PCHO/DOC; in %) was assessed for each mesocosm individually at the start of the experiment (day 0) and at the end of the bloom (see above).

Statistical analysis.

Treatment effects between P- and N-deficient microcosms were tested using One-way ANOVA (Statistica). Temperature effects were assessed by linear regression analysis (Sigmaplot). A statistical significance level of $p < 0.05$ was applied to all tests.

RESULTS

Initial nutrient availability and algal bloom development

After the addition of inorganic nutrients and the successful establishment of two differing nitrogen:phosphorus (N:P) regimes of N:P = 29:1 ("P-limit" treatments) and N:P = 8:1 ("N-limit" treatments), respectively, the development of distinct phytoplankton blooms was observed in all treatments. The onset of algal growth was marked by a rapid decline in inorganic nutrients and a simultaneous increase in Chl a concentration (Fig. 2). Rising temperature clearly affected the temporal development of the bloom with a doubling of algal growth rates from 0.2 at 4°C to 0.4 µg Chl a day^{-1} at 12°C, an accelerated uptake of inorganic nutrients and a forward shift of the bloom peak by approximately 1.75 days $°C^{-1}$ (Tab. 1). In the P-limit and N-limit treatments, maximum Chl a concentrations of 16.2 ± 1.9 and 7.8 ± 1.8 µg Chl a L^{-1}, respectively, were achieved (Fig. 2A, B), yielding a

biomass enhancement factor of 2.1 between the nutrient treatments. In contrast, a notable effect of temperature on maximum Chl a was not observed.

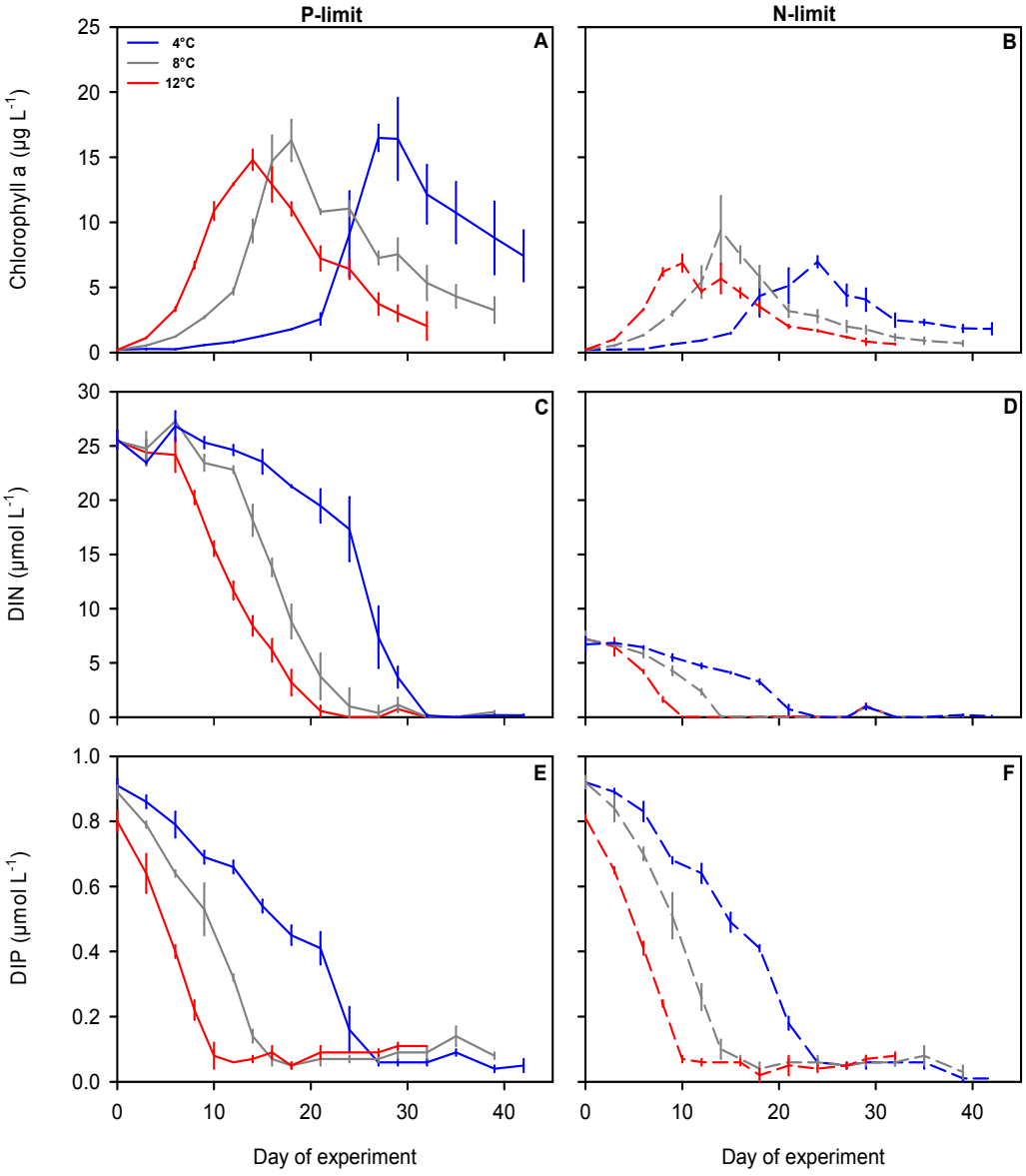

Figure 2. Temporal development of chlorophyll a (Chl a), dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP). The different colours represent the three temperature treatments: 4°C (blue), 8°C (grey), and 12°C (red). The left panel displays Chl a, DIN, and DIP concentrations in the P-limit treatments (solid line), the right panel depicts the respective N-limit treatments (dashed line). Lines represent the average of 3 replicate microcosms ± SD.

Despite the differing types of nutrient limitation, DIN and DIP were both scavenged until the detection limit in all microcosms (Fig. 2C-F). A substantial phosphorus deficiency of the P-limit treatments is, however, indicated by a pronounced increase in the maximum hydrolytic activity of alkaline phosphatase (v_{max} APA) throughout the experiment (Fig.

3A). Moreover, rising temperature seemed to further enhance the degree of phosphorus limitation as the average v_{max} APA was significantly higher in the 12°C replicates compared to the treatments at 8 and 4°C ($p < 0.03$). In comparison, v_{max} APA remained substantially lower in the N-deficient microcosms (Fig. 3B) and did not display an effect of temperature.

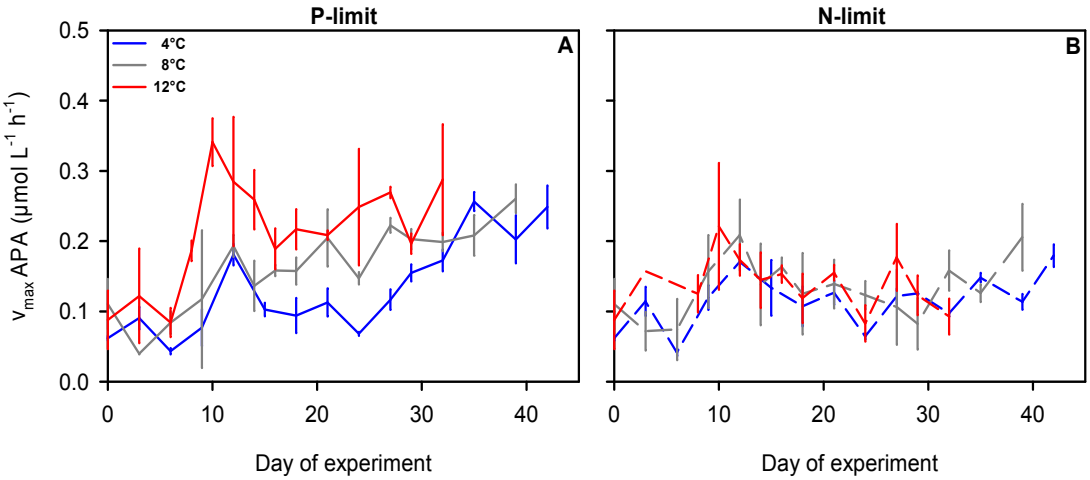

Figure 3. Temporal development of maximum hydrolytic activity of alkaline phosphatase (v_{max} APA). Lines represent the average of 3 replicate microcosms ± SD. Colour code is as in Fig. 2. Data were kindly provided by P. Breithaupt.

Build-up of particulate and dissolved organic matter pools

The increase in Chl a was followed by the build-up of particulate organic carbon (POC), nitrogen (PON), and phosphorus (POP). POC concentrations increased from initially 4.1 ± 0.5 µmol C L^{-1} to maximum values of 438.9 ± 51.6 and 283.2 ± 53.9 µmol C L^{-1} under P- and N-deficiency (Fig. 4A, B). Thus, the build-up of POC exceeded the expected carbon production, as based on the initially available nutrients and the Redfield ratio of C:N:P = 106:16:1 ((Redfield et al. 1963); P-limit: 0.87 µmol DIP L^{-1} * 106 = 92.2 µmol C L^{-1}, N-limit: 7.0 µmol DIN L^{-1} * 6.6 = 46.2 µmol C L^{-1}), by a factor of approximately 4.8 and 6.1 in the respective nutrient treatments. Similarly, a relatively higher POC production of the N-deficient treatments for a given amount of inorganic nutrients is also suggested by the ratio between the maximum net accumulation of POC (ΔPOC) in the P- and in the N-limit microcosms, yielding a biomass enhancement factor of 1.6. POC accumulation continued for several days after the end of exponential growth and the peak in Chl a in all treatments. While POC concentrations reached a plateau and remained high until the end of the experiment in the P-limit treatments at 4 and 8°C, a pronounced decrease in POC was observed at 12°C from day 24 onward (Fig. 4A), yielding a net loss of approximately 160 µmol C L^{-1}. In contrast, a similar degradation signal was not observed in the respective N-deficient treatment.

		4°C	8°C	12°C	m	R^2	p
$\mu_{Chl\ a}$	**P-limit**	0.20 ± 0.0	0.27 ± 0.01	0.43 ± 0.0	0.02	0.96	<0.0001
	N-limit	0.18 ± 0.0	0.27 ± 0.02	0.42 ± 0.01	0.02	0.83	'0.001
DIN	**P-limit**	1.1 ± 0.1	1.4 ± 0.3	1.3 ± 0.1	0.03	0.21	0.22
	N-limit	0.3 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.05	0.93	<0.0001
DIP	**P-limit**	0.03 ± 0.0	0.05 ± 0.0	0.08 ± 0.01	0.01	0.95	<0.0001
	N-limit	0.03 ± 0.0	0.05 ± 0.0	0.08 ± 0.0	0.01	0.94	<0.0001
bloom peak	**P-limit**	28 ± 1	18 ± 0	14 ± 0	-1.71	0.94	<0.0001
	N-limit	24 ± 0	14 ± 0	9 ± 1	-1.83	0.95	<0.0001

Table 1. Autotrophic growth and temporal development of the bloom. Algal growth rates ($\mu_{Chl\ a}$; µg Chl a day^{-1}) were determined during exponential growth based on chlorophyll a (Chl a) measurements. Uptake rates of DIN and DIP (µmol DIN or DIP day^{-1}) were calculated as the daily change in DIN or DIP concentration from day 0 of the experiment until the day of nutrient limitation. The bloom peak describes the day of maximum Chl a concentration. Values represent the average of 3 replicate microcosms ± SD. The effect of temperature on the various parameters was assessed using linear regression analysis with the slope describing the direction and magnitude of change with increasing temperature. * indicates significant temperature effects.

		4°C	8°C	12°C	m	R^2	p
∆ POC	**P-limit**	435.3 ± 53.0	459.8 ± 75.2	409.2 ± 16.7	-3.30	0.05	0.57
	N-limit	294.6 ± 46.4	297.1 ± 83.0	245.7 ± 7.6	-6.10	0.16	0.30
∆ PON	**P-limit**	24.7 ± 0.4	23.5 ± 2.4	22.6 ± 0.6	-0.27	0.35	0.09
	N-limit	10.8 ± 3.0	9.1 ± 0.9	8.4 ± 1.3	-0.30	0.26	0.16
∆ POP	**P-limit**	0.68 ± 0.06	0.61 ± 0.01	0.61 ± 0.02	-0.01	0.47	0.04*
	N-limit	0.71 ± 0.03	0.71 ± 0.07	0.68 ± 0.05	0.00	0.06	0.54

Table 2. Maximum net build-up of particulate organic carbon (∆POC), nitrogen (∆PON), and phosphorus (∆POP). Values represent the average of 3 replicate microcosms ± SD. The effect of temperature on ∆POC, ∆PON, and ∆POP was assessed using linear regression analysis with the slope describing the direction and magnitude of change with increasing temperature. * indicates significant temperature effects.

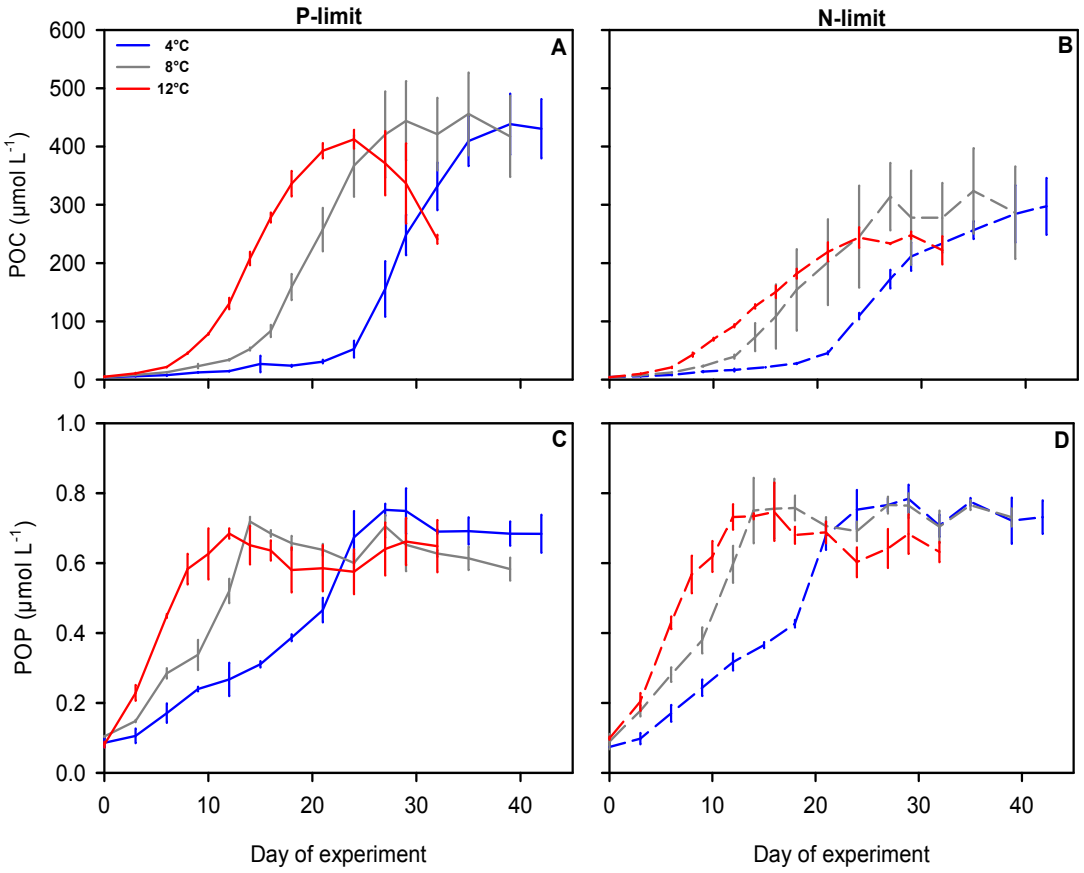

Figure 4. Build-up of particulate organic carbon (POC) and phosphorus (POP). Lines represent the average of 3 replicate microcosms ± SD. Colour code is as in Fig. 2.

The temporal development of particulate organic nitrogen (data not shown) and phosphorus (Fig. 4C, D) concentrations closely followed the drawdown of DIN and DIP. The maximum net build-up of PON (ΔPON) ranged between 22.6 ± 0.6 (12°C) and 24.7 ± 0.4 µmol N L^{-1} (4°C) in the P-limit treatments and between 8.4 ± 1.3 (12°C) and 10.8 ± 3.0 µmol N L^{-1} (4°C) in the N-limit treatments, hence showing a weak decrease with rising temperature ($p < 0.1$; Tab. 2). Under N-deficient conditions, the net build-up of PON even exceeded the initially available amount of DIN. For the build-up of POP between 0.61 and 0.71 µmol P L^{-1} (ΔPOP) of the initially available 0.87 µmol DIP L^{-1} were found in the particulate fraction, with significantly ($p = 0.01$) lower values of ΔPOP under P-deficiency (Tab. 2). Moreover, rising temperature led to a significant decrease of ΔPOP in the P-limit treatments ($p < 0.05$). After the phase of exponential algal growth, both PON and POP concentrations remained high until the end of the experiment. A degradation signal, as observed for POC, was not detected in any of the treatments.

In the P-limit microcosms, a distinct increase in the concentration of dissolved organic carbon (DOC) occurred shortly after the onset of phosphate depletion (Fig. 5A).

Warming clearly stimulated the accumulation of DOC, as indicated by a significant increase in the average DOC concentration (ø DOC) with rising temperature ($p = 0.02$; Table 3). In contrast, DOC concentrations increased only weakly in the N-limit treatments and were not notably affected by elevated temperature (Fig. 5B). The pools of dissolved organic phosphorus (DOP) and nitrogen (DON) ranged initially at 0.22 ± 0.09 µmol P L^{-1} (Fig. 5C, D) and 23.7 ± 1.8 µmol N L^{-1} (data not shown), respectively. DON concentrations displayed a weak decrease of on average 4.5 ± 0.7 µmol N L^{-1} in the course of the experiment, whereas DOP remained fairly constant. A clear response of the DON and DOP pools to either rising temperature or nutrient regime was not observed.

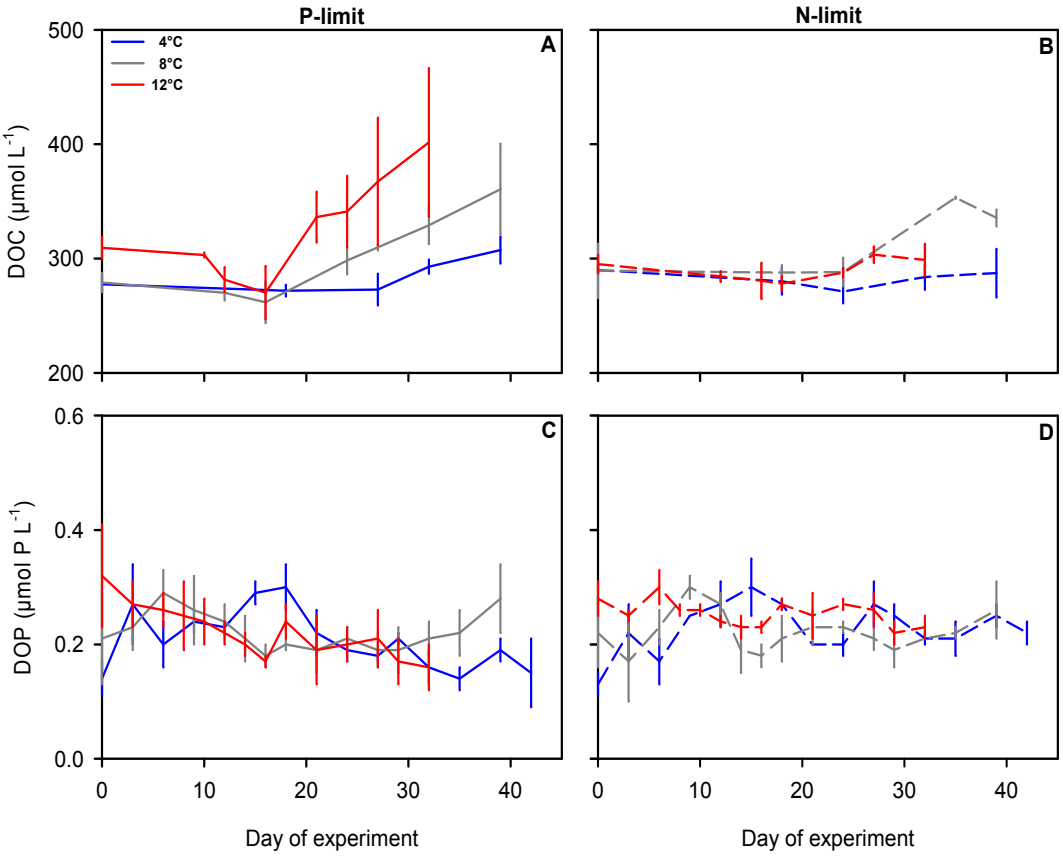

Figure 5. Build-up of dissolved organic carbon (DOC) and phosphorus (DOP).
Lines represent the average of 3 replicate microcosms ± SD. Colour code is as in Fig. 2.

Elemental stoichiometry of particulate organic matter

Initial molar ratios of POC to PON ($C:N_{part}$) and POP ($C:P_{part}$) were on average 3.6 ± 0.6 (Fig. 6A, B) and 48.0 ± 12.4, respectively (Fig. 6C, D). With the onset of nutrient deficiency, a rapid increase in $C:N_{part}$ and $C:P_{part}$ was observed under both nutrient regimes, resulting in a pronounced deviation from the Redfield ratio. The magnitude of the stoichiometric response depended, however, on the kind of nutrient limitation. Thus, $C:N_{part}$ showed the strongest divergence in the N-limit treatments with a maximum of 37.8 ± 10.2 compared to a value of 19.3 ± 2.6 under P-deficiency, whereas the deviation of $C:P_{part}$ was most pronounced in the P-limit microcosms with a maximum of 709.7 ± 108.2 compared to 411.2 ± 65.7 under N-deficient conditions. Although maximum $C:P_{part}$ values tended to increase with rising temperature in the P-limit treatments, a significant effect of warming on the stoichiometric composition of POM could not be detected.

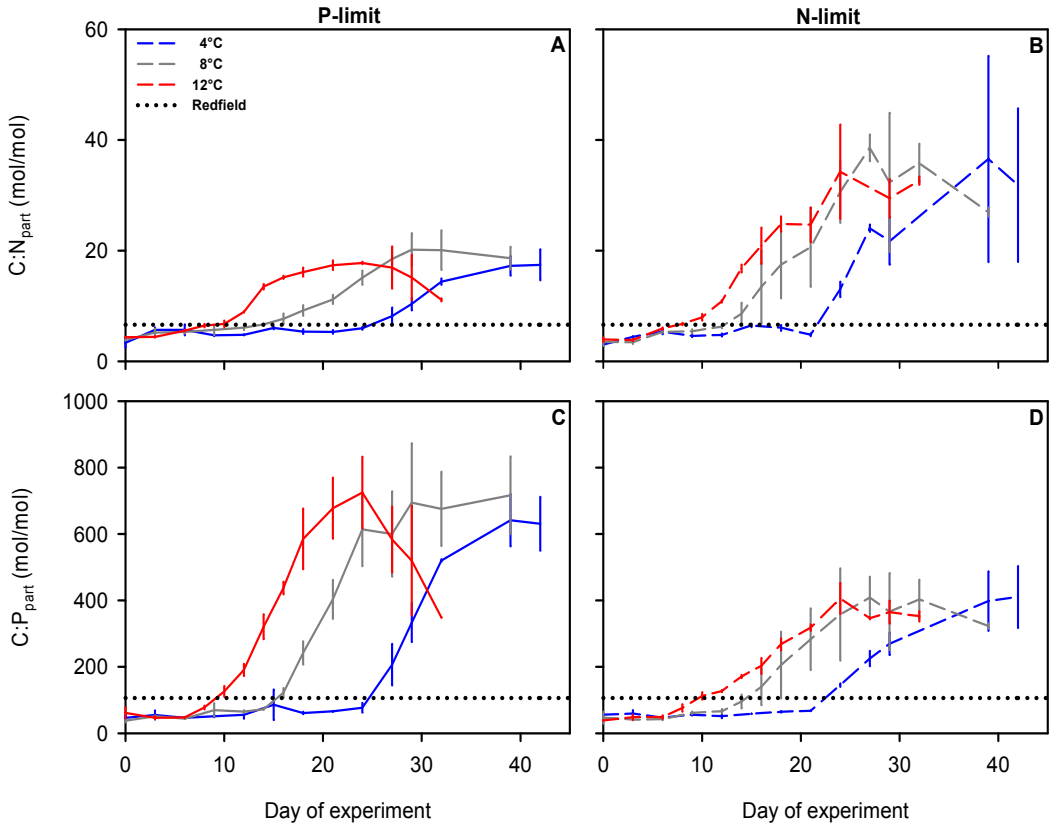

Figure 6. Elemental stoichiometry of particulate organic matter. Given are the ratios of POC to PON ($C:N_{part}$) and of POC to POP ($C:P_{part}$). Lines represent the average of 3 replicate microcosms ± SD. The dotted black lines indicate the Redfield ratio of C:N = 6.6:1 and C:P = 106:1. Colour code is as in Fig. 2.

Composition of dissolved organic matter

At the start of the experiment, dissolved polysaccharides (PCHO) were present at a concentration of 17.4 ± 2.7 µmol C L^{-1} (Fig. 7A, B). Concomitant to the accumulation of DOC at the time of nutrient depletion, a pronounced increase in PCHO concentration occurred in the P-deficient microcosms (Fig. 7A). Rising temperature significantly enhanced the rate of polysaccharide accumulation, as illustrated by the slope of PCHO increase (p = 0.02; Fig. 8 A, Tab. 3). In contrast, the N-limit treatments displayed only a weak increase in PCHO (Fig. 7B), and did not respond significantly to rising temperature (p = 0.29; Fig. 8A).

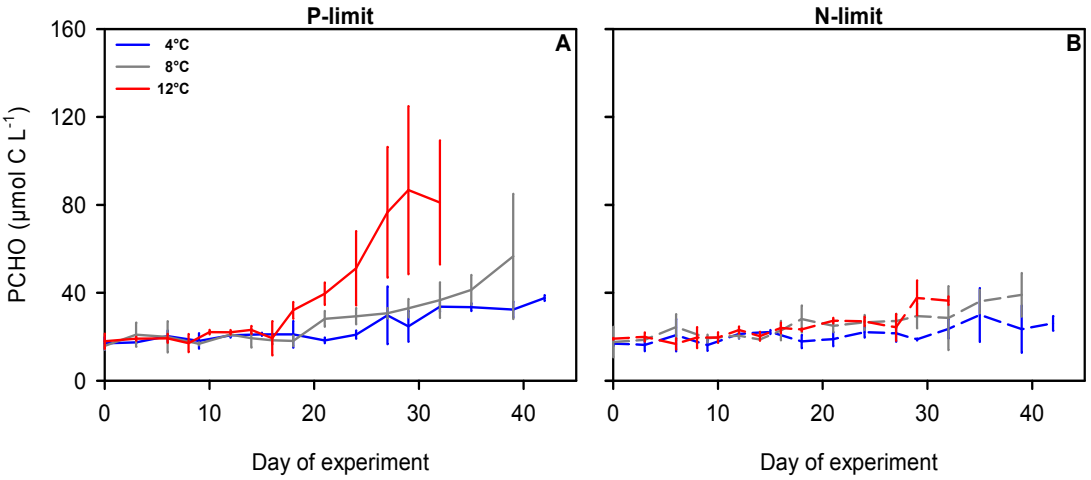

Figure 7. Dissolved polysaccharide (PCHO) concentration over the experimental period. Lines represent the average of 3 replicate microcosms ± SD. Colour code is as in Fig. 2.

		4°C	8°C	12°C	m	R^2	p
ø DOC	**P-limit**	10.5 ± 0.9	11.2 ± 2.5	20.4 ± 4.7	3.6	0.55	0.02*
	N-limit	8.0 ± 3.4	10.9 ± 1.4	9.4 ± 0.8	0.82	0.07	0.48
v_{incr} (PCHO)	**P-limit**	0.8 ± 0.3	1.4 ± 0.7	3.2 ± 1.6	0.30	0.57	0.02*
	N-limit	0.4 ± 0.1	0.6 ± 0.5	0.7 ± 0.2	0.03	0.16	0.29
Δ PCHO/Δ POC	**P-limit**	4.8 ± 0.9	4.5 ± 1.5	14.4 ± 7.6	1.2	0.45	<0.05*
	N-limit	3.1 ± 1.0	4.2 ± 2.2	3.2 ± 0.8	0.01	0.0008	0.94

Table 3. Dissolved organic carbon (DOC) and dissolved polysaccharides (PCHO). Given are the average DOC concentration (ø DOC; in µmol C L^{-1}), the rate of PCHO accumulation (v_{incr} (PCHO); in µmol C L^{-1} d^{-1}), both calculated for the period of beginning nutrient deficiency to the end of the experiment, as well as the ratio between maximum net PCHO build-up and maximum net accumulating biomass (ΔPCHO/ΔPOC; in %). Values represent the average of 3 replicate microcosms ± SD. The effect of temperature on these variables and parameters was assessed by using linear regression analysis. The slope describes the direction and magnitude of change with increasing temperature. * indicates significant temperature effects.

Normalising the net accumulation of PCHO to the respective build-up of POC (ΔPCHO/ΔPOC) revealed a significant positive trend with increasing temperature in the P-deficient microcosms ($p < 0.05$, Tab. 3), whereas the N-limit treatments remained unaffected. Dissolved polysaccharides initially constituted 5.7 ± 1.7 % of total DOC concentration. Along with the accumulation of PCHO in the course of the experiment, a shift in their contribution to the bulk DOC pool occurred. Thus, the PCHO fraction constituted between 4.2 and 25.8 % of DOC at the end of the bloom. While this was clearly temperature-dependent in the P-limit treatments, as illustrated by a significant increase in the percentage from 10.5 ± 0.9 at 4°C to 20.4 ± 4.7 at 12°C ($p = 0.01$; Fig. 8B), the PCHO fraction accounted on average for 9.3 ± 2.3 % of total DOC in N-deficient microcosms and was not notably affected by temperature (Fig. 8B).

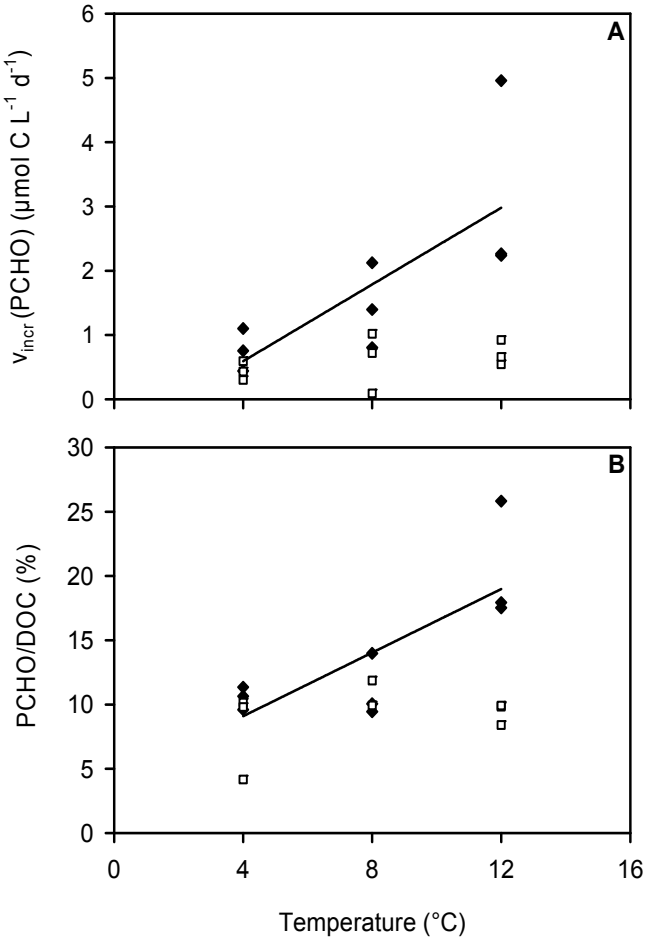

Figure 8. Temperature-dependency of polysaccharide (PCHO) accumulation and the contribution of PCHO to the bulk DOC pool. (A) Rate of polysaccharide accumulation (v_{incr} (PCHO); µmol C L^{-1} d^{-1}), (B) proportion of PCHO to total DOC concentration (%) at the end of the post-bloom phase. (♦) P-limit treatments, (□) N-limit treatments. Solid lines depict significant ($p < 0.05$) linear regressions (n=9; P-limit: v_{incr} (PCHO) = 0.3T – 0.6, $R^2 = 0.57$, $p = 0.019$; PCHO/DOC = 1.24T + 4.14, $R^2 = 0.61$, $p = 0.013$; N-limit: n.s.).

At three characteristic stages of bloom development, the monomeric compounds constituting the dissolved polysaccharide pool were analysed chromatographically. In addition to the findings on bulk PCHO concentration, this analysis revealed distinct changes in the monomeric composition of the PCHO pool (Fig. 9).

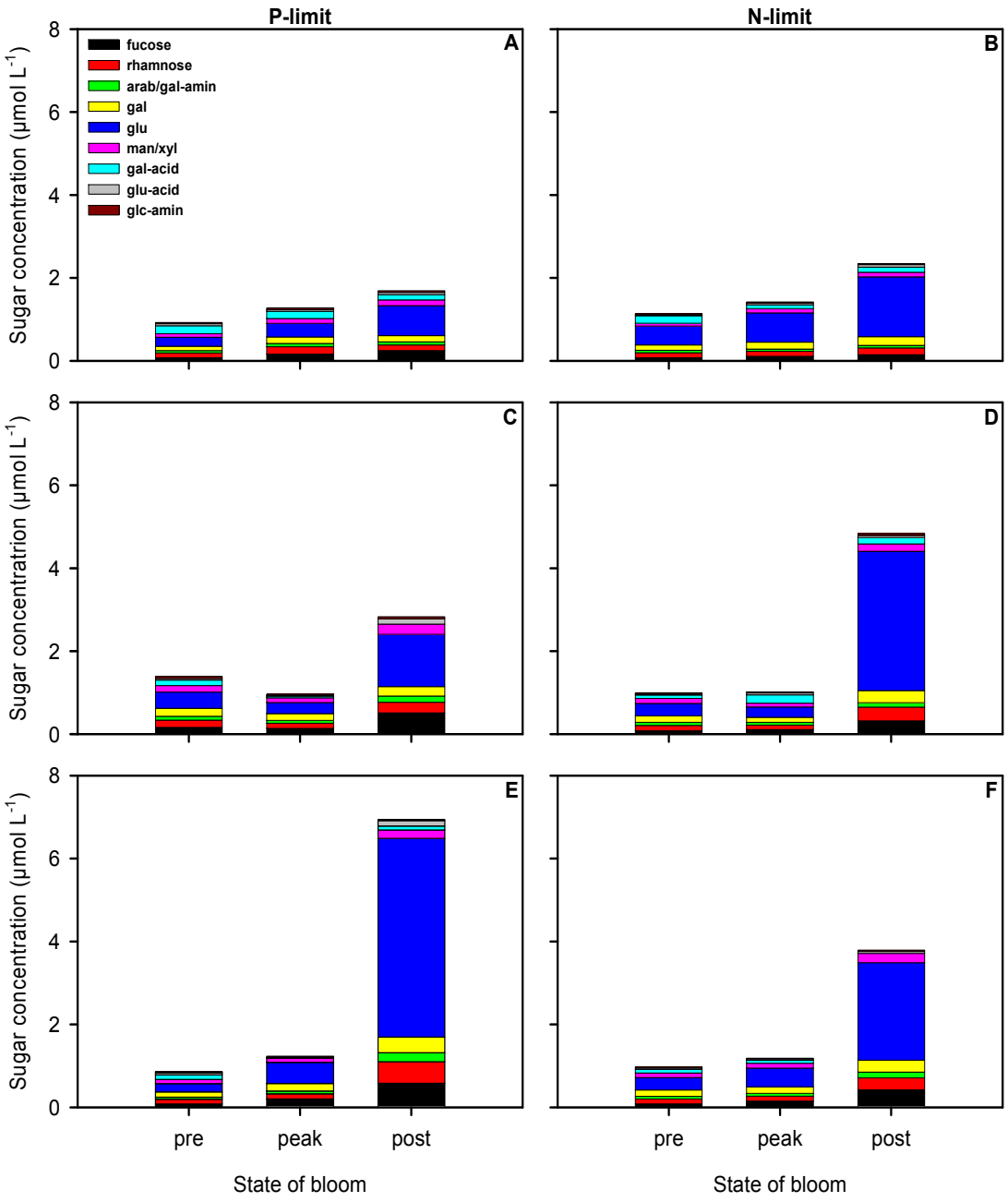

Figure 9: Monomeric composition of dissolved polysaccharides at three characteristic stages of the algal bloom (pre-bloom, peak and post-bloom) at the three experimental temperatures 4°C (A, B), 8°C (C, D), and 12°C (E, F) and under the two contrasting nutrient regimes P-limit (left) and N-limit (right). Arab/gal-amin = arabinose/galactosamine; gal = galactose; glu = glucose; man/xyl = mannose/xylose; gal-acid = galacturonic acid; glu-acid = glucuronic acid; glc-amin = glucosamine.

While polysaccharides consisted of a diverse mixture of carbohydrate monomers with a summed concentration of 1.05 ± 0.31 µmol L^{-1} at the start of the experiment ("pre-bloom"), first indications for a shift in the relative contribution of individual monomers

emerged with the onset of algal growth, albeit a fairly constant total concentration ("bloom peak"). Thus, a considerable increase in glucose concentration became visible in most of the treatments. Approximately two weeks after the respective bloom peak ("post-bloom"), glucose was by far the most abundant compound, accounting for on average 48 ± 21 and 56 ± 15 % in the P- and N-limit treatments, respectively. In addition, a marked increase in the abundance of the deoxy sugars fucose and rhamnose over the experimental period was detected in the P-limit treatments with final concentrations of 0.38 ± 0.05, 0.77 ± 0.16, and 1.63 ± 0.40 µmol L^{-1} at 4, 8, and 12°C, respectively.

Microbial activities

In close temporal connection to the development of algal biomass, an increase in bacterial secondary production (BSP) was observed (Fig. 10). Here, already during the first few days of the experiment, a clear temperature-response appeared with higher BSP rates at elevated temperatures. While they further increased in the P-limit treatments thereafter (Fig. 10A), BSP rates levelled off quickly after the first impulse in the N-limit treatments and remained stable at low activities until the end of the experiment (Fig. 10B).

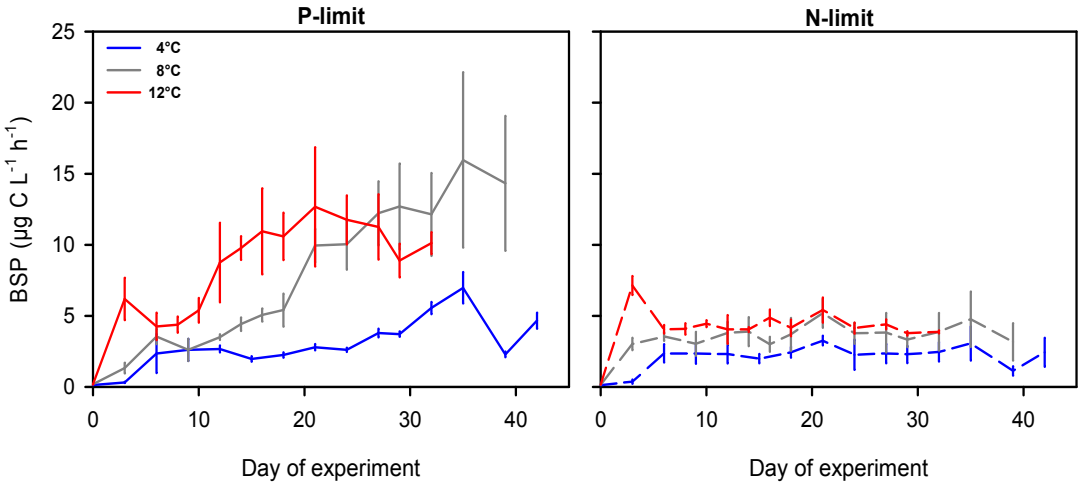

Figure 10. Temporal development of bacterial secondary production (BSP). Lines represent the average of 3 replicate microcosms ± SD. Colour code is as in Fig. 2. Data were kindly provided by P. Breithaupt.

A weak decrease in BSP rates was, however, also observed in the warmest P-limit treatments from day 21 onwards. Time averages calculated for each replicate during the post-bloom phase (see *Materials and Methods* for details on calculations) revealed a significant response of BSP rates to rising temperature for both P- and N-deficient

treatments (Fig. 10). In general, the average BSP rates showed a stronger relation to temperature and were markedly higher in the P-limit compared to the N-limit treatments. Thus, with an increase in temperature of 8°C, average BSP rates increased by a factor of approx. 2.5 from 4.5 ± 0.2 to 11.2 ± 1.9 µg C L^{-1} h^{-1} under P-deficient conditions, whereas they hardly doubled from 2.3 ± 0.7 to 4.4 ± 0.4 µg C L^{-1} h^{-1} in N-deficient treatments (Fig. 11A; P-limit: p=0.009; N-limit: p=0.008).

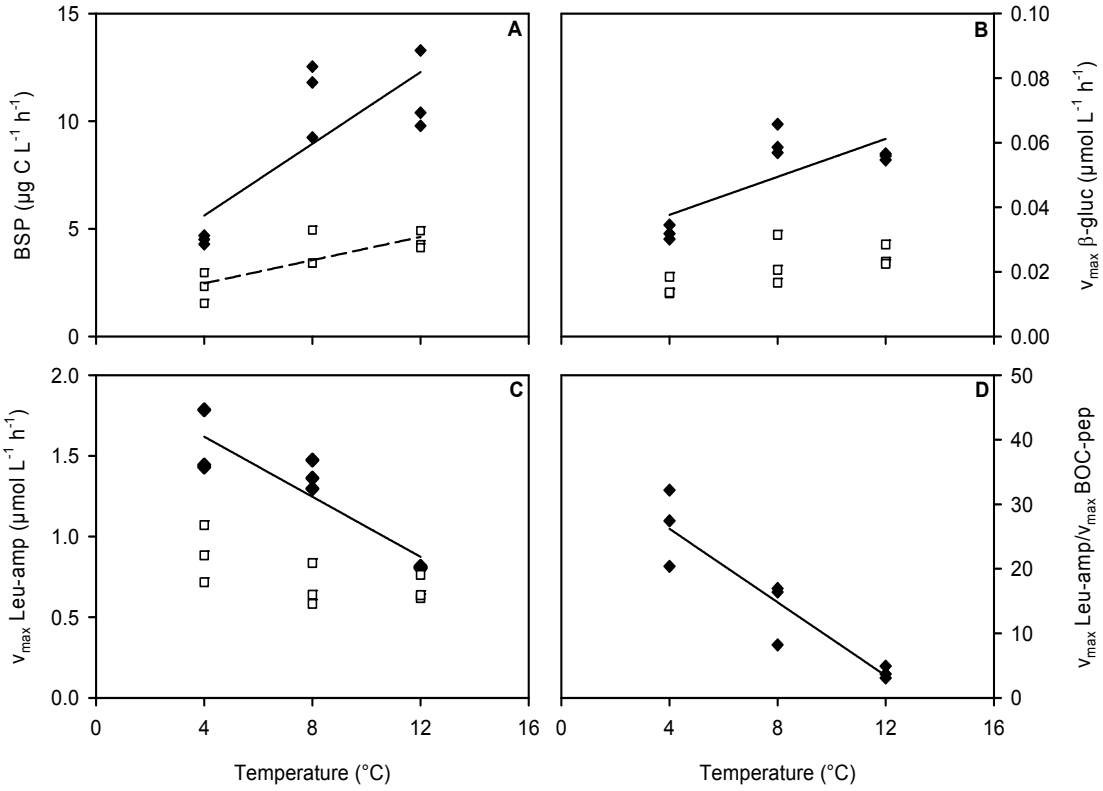

Figure 11. Temperature-dependency of BSP and bacterial hydrolytic enzymes during the post-bloom phase. (A) BSP, (B) v_{max} of ß-D-glucosidase (ß-D-gluc), (C) v_{max} of leucine-aminopeptidase (Leu-amp), and (D) the ratio of v_{max} Leu-amp to v_{max} BOC-peptidase. (♦) P-limit treatments, (□) N-limit treatments. Solid lines represent significant ($p < 0.05$) linear regressions (n = 9; P-limit: BSP = 0.83T + 2.28, $R^2 = 0.65$, p = 0.009; ß-D-gluc = 0.003T – 0.026, $R^2 = 0.58$, p = 0.017; Leu-amp = -0.09T + 1.99, $R^2 = 0.83$, p = 0.001; Leu-amp/BOC-pep = 0.012T + 0.07, $R^2 = 0.44$, $p < 0.05$; N-limit: BSP = 0.27T + 1.39, $R^2 = 0.66$, p = 0.008; ß-D-gluc, Leu-amp n.s.; Leu-amp/BOC-pep n.d.). Data were kindly provided by P. Breithaupt.

Enhanced microbial activity in the P-limit treatments was also indicated by rate measurements of the bacterial extracellular enzymes β-D-glucosidase, and leucine-aminopeptidase (Fig. 11 B, C, D). Here, average rates of maximum hydrolytic velocity (v_{max}), calculated as a time mean for the post-bloom phase, revealed significantly higher values of v_{max} for both β-D-glucosidase (β-gluc; Fig. 11B; $p < 0.0001$) and leucine-

aminopeptidase (leu-amp; Fig. 11C; p = 0.002) at P-deficiency compared to the N-limit treatments. Moreover, a distinct, although not uniform, response in the maximum hydrolytic activity of these enzymes to rising temperature was observed in the P-limit treatments. Thus, v_{max} rates of β-gluc increased significantly with rising temperature from 0.032 ± 0.002 at 4°C to 0.056 ± 0.001 µmol L^{-1} h^{-1} at 12°C (p = 0.02). In contrast, the v_{max} of leu-amp showed a significant decline by a factor of approx. 2 from 1.55 ± 0.20 at 4°C to 0.81 ± 0.01 µmol L^{-1} h^{-1} at 12°C (p < 0.001). Analogous to this negative correlation with temperature also the ratio of leucine-aminopeptidase to BOC-peptidase, being an indicator for the relative use of short-chained over more complex, long-chained peptides, showed a significant decrease with rising temperature from 26.7 ± 5.9 at 4°C to 3.9 ± 0.9 at 12°C (Fig. 11D; p = 0.007). In contrast to P-deficient microcosms, the maximum hydrolytic activity of β-gluc and leu-amp remained unaffected by rising temperature under N-deficient conditions with average values of 0.021 ± 0.006 and 0.75 ± 0.16 µmol L^{-1} h^{-1}, respectively.

DISCUSSION

Effects of temperature and nutrient availability on autotrophic growth

After the addition of inorganic nutrients and the successful establishment of two differing nitrogen:phosphorus (N:P) regimes, with N:P ratios being markedly higher (i.e., the "P-limit" treatments) or lower (i.e., the "N-limit" treatments) than the classical Redfield ratio of 16:1 (Redfield *et al.* 1963), distinct blooms of *Skeletonema costatum* developed in all treatments. Increasing the temperature by 4 to 8°C clearly affected the timing of the bloom as evidenced by a doubling of algal growth rates, an accelerated uptake of inorganic nutrients and a forward shift of the bloom peak by approximately 1.75 days $°C^{-1}$. The magnitude of algal growth, as depicted by maximum Chl a concentration, was independent of ambient temperature, but displayed approximately two-fold higher values in P-limit compared to N-limit treatments, which is in accordance with the predicted biomass production based on the available amount of the limiting nutrient (i.e. 0.87 µmol DIP L^{-1} for P-limit and 7.0 µmol DIN L^{-1} for N-limit treatments) and the Redfield ratio of C:N:P = 106:16:1. The lack of a pronounced response of autotrophic growth parameters to experimental warming is consistent with findings from a previous indoor-mesocosm study using a natural plankton community, where an increase in temperature of 2-6°C led to an advancement of the spring bloom by 1.0 day $°C^{-1}$ (see also *Chapter 1*).

The build-up and elemental composition of particulate organic matter

Concomitant to the rapid decline in dissolved inorganic nutrients and the rise in Chl a concentration, a pronounced increase in the POC, PON and POP pools occurred in all treatments. While the accumulation of PON and POP ceased within 2 days after the exhaustion of the respective inorganic nutrients, that of POC continued for approximately 13 to 18 days, suggesting an overconsumption of inorganic carbon in excess of the prevailing limiting nutrient (i.e., DIN or DIP). This is also reflected in the maximum net production of POC (∆POC), which exceeded the predicted values considerably by a factor of 4.8 and 6.1 under P- and N-deficient conditions. Similar to maximum Chl a, ∆POC was higher in the P-limit than in the N-limit treatments, although this nutrient-driven biomass enhancement was less pronounced with a factor of 1.6 compared to 2 for Chl a.

The maximum net accumulation of PON and POP closely mirrored the availability of the respective dissolved inorganic nutrients. Thus, ∆PON accounted for 92.6 ± 6.1% of initially available DIN in the P-limit treatments, whereas it even accounted for 134.8 ± 28.7 % in the N-limit treatments. The excess build-up of PON under N-deficient conditions clearly suggests that also dissolved organic nitrogen compounds were used for the synthesis of new biomass. The observed decrease in DON concentrations by approximately 4.5 ± 0.7 µmol N L^{-1} further confirmed this idea. Taking these "extra" nutrients into account, the above reported ratio of observed to predicted carbon production hence declined from a factor of 6.1 to 4.6 and was thus of comparable size to that observed in the P-limit treatments. Consequently, the observed Chl a-based biomass ratio of 2 between P- and N-deficient treatments is bound to rather indicate a shift in the production of Chl a per biomass under the respective nutrient conditions. ∆POP accounted for 73.1 ± 5.5 and 80.4 ± 5.6 % of available DIP in the P- and N-limit treatments, respectively, hence being higher under N- compared to P-deficient conditions. Moreover, ∆POP significantly decreased with rising temperature in the P-limit treatments. In principle, two processes may account for the observed response of ∆POP to both nutrient availability and experimental warming: (i) a shift in the competition of algae and bacteria for inorganic nutrients, and/or (ii) an enhanced bacterial degradation of organic matter. The substantially higher net accumulation of POP under N- compared to P-deficient conditions, albeit an initially equal availability of DIP, may, in fact, suggest a pronounced competition of algae and bacteria for phosphorus in the latter treatments. With an average molar C:N:P ratio of 50:10:1 (Fagerbakke *et al.* 1996), bacterial cells typically display substantially higher requirements for phosphorus than algal cells (Vadstein 2000) and have been suggested to be superior competitors for inorganic nutrients (Currie and Kalff 1984). The

stimulating effect of rising temperature on bacterial activities, as observed in this study, may have enhanced this competition even further, thereby leading to the temperature-dependent decrease in ΔPOP. An enhanced demand for phosphorus was also indicated by the activity of APA, an enzyme that is produced by both algal and bacterial cells (Coleman 1987) and cleaves organic phosphorus compounds into utilisable phosphate units. Maximum turnover rates of APA were markedly higher in the P-limit compared to the N-limit treatments throughout the experiment. In addition, a pronounced response of APA to rising temperature was observed under the former conditions, in particular during the build-up phase of the bloom. However, Vadstein (2000) suggested in his review on the role of heterotrophic bacteria in aquatic phosphorus cycling that the bacterial superiority in nutrient acquisition may be substantially weakened in high-nutrient situations with a pulsed supply of the limiting resource, as investigated here, due to low cellular storage capacities and hence a reduced ability to efficiently retain the acquired nutrients. In comparison, diatoms have evolved large vacuoles that enable them to store inorganic nutrients in sufficient amounts for less favourable conditions, and hence to deprive their competitors of these resources (Falkowski *et al.* 2004). These findings suggest that nutrient competition between *Skeletonema costatum* and the natural bacterial community was most likely not the dominating process in our study. Alternatively, the observed decrease in ΔPOP in response to nutrient stoichiometry and rising temperature may also result from shifts in the heterotrophic degradation of organic compounds. In line with this idea, the P-limit treatments displayed distinctly higher bacterial activities than the N-deficient treatments throughout the experiment, as depicted by the rates of BSP and extracellular enzymes. Moreover, these activities were also clearly stimulated by warming under P-deficient conditions, whereas the response to rising temperature was substantially dampened or even nonexistent in the respective N-treatments.

The observed changes in heterotrophic activities in response to warming and depending on the prevailing type of nutrient deficiency were also reflected by changes in the biogeochemical pools of organic matter. Thus, POC concentrations displayed a marked decrease at the most elevated temperature of the P-limit treatments, thereby indicating bacterial decomposition of the POC pool, whereas this was not observed in the respective N-limit treatments. In contrast to POC, the concentration of POP remained high until the end of the experiment in both P- and N-limit treatments. While the lack of a clear heterotrophic degradation signal under N-deficient conditions is in accordance with the overall reduced degradation activity of bacteria, the absent decrease in POP concentration under P-deficient conditions may rather indicate a rapid remineralisation and efficient reutilisation of phosphorus compounds, as the cycling of

phosphorus is generally acknowledged to be faster than that of carbon. In line with this, a preferential remineralisation of phosphorus compounds has been reported for both particulate (Shaffer *et al.* 1999) and dissolved organic matter (Clark *et al.* 1998).

Partitioning of carbon between dissolved and particulate organic matter

In our study, distinct effects of both rising temperature and the prevailing kind of nutrient limitation on the accumulation of dissolved organic compounds were observed. Thus, DOC concentrations displayed a distinct increase in the P-limit treatments after the onset of nutrient deficiency, whereas this was considerably reduced under N-deficient conditions. The timing of the accumulation suggests that algal exudation due to a cellular overflow of excess photosynthates was the primary source and similar observations have been made frequently both in laboratory and mesocosm studies (Myklestad 1995; Norrman *et al.* 1995; Obernosterer and Herndl 1995). Moreover, the increase in DOC under P-deficient conditions was accompanied by a substantial accumulation of polysaccharides, which have been shown to comprise a major fraction of freshly produced algal exudates (Benner *et al.* 1992; Biddanda and Benner 1997; Biersmith and Benner 1998). In accordance with DOC data, the increase in polysaccharides in the respective N-limit treatments was markedly lower. These differences in bulk accumulation of DOC and PCHO may be partly explained by the overall higher production of biomass in the P- compared to the N-deficient microcosms. To account for such differences in biomass development, the net increase in polysaccharides (ΔPCHO) was normalised to the respective maximum biomass accumulating in the water column, i.e. ΔPOC. This revealed that ΔPCHO accounted for 3.1 to 4.2% of ΔPOC under N-deficient conditions and was independent of the ambient temperature. In comparison, the proportion of ΔPCHO on ΔPOC substantially increased in response to warming under P-deficient conditions from 4.8% at 4°C, being comparable to the N-limit treatments, up to 14.4% at 12°C. Moreover, increasing the temperature by up to 8°C also markedly accelerated the rate, at which polysaccharides accumulated, by a factor of 4 in the P-limit treatments, whereas the N-limit microcosms remained apparently unaffected. Together, these findings strongly suggest a distinct, temperature-driven shift in the partitioning of organic carbon between particulate and dissolved pools, which is, however, only detectable under P-deficient conditions.

Although the bacterial community in the P-limit treatments exhibited a clear stimulation by rising temperature in terms of increasing biomass production rates and hydrolytic extracellular enzyme activities, it was apparently not able to counterbalance the release of DOC and polysaccharides. A seasonal accumulation of DOC in the surface ocean, in particular at the end of phytoplankton blooms, has also been reported

from various oceanic areas (Carlson *et al.* 1994; Copin and Avril 1993), where it is generally attributed to a temporal decoupling of production and consumption processes. Thus, while the autotrophic release of dissolved carbon-rich material is often stimulated by nutrient deficiency at the end of phytoplankton blooms due to continuing photosynthetic carbon uptake, the microbial consumption of these compounds may be limited by several processes. Here, both a partly high resistance of the accumulating matter to rapid microbial decomposition (Fry *et al.* 1996), and an inefficient bacterial utilisation of these compounds due to nutrient limitation (Thingstad *et al.* 1997) have been suggested as possible mechanisms. The chromatographic analysis of monomeric components, which constitute the polysaccharide pool, showed that glucose represented the most abundant polysaccharide-subunit. While free dissolved glucose has been shown to support a substantial fraction of bacterial carbon demand of > 30% in the ocean (Rich *et al.* 1996) and thus to be labile, the opposite may apply for combined glucose bound in dissolved polysaccharides (Kirchman *et al.* 2001). Hence, the observed predominance of glucose in the PCHO pool during our study may potentially indicate a reduced bioavailability of dissolved polysaccharides. In addition, a temperature-dependent shift in the structural complexity of the accumulating material was suggested by a pronounced decrease in the activity of leucine-aminopeptidase, targeting short-chained peptides, relative to BOC-peptidase, an endopeptidase that splits more complex, long-chained peptides.

Prevailing carbon limitation of bacterial growth has been one of the central paradigms of microbial ecology. This notion has been challenged in recent years by a rising number of empirical studies showing that bacteria may in many cases be limited by the availability of phosphorus or nitrogen rather than carbon, hence causing the accumulation of otherwise degradable DOC compounds due to a lowered uptake efficiency (Rivkin and Anderson 1997; Zweifel *et al.* 1993). Similarly, a phosphorus deficiency of the mixed algal-bacterial community was clearly indicated in our study by (i) the enhanced activity of the DOP-cleaving enzyme alkaline phosphatase (APA), which may originate from both phytoplankton and bacteria, and (ii) a marked increase in the particulate C:P ratio, being on average approximately 4 times higher than the Redfield ratio C:P = 106:1. Yet, the POP pool displayed signs of a rapid remineralisation and efficient reutilisation of phosphorus compounds, which may have provided the community with short pulses of phosphate.

As the rates of particulate primary production in the P-limit treatments did not differ significantly between temperature treatments, and dissolved primary production was as low as under N-deficient conditions (P. Breithaupt, *pers. comm.*), the described mechanisms would only result in an accumulation of the released material, with the

initiation of DOC accumulation being dependent on the onset of nutrient limitations. This clearly indicates the presence of another, temperature-sensitive process contributing to the enhanced accumulation of DOC and PCHO at elevated temperature. A number of studies have reported that bacteria may themselves produce copious amounts of slow-to-degrade DOC (Brophy and Carlson 1989; Kawasaki and Benner 2006; Ogawa *et al.* 2001). The potential mechanisms of bacterial DOC release include the partial hydrolysis of polymeric substances (Azam 1998), as well as the release of a polysaccharide-rich matrix, which is often surrounding bacterial cells (Heissenberger *et al.* 1996) and has been shown to be constantly renewed during growth (Stoderegger and Herndl 1998). Metabolically active bacteria may thus rather contribute to the accumulation of DOC than to its consumption. Consequently, the observed stimulation of bacterial activities by rising temperature may have been responsible for the temperature-dependent increase in DOC and PCHO concentrations in our study.

Interactive effects of rising temperature and nutrient availability, and their implications for the future marine carbon cycle

In the present study, the interactive effects of rising temperature and nutrient availability in the form of either N- or P-deficient growth conditions exerted a strong impact on the build-up and partitioning of organic matter, as well as on its utilisation by heterotrophic bacteria. Most strikingly, the mixed communities displayed considerably different response patterns to rising temperature, when grown under either N- or P-deficient conditions, with a clear stimulation of nearly all monitored parameters under P-deficiency, and an either absent or at least substantially dampened reaction under N-deficiency. Thus, rising temperature caused, for instance, marked shifts in the magnitude and composition of the DOC pool in P-deficient microcosms, whereas the respective N-deficient treatments remained unaffected. Likewise, warming also stimulated the bacterial production of biomass and the activities of extracellular enzyme under P-deficient conditions. In contrast, the bacterial community in the N-limit microcosms displayed only a weak increase with rising temperature (i.e. BSP) or even no response at all (i.e. extracellular enzyme activities). This ubiquitous difference between the two nutrient treatments in their sensitivity to experimental warming clearly raises questions regarding the underlying mechanisms.

It is well known that phosphorus compounds are cycled very rapidly within the marine environment (Dyhrman *et al.* 2007), and evidence is increasing that P may seasonally become limiting for both autotrophic and heterotrophic growth in certain ocean areas (e.g., freshwater-influenced coastal zones and marginal seas, e.g the Baltic Sea (Zweifel *et al.* 1993), the North Pacific (Karl *et al.* 2001) and Atlantic subtropical gyres

(Ammerman *et al.* 2003), and the Mediterranean Sea (Thingstad *et al.* 2005). The high demand of microorganisms for phosphorus is further underscored by the observed preferential remineralisation of phosphorus compounds in sinking particulate organic matter (Knauer *et al.* 1979; Shaffer *et al.* 1999) and the pool of dissolved organic matter (Clark *et al.* 1998). Similarly, the net build-up of POP displayed a pronounced decrease with rising temperature under P-deficient conditions in our study. In concert with the observed stimulation of bacterial biomass production and bacterial exoenzyme activities by warming, this strongly suggests an elevated remineralisation of phosphorus compounds under P-deficient conditions, which possibly provided the mixed algal-bacterial community with short pulses of phosphorus throughout the experiment. Additionally, high rates of APA also suggest intermittent phosphorus availability through cleaving of DOP. In contrast, a slower remineralisation of nitrogenous compounds, due to low heterotrophic activities, may have caused a more persistent nutrient-deficient situation in the N-limit communities, hence impeding any temperature-driven stimulation of cellular processes.

This notion is further corroborated, if we consider the cellular function and utilisation of phosphorus and nitrogen compounds. Nitrogen in cells is mostly present in the form of proteins, whereas the major phosphorus-containing components are nucleic acids (i.e., RNA, DNA), and phospholipids (i.e., the building block of cell membranes). RNA is the most P-rich cellular fraction and constitutes a major fraction of ribosomes, the cellular organelles responsible for protein synthesis and thus for growth (Geider and LaRoche 2002). However, ribosomes also contain by weight approximately one third protein (Geider and LaRoche 2002). From a simplistic point of view, the cellular allocation of nutrients can be divided into (i) an assembly machinery (i.e. ribosomes), being rich in phosphorus, but also containing nitrogen, and (ii) a resource acquisition machinery (e.g. proteins for nutrient uptake enzymes or photosynthetic pigments), which is rich in nitrogen, but contains little or no phosphorus. The acquisition machinery thus depends on protein supply by the assembly machinery. These fundamental differences in the cellular allocation of phosphorus and nitrogen may help to explain the observed discrepancies in the response of the P- and N-limit communities to rising temperature. Thus, under P-deficient conditions microorganism growth would be limited due to an inability to sufficiently produce RNA for the synthesis of new ribosomes. On the other hand, as discussed earlier, the stimulation of bacterial activities probably led to increased remineralisation of phosphorus compounds, which, together with an efficient uptake of the recycled material, may have refuelled the algal-bacterial community with short phosphorus pulses. In contrast, an organism experiencing nitrogen limitation may be hindered in two ways: it may either allocate nitrogen sources to the synthesis of

ribosomes, which are in turn a prerequisite for the assembly of proteins and enzymes necessary for various cellular functions, or to the resource acquisition machinery, which is, for instance, responsible for energy supply via light-harvesting pigments. The high Chl a-ratio between the P-limit and N-limit treatments, yielding a biomass factor of 2 compared to POC- value of 1.6, indeed suggests that N-deficient cells allocated fewer resources per biomass into the production of pigments and the acquisition of energy than P-deficient cells.

In concert, the observed findings indicate pronounced changes in the partitioning and fate of organic carbon in response to the prevailing type of nutrient limitation and increasing sea surface temperature, with likely consequences for food web structure and the biological carbon sequestration to depth. Thus, organic carbon accumulating in the water column was mostly present in particulate form under N-deficient growth conditions, whereas the concentration of DOC remained fairly low. Moreover, also the bacterial utilisation of these organic pools was markedly reduced and responded only weakly to experimental warming. Overall, the prevailing accumulation of POC may, together with the restricted microbial degradation, facilitate the efficient transport of organic carbon from the surface ocean to depth. In contrast, rising temperature clearly shifted the partitioning of organic carbon between the particulate and dissolved pools towards the latter under P-deficient conditions. Moreover, warming clearly stimulated the bacterial degradation of organic carbon, hence indicating increased carbon flow through the microbial loop. Together, these changes in the cycling of organic matter in the water column when shifting communities from N- to P-limitation may affect food web structure and reduce the availability of organic matter fro transport to depth via the biological carbon pump, with likely consequences for the net air-sea flux of CO_2.

Although nitrogen is believed to be the major growth limiting nutrient throughout most of the ocean, several marine habitats have been shown to be at least seasonally limited by the availability of phosphorus. The projected changes in precipitation and nutrient loading are likely to further increase the potential for P-limitation in these areas. This may in particular affect coastal ecosystems, which rank amongst the most productive oceanic regions and provide important ecosystem services (e.g. food supply, recreational value), due to their close vicinity to land and the associated large influence of river runoff and atmospheric dust deposition. According to our findings, shifting such systems from N- to P-limitation may significantly alter the biogeochemical cycling of organic matter in the ocean and overall ecosystem functioning.

V.
Synthesis & Future Perspectives

V. SYNTHESIS AND FUTURE PERSPECTIVES

Planktonic food web processes play a pivotal role in the biogeochemical cycling of carbon, nitrogen, phosphorus and other elements in the ocean, provide the base for global fisheries, and contribute to the net air-sea flux of CO_2. Despite this overarching importance, the current knowledge on their response to global climate change and the associated chemical and physical perturbations of the marine environment is still in its infancy. This thesis aims at elucidating the effects of rising sea surface temperature and altered nutrient stoichiometry on the biogeochemical cycling and fate of organic matter in the surface ocean. The first two chapters of this thesis explored the response of a natural plankton community to a range of four different temperatures, corresponding to the *in situ* condition and three elevated temperature scenarios as projected by the IPCC until the end of the 21st century. In the third chapter, model algal-bacterial communities were exposed to three temperatures and two different dissolved inorganic nitrogen:phosphorus-ratios in a factorial combination, simulating weak to strong warming as projected for the Baltic Sea region during winter as well as changes in the anthropogenic nutrient loading of coastal ecosystems. The P-deficient situation described in this latter chapter is comparable to the naturally occurring growth conditions of the western Baltic Sea in springtime as seen in *Chapter 1* and *2*, and is hereafter referred to as "*Chapter 3-P*".

In this *Synthesis*, the observed biotic responses to these perturbations will be discussed and integrated into the present state of knowledge on climate change. This will be followed by a perspective on future research directions, which emerge from this thesis.

1. The use of microcosm and mesocosm set-ups to investigate the cycling of organic matter in planktonic food webs: possible advantages and disadvantages

The studies described in this thesis have made use of different experimental approaches: a novel indoor-mesocosm set-up, in which natural plankton communities were investigated (*Chapter 1, 2*), as well as a microcosm experiment with a model algal-bacterial assemblage (*Chapter 3*). This section aims at discussing the advantages, but also the potential drawbacks of these approaches.

Since the 1980s, mesocosms have been a widely used tool in the aquatic sciences to investigate, for instance, food web interactions (e.g. Christoffersen *et al.* 1993; Larsen *et al.* 2001; Riemann 1985; Stibor *et al.* 2004; Zöllner *et al.* 2009), nutrient enrichment effects (e.g. Olsen *et al.* 2006; Øvreås *et al.* 2003), or particle formation

mechanisms (Dam and Drapeau 1995; Engel *et al.* 2002; Mopper *et al.* 1995). Due to their size of usually several hundreds of liters up to the m^3-scale, they are capable of harbouring whole plankton communities up to the level of planktivorous fish. In contrast to laboratory-based microcosm or chemostat experiments, in which generally individual species or selected assemblages are kept under controlled, but often artificial environmental settings, the use of mesocosms provides the unique opportunity to manipulate natural communities under close to *in situ* conditions. In addition, the mesocosm approach allows to expose these communities to a gradual manipulation of an environmental factor or even to test their response to a factorial combination of several stressors, as well as to replicate the individual treatments. Together, these characteristics make mesocosm studies a valuable tool that bridges the gap between artificial laboratory and *in situ* field studies, in which the effects of the various environmental stressors often cannot be disentangled properly.

More recently, mesocosms have also been adopted to study the effects of climate change on aquatic food web processes and the biogeochemical cycling of organic matter (e.g. ocean acidification: Engel *et al.* 2005; Riebesell *et al.* 2008; Riebesell *et al.* 2007; vertical mixing depth: Berger *et al.* 2007; surface ocean warming: Hoppe *et al.* 2008; Sommer *et al.* 2007). In the study described in *Chapters 1* and *2*, investigating the effects of rising sea surface temperature on the development of a typical spring bloom and the associated cycling of organic matter within, use was made of a novel indoor-mesocosm set-up (see also *Chapter 1* and Sommer *et al.* 2007) for technical details). In brief, eight tanks, made of food-safe polyethylene and with a volume of 1.4 m^3 each, were deployed in temperature-controlled climate chambers. Light benches above each of the mesocosms, equipped with full-spectrum light tubes covering the range of photosynthetically active radiation (PAR), provided close-to-natural light conditions with a computer-generated diel 12:12 hours light:dark-cycle. Unfiltered seawater, containing a natural plankton community, was pumped from 6 m depth from the Kiel Fjord and was simultaneously distributed onto the eight mesocosms. The water column was gently mixed by means of a propeller, thereby keeping algal cells and smaller particles in suspension, whereas larger particles that formed during the bloom sedimented out of the water column and accumulated at the bottom of the enclosures.

A first feasibility study of the experimental set-up during spring 2005 showed that the system is indeed capable to reproduce the typical succession of plankton organisms as observed during the spring bloom of temperate ocean areas, starting with the development of phytoplankton biomass up to the emergence of the next mesozooplankton generation towards the end of the bloom (Sommer *et al.* 2007).

However, the experiment also revealed several drawbacks of the set-up, which should be taken into account for future studies. Besides the fact that the removal of a water mass from its natural surroundings and the release from other external forces, e.g. vertical mixing or lateral advection, creates in itself a to a certain degree artificial situation, the development of biofilms is one of the most frequent problems occurring in mesocosm experiments. Here, in particular a high ratio of wall area to total volume (Berg *et al.* 1999) and the application of low light intensities can promote wall growth. While the wall area:volume-ratio of the here described mesocosms was even lower than the one recommended by Berg *et al.* (1999) for pelagic process studies, apparently too low light intensities, corresponding to ~16% of ambient surface irradiance, led to a slow development of phytoplankton biomass and provided a niche for benthic microalgal growth in the 2005 experiment. While this did not perturb the successional pattern of plankton organisms (Sommer *et al.* 2007), it created an unfavourable situation for biogeochemical and microbiological investigations of organic matter pools and fluxes. Thus, the temporal advantage of low-light adapted benthic over pelagic species led to a considerable loss of inorganic nutrients from the water column, which, in turn, reduced the overall pelagic production of organic matter. The net build-up of suspended POP, for instance, accounted only for ~15-20 % of initially available DIP. This set-up artefact posed severe difficulties for the calculation of mass balances for carbon, nitrogen, and phosphorus from measurements of the respective dissolved inorganic and particulate and dissolved organic pools, as it was in particular impossible to attribute changes in the dissolved compartments to either the benthic or the pelagic phase. As dissolved organic compounds also provide important carbon and nutrient sources for bacterial growth, the strong biofilm development inextricably also perturbed measurements of bacterial biomass production, respiration, and of extracellular enzyme activities. Based on the results obtained during this feasibility study, a maximum duration of 7-8 weeks was suggested for future experiments (Sommer *et al.* 2007). Consequently, this prohibits the use of this mesocosm set-up for investigations of longer-term effects of climate change stressors on plankton communities and of potential evolutionary adaptation mechanisms. Unfortunately, a similarly strong appearance of benthic microalgae also impeded the evaluation of results gained in a later study in spring 2007, where a light intensity of 32% of ambient surface irradiance was applied. The results obtained during the experiments in 2005 and 2007 were hence not incorporated into this thesis.

In the mesocosm study in spring 2006 (*Chapter 1, 2*), the problem of low-light induced biofilm development was avoided by supplying the pelagic community with a higher daily light dose, closely matching with the one reported for the North Atlantic spring bloom (Siegel *et al.* 2002). This led to a rapid development of distinct

phytoplankton blooms in all treatments and a subsequent decline to initial biomass values within 4 weeks. In contrast to the above described experiments, a development of wall growth was not observed in this study. The build-up of POP accounted for ~63-77% of the initially available amount of DIP (*Chapter 1*), and the loss of organic matter from the water column was dominated by sinking of particulate organic matter (POM) to the bottom of the mesocosms (*Chapter 2*).

While the experiment was highly successful and allowed us to closely follow the build-up and degradation of the bloom and the associated fluxes between dissolved inorganic and dissolved and particulate organic matter pools, the bottom accumulation of POM may, however, also constitute another potential drawback of the set-up. The shallow depth of the mesocosms unfortunately does not allow to physically separate the sedimented material from the upper water column, for instance by means of a density gradient creating an upper mixed and a lower unperturbed layer. In the experiment in 2006, this has likely led to the intrusion of a respiration signal at the very end of the bloom, which may have originated from degradation processes occurring at the bottom of the mesocosms. In order to exclude potential effects of such set-up artefacts on mass balance calculations, the respective calculations were restricted to the period of bloom-build-up.

In the study described in *Chapter 3*, a microcosm approach was applied to investigate the combined effects of rising sea surface temperature and altered nutrient stoichiometry on a model algal-bacterial community in a factorial design. The microcosms consisted of polycarbonate bottles with a volume of 25 L each. Natural seawater was collected in late spring and was left to age in the dark for several weeks. Prior to the experiment, the water was filtered in order to retain only the bacterial community and inoculated with an axenic culture of *Skeletonema costatum*, which was the dominant algal species in the previous mesocosm experiments. Albeit such laboratory-based investigations with single key species or mixed assemblages certainly possess a more artificial character than, for instance, mesocosm studies with natural plankton communities, they are still a useful tool to investigate individual components and distinct processes of the complex marine food web. Moreover, this smaller-scale set-up allowed us to apply a statistically appropriate replication of each factor combination, which would not have been possible with the limited number of available mesocosms.

2. Shifting the balance – Effects of sea surface warming and altered nutrient stoichiometry on the biogeochemical cycling and fate of organic matter

The complex interplay between the photosynthetic production of organic matter by phytoplankton cells and its consumption and respiration through heterotrophic processes in the surface ocean not only determines the flow of energy and matter through the planktonic food web and the total productivity of marine systems, but also critically controls the biological sequestration of climatically important elements, i.e. carbon, to the ocean interior, and hence the ocean's capacity to take up and store atmospheric CO_2. Accordingly, changes in the export of photosynthetically bound carbon from the sunlit surface layer to the deep sea via the biological carbon pump have been suggested as a potential mechanism contributing to the glacial-interglacial changes in atmospheric CO_2 (Sigman and Boyle 2000).

The studies presented in this thesis revealed that the projected warming of the surface ocean due to anthropogenic climate change may also significantly alter the biogeochemical cycling of organic matter by shifting the balance between the autotrophic production and heterotrophic consumption processes, and could hence provide an important feedback mechanism to the Earth's climate system.

Temperature is an important environmental factor that directly controls the rate of biological processes. Their individual temperature-sensitivities can, however, display a large variability, with autotrophic processes being in general less dependent on ambient temperature than heterotrophic ones. In line with this, different parameters characterising autotrophic growth and photosynthetic build-up of organic matter, i.e. changes in chlorophyll a (Chl a) concentration and primary production rates, remained fairly insensitive to rising temperature during the meso- and the microcosm studies, resulting only in a weak acceleration of the bloom onset by 1-1.75 days $°C^{-1}$ (*Chapters 1-3*). While this is in good accordance with the widely acknowledged temperature-independence of phytoplankton production under light-limited conditions (Tilzer *et al.* 1986), it underscores the importance of other environmental factors in determining the timing and magnitude of phytoplankton spring blooms in the field, e.g. light and nutrient availability. In line with this, a ~30% increase in POC-normalised maximum values of Chl a and primary production rates was observed under phosphorus- compared to nitrogen-deficient growth conditions (*Chapter 3*), whereas increasing the ambient light intensity from 16% to 64% of surface irradiance led to a temporal advancement of the spring bloom of up to 1.5 months (Sommer and Lengfellner 2008).

In contrast to the autotrophic food web compartment, rising temperature exerted a distinctly stronger control on the activity and timing of various heterotrophic processes driving organic matter loss in the surface ocean. Thus, already at the peak of the

phytoplankton bloom, rates of bacterial secondary production (BSP) displayed 2- to 3-fold higher values at elevated temperature (*Chapters 2, 3-P*), whereas the activity of the bacterially-derived extracellular enzyme β-D-glucosidase increased by a factor of 1.75 over the applied temperature range (*Chapter 3-P*). Interestingly, measurements of bacterial respiration did not show a pronounced effect of warming, which is in contrast to the reported decrease in bacterial growth efficiency with rising temperature (Rivkin and Legendre 2001). The lacking response in the bacterial fraction was, however, compensated by a two-fold increase of community respiration rates in the size fraction > 3 µm (*Chapter 2*), likely indicating a transfer of bacterially-consumed carbon to higher trophic levels through, for instance, protist grazing. Considering that rising temperature will also affect algal metabolic rates and that phytoplankton biomass presumably dominated in this larger size fraction, it is likely that the observed respiration signal was also partly caused by enhanced algal respiration.

The marked differences in the response patterns of the autotrophic and heterotrophic food web components to experimental warming further mediated pronounced changes in the build-up and composition of particulate and dissolved organic matter pools. While the initial phase of the developing spring bloom was dominated by autotrophic processes, as characterised by a rapid decline in dissolved inorganic nutrients and a concomitant increase in Chl a and particulate organic matter concentrations, the stimulation of heterotrophic activities by warming led to an enhanced and accelerated consumption and respiration of organic matter, which became visible already at the peak of the bloom. This resulted in a significant decline in the net drawdown of dissolved inorganic carbon (DIC) by the plankton community, and reduced the sinking loss of particulate organic carbon from the water column (*Chapter 2*). Moreover, a pronounced decrease in the net build-up of POP was observed with rising temperature, which likely indicates a preferential remineralisation of the growth-limiting element by heterotrophic microbes (*Chapter 1* and *3-P)*.

Experimental warming also affected the partitioning of organic carbon between the particulate and dissolved pools by stimulating a substantially higher accumulation of dissolved carbon-rich compounds (*Chapter 1, 3-P*). The timing of the increase in dissolved organic carbon (DOC) concentration, i.e. at the onset of inorganic nutrient limitation, and the high proportion of dissolved polysaccharides, which have been shown to compose a major fraction of fresh phytoplankton exudates (Biddanda and Benner 1997; Biersmith and Benner 1998), strongly suggested a predominantly algal origin of the accumulating material. The release of carbon-rich photosynthates by phytoplankton cells is a phenomenon that frequently occurs at the end of phytoplankton blooms (Carlson *et al.* 1998; Carlson *et al.* 1994; Copin and Avril 1993) and has been proposed

to represent an overflow mechanism at times, when photosynthetic carbon fixation continues, but the lack of inorganic nutrients impedes the synthesis of biomass (Fogg 1966; Wood and Van Valen 1990).

Rising temperature did not only increase the absolute concentrations of DOC and polysaccharides, but also significantly accelerated their accumulation. However, as both particulate and dissolved primary production rates displayed a fairly temperature-independent behaviour in the here described studies (P. Breithaupt, *pers. comm.*; see also *Chapter 1*), hence not supporting enhanced algal exudation in response to warming, it becomes clear that another process most likely contributed to this temperature-dependent behaviour. Heterotrophic bacteria are generally perceived as the major consumers of DOM in seawater (e.g. Azam *et al.* 1983). However, in recent years evidence has grown that they can also be active producers of DOC (Azam 1998; Brophy and Carlson 1989; Heissenberger and Herndl 1994; Ogawa *et al.* 2001). Potential mechanisms of bacterial DOC release include the partial hydrolysis of biodegradable polymeric matter through non-specific reactions of bacterial extracellular enzymes (O'Brien and Herschlag 1999; Ogawa *et al.* 2001; Williams 2000), and the release of capsular material, which surrounds bacterial cells, during high metabolic activities. DOC of bacterial origin has been shown to be highly resistant to rapid microbial utilisation over time scales of up to >1 year, and only 10-15% were identified as hydrolysable amino acids and sugars in a recent study by Ogawa et al. (2001). According to these findings, the stimulation of both bacterial biomass production and extracellular enzyme activities by rising temperature, observed in *Chapters 2* and *3-P*, may have contributed substantially to the temperature-dependent accumulation of presumably biorefractory DOC in the water column.

The consumption of the dissolved fraction of primary produced organic matter in the surface ocean largely occurs via the microbial loop, where dissolved compounds are either incorporated into bacterial biomass and thereby transferred to higher trophic levels (i.e. a trophic link) or where they are respired back into their inorganic constituents (i.e. a trophic sink) (Azam *et al.* 1983; Ducklow *et al.* 1986). Another pathway leading to the transformation of dissolved organic matter into particulate form, hence making it available for utilisation by higher trophic levels again, is the abiotic aggregation of dissolved compounds to so-called transparent exopolymer particles (TEP). The formation of TEP from dissolved polysaccharide-rich precursor material via abiotic aggregation has been shown both in laboratory (Chin *et al.* 1998; Kerner *et al.* 2003) and mesocosm studies (Engel *et al.* 2004). Due to their surface-active nature TEP play an important role in the aggregation and sedimentation of phytoplankton blooms (Engel 2004; Passow and Alldredge 1994). In *Chapter 2*, two distinct phases of TEP production

were observed, which differed in their sensitivity to warming. Thus, an initial rise in TEP concentration was observed during the build-up phase of the bloom with highest values at *in situ* temperature. While TEP concentrations declined thereafter in the colder treatments, a second increase occurred at the end of the bloom in the most elevated temperature treatments, which exceeded the first peak by several-fold. Concomitant to the second rise in TEP concentration, a decline in the polysaccharide pool was observed, hence suggesting the abiotic aggregation of these compounds into TEP. Enhanced bacterial activities at elevated temperature may have further promoted TEP-formation through the release of persistent DOC compounds and/or an overworking and selective utilisation of the present material, which has been shown to affect the hydrophobic nature of DOM (Giroldo and Vieira 2003). However, the role of TEP-formation in the aggregation and sedimentation of POM in the here presented study remains elusive, as the increase occurred when particulate organic matter concentration had already decreased to almost pre-bloom levels.

Chapter 3 of this thesis investigated the interactive effects of rising sea surface temperature and differing nutrient stoichiometry, i.e. in the form of either phosphorus- or nitrogen-deficient growth conditions, on the cycling of organic matter within a model algal-bacterial community. Contrasting to the above described observations under phosphorus-deficient conditions, low nitrogen availability caused distinctly different community response patterns to rising temperature with, for instance, a markedly weaker stimulation of heterotrophic activities. In addition, also the partitioning of organic carbon between particulate and dissolved matter remained unaffected by warming. Overall, nitrogen-deficient growth conditions may hence facilitate an efficient transport of organic carbon to depth due to a restricted microbial utilisation and a predominant accumulation of particulate organic carbon.

While nitrogen is widely accepted as the major nutrient limiting marine primary production (Falkowski 1997; Tyrrell 1999), a (seasonal) limitation by phosphorus has been reported for several ocean areas (Dyhrman *et al.* 2007; Elser *et al.* 2007). Moreover, expected changes in the nutrient supply to the marine environment through increased vertical stratification and altered inputs of allochthonous nutrients due to anthropogenic climate change may further enhance the potential for a phosphorus limitation of ocean productivity (Dyhrman *et al.* 2007; Jickells 1998).

Given these projections, the above described shift in the balance between autotrophic production and heterotrophic consumption processes of organic matter in response to simulated surface ocean warming under phosphorus-deficient conditions has a strong potential to significantly alter the biogeochemical cycling of organic matter in the upper ocean, with likely consequences for the structure and functioning of the

marine food web and the biological sequestration of carbon to depth. Illustrating the observed effects in a simplified sketch of the involved key processes (Fig. 1), rising temperature led in particular to (I) an enhanced and accelerated consumption and respiration of organic matter, likely due to a pronounced channelling through the microbial loop, and (II) an increased accumulation of dissolved relative to particulate organic carbon. These alterations resulted in (III) a reduced net community drawdown of DIC in the water column and (IV) lowered the availability of organic carbon for export processes. Ultimately, the observed changes in organic matter flow through the planktonic food web may significantly reduce the transfer of energy and matter to higher trophic levels and weaken the efficiency of the biological carbon pump, thereby providing a positive feedback mechanism to Earth's climate system.

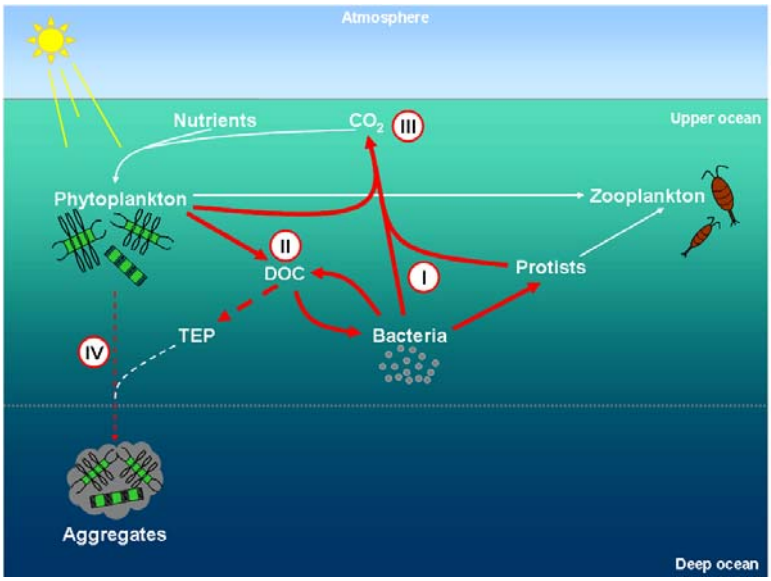

Figure 1: Simplified sketch of upper ocean food web processes driving organic matter cycling. Processes investigated in this thesis, which were found to be sensitive to surface ocean warming, are marked in red. According to the here observed temperature effects on individual food web components with (I) an enhanced heterotrophic consumption and respiration of organic matter relative to its autotrophic production, with a presumably higher fraction of primary production passing through the microbial loop, and (II) a pronounced shift in the partitioning of organic carbon between its particulate and dissolved pools towards the latter, sea surface warming may significantly (III) reduce the net community drawdown of DIC in the surface ocean and (IV) lower the availability of organic carbon for export processes. To what extent enhanced TEP formation may have promoted organic matter aggregation (white dashed line) remains elusive, as TEP concentrations only increased at the very end of the spring bloom. In concert, these alterations in the interplay of food web processes and the associated biogeochemical cycling of organic matter have a strong potential to reduce the transfer of energy and matter to higher trophic levels and to weaken the efficiency of the biological carbon pump.

3. Future perspectives

In the following, I would like to highlight three potential lines of future research, emerging both from observations made in this thesis and from personal interests.

As discussed in the first synthesis section, the AQUASHIFT mesocosm set-up was found to be not perfectly ideal from a biogeochemical perspective, as their shallow depth impeded to separate the water column into an upper mixed and a lower unperturbed water layer, for instance through the establishment of a density gradient. While in natural bloom situations aggregated organic matter, sinking below the upper mixed layer, is removed from processes in the surface, the sedimented material accumulating at the bottom of the mesocosms was not properly separated from the upper water column. This bears a high risk of a resuspension of sedimented matter, and also of an intrusion of, for instance, degradation signals, which originate from the bottom-accumulated matter. Moreover, the set-up did not allow to install sediment traps and hence impeded a thorough quantification of organic matter loss through sinking of particulate matter. As the vertical transport of biologically fixed carbon from the surface ocean to depth via the biological pump is one of the key processes driving the net air-sea flux of CO_2, thereby contributing to the mitigation of climate change, it is imperative to quantify changes in the export flux of organic carbon in response to a changing marine environment. Hence, future studies investigating the effects of environmental perturbations, such as rising sea surface temperature, on pelagic surface ocean processes will require mesocosms of larger size, ideally both in depth and in width, for a more realistic representation of upper ocean processes. Having large and deep mesocosms, a separation of the water column into an upper mixed and a lower unperturbed layer would then allow to quantify organic matter vertical fluxes by use of sediment traps, which are installed in the lower water layer and collect the material that sinks down from above, and would also minimise the risk of lateral advection of resuspended material from the bottom and side walls of the mesocosm. However, increasing the mesocosm size will logically result in several new difficulties. Due to the spatial restrictions of climate chambers, the application of larger-size mesocosms would require to move again from an indoor- to an outdoor-environment. While this is, to some extent, a step forward towards more realistic conditions, providing for instance natural daylight, it may prove particularly difficult to simulate different degrees of sea surface warming, as done in the here presented mesocosm study. Heating large water bodies electrically is very energy-intensive and needs a continuous supply with electricity and thus a connection to land. This may, however, still be feasible for land-based medium-sized mesocosm systems. Alternatively, Berger and co-workers (2007) conducted a lake mesocosm experiment (mesocosm diameter 0.95 m, maximum mesocosm depth 8m,

corresponding to a maximum volume of ~ 5.7 m^3) in which they created different water temperatures by surrounding some of the enclosures with a second bag, which was larger in overall size and depth. The water within this outer bag was kept well-mixed, thereby creating a cooling water bath for the inner mesocosms. Although certainly not easy to be done, this would provide a energy-saving possibility to create different water temperatures, and may hence also be suitable for larger-scale mesocosms in marine environments. Similarly, surrounding such mesocosms individually with water hoses that continuously circulate cold water from greater depth by use of a water pump, thereby cooling down the inside water column of the mesocosm, may also have some potential. In conclusion, the appropriate design of such mesocosm studies depends on the individual requirements and research tasks. With a suitable and feasible experimental set-up at hand, new research tasks, such as the following, can be approached.

The studies presented in this thesis have focussed on the direct effects of rising sea surface temperature on the various biological processes involved in the cycling of organic matter through the planktonic food web. In the future ocean, these will further interact with indirect temperature effects, i.e. increasing light availability and decreasing nutrient supply from deeper waters due to enhanced vertical stratification of the upper water column, as well as other anthropogenically induced perturbations of the ocean's chemical and physical properties, such as CO_2-induced ocean acidification, a reduction of the oceanic mixed layer depth through changes in the hydrological cycle, or alterations in allochthonous nutrient loading (Solomon *et al.* 2007). While several of these co-occurring climate change stressors have already been shown to independently affect life in the pelagic realm (e.g. Orr *et al.* 2005; Riebesell 2000; Riebesell *et al.* 2007), our knowledge of their interactive effects, likely yielding complex and non-linear responses of marine pelagic communities, is still rudimentary. The study described in *Chapter 3*, for instance, clearly showed that a change in the initial ratio of inorganic nitrogen and phosphorus availability can cause pronounced shifts in the reaction of a mixed algal-bacterial community to rising temperature. While both the autotrophic and heterotrophic components of the food web remained fairly insensitive to experimental warming under nitrogen-deficient conditions, it stimulated particularly the heterotrophic consumption and respiration of organic matter in the respective phosphorus-deficient treatments (*Chapters 2,3-P*). In the latter situation, this reduced both the net community uptake of dissolved inorganic carbon (DIC) and the availability of particulate organic carbon for export processes and may thereby provide a positive feedback to climate change (*Chapter 2*).

Moreover, Litchman and co-workers (2009) recently showed, using an evolutionary game theoretical model, that climate change-driven shifts from nitrogen- to phosphorus-

limitation and/or a shallowing of the mixed layer depth can lead to a decrease in diatom cell size, thereby further adding to the reduced transfer of carbon and energy to higher trophic levels and the lowered sequestration of carbon to depth due to decreased sinking under future ocean conditions. In contrast to these findings, rising atmospheric CO_2 concentrations were shown in a large-scale mesocosm study to increase the net community uptake of DIC by a natural plankton community and to promote an efficient transport of this extra carbon to depth (Riebesell *et al.* 2007), hence potentially providing a negative climate feedback.

Given these examples of possible effects of individual climate change stressors on marine biota and the biological sequestration of climatically active elements to the ocean interior, it is imperative to gain a better understanding of their combined synergistic or antagonistic strengths in order to predict potential biotic feedbacks to Earth's climate system and how the ocean's capacity to mitigate climate change will develop in the future. This will require a multiple approach of small-scale laboratory studies, focussing on a mechanistic understanding of underlying food web processes using key species and mixed assemblages, to larger-scale mesocosm and field studies, investigating climate change effects at the community and ecosystem level. Together, these combined efforts will help to improve the incorporation of currently underrepresented biological processes into global atmosphere-ocean models and hence to develop realistic simulations of future climate scenarios.

In recent years, our perception of seawater as an environment for pelagic microorganisms and microbially-driven processes has changed drastically. While the organic matter pool used to be separated into a dissolved and a particulate fraction by an arbitrary choice of filter pore sizes, it is now widely accepted that organic matter in seawater exists in a continuum of size classes from single macromolecules over colloids to truely particulate matter. Moreover, it was discovered that a large fraction of the formerly "dissolved" material may be present in the form of gel-like networks, which physically structure the seawater fluid (Verdugo *et al.* 2004 and references therein). Thus, up to 10% of surface DOC may belong to this marine gel phase (Chin *et al.* 1998; Guo and Santschi 1997; Stordal *et al.* 1996). These nanoscale habitats have been suggested to provide microorganisms with "hot spots" of high substrate and nutrient concentration, and to serve as attachment sites or as refuges from predation (Verdugo 2004), and may hence display important interfaces of intense organismic interactions as well as organic matter turnover.

In the here described studies, rising sea surface temperature led to a pronounced increase in the release and accumulation of dissolved carbon-rich compounds of both autotrophic and heterotrophic origin into the water column (*Chapter 1, 3-P*). Moreover, it

also stimulated the subsequent aggregation of this material to so-called transparent exopolymer particles (TEP; *Chapter 1*), which possess gel-like properties. Thus, surface ocean warming not only affected the amount of organic matter, serving as a substrate for microbial growth and respiration, but likely also altered the microenvironment in which the turnover of organic matter occurs. In order to understand climate-change driven shifts in microbial activities in the bulk seawater medium, it may prove essential to investigate the interactions between microorganisms themselves and their surrounding environment on the nanoscale more closely. This will require new methodological approaches combining classical activity measurements with molecular tools to identify key members and their ecological function, as well as with microscopical and biochemical techniques to make the structure and composition of the gel-matrix visible.

Remaining at the nanoscale, another important aspect of organic matter processing in the pelagic realm that has long been neglected is the role of marine viruses in mediating biogeochemical fluxes of carbon and other biologically important elements. Viruses are major agents of both autotrophic and heterotrophic mortality in the ocean (Brussaard 2004; Thingstad 2000; Weinbauer 2004) and may affect the biogeochemical cycling of organic matter in the surface ocean through a myriad of ways. Due to a high host-specificity of most viruses, they actively shape the composition and diversity of microbial communities (Bouvier and Del Giorgio 2007; Weinbauer 2004; Weinbauer and Rassoulzadegan 2004; Wommack and Colwell 2000), thereby also controlling ecological functions of microbes in the marine food web. Moreover, viral lysis of living cells and organisms leads to the release of dissolved and particulate organic compounds (e.g. Middelboe and Lyck 2002; Middelboe *et al.* 2003). As this material is largely circuited into the microbial food web, where it is either incorporated again into microbial biomass or respired back into CO_2 and inorganic nutrients, viral lysis may decrease the trophic transfer of energy and matter to higher trophic levels and reduce the availability of particulate organic matter for export via the biological pump (Suttle 2005). However, it has also been suggested that viral activities may at times increase the efficiency of biological carbon export by altering the stoichiometry of the lysed material (Suttle 2007) or by increasing sinking rates of certain phytoplankton species through infection (Lawrence and Suttle 2004). As up to one fourth of annual primary production can pass through the so-called viral shunt (Wilhelm and Suttle 1999), it becomes clear that virus-mediated fluxes of organic matter are an important component of the pelagic realm and should be incorporated into global carbon cycle models. Despite the progress that has been made in recent years, unraveling many virus-driven processes in marine food webs, current knowledge on the response potential of the virosphere and associated alterations of organic matter fluxes to changing

environmental conditions caused by anthropogenic climate change are yet completely unknown. As we have seen in this thesis, it is in particular the microbial communities that will respond strongly to changing environmental conditions, thereby also altering biogeochemical fluxes of carbon and other elements in the surface ocean. In order to improve our understanding of the biotic processes in the marine carbon cycle and the potentially resulting feedbacks to the climate system, it is indispensable to also include viruses and their role in marine food webs in upcoming studies on the future ocean.

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PARTS OF THIS THESIS HAVE BEEN PUBLISHED IN PEER-REVIEW JOURNALS OR ARE READY FOR SUBMISSION:

PUBLISHED ARTICLES:

- **Chapter 2**

  Julia Wohlers, Anja Engel, Eckart Zöllner, Petra Breithaupt, Klaus Jürgens, Hans-Georg Hoppe, Ulrich Sommer, Ulf Riebesell (2009). Changes in biogenic carbon flow in response to sea surface warming. *Proc. Nat. Acad. Sci.* 106:7067-7072

  Additionally, supplemental information (SI) is available online at *www.pnas.org/cgi/content/short/0812743106*. The SI contains material from **Chapter 1**.

MANUSCRIPTS TO BE SUBMITTED:

- **Chapter 3:**

  Julia Wohlers, Petra Breithaupt, Katja Walther, Anja Engel, Peter Fritsche, Nicole Händel, Hans-Georg Hoppe, Klaus Jürgens, Andrea Ludwig, Eckart Zöllner, Ulf Riebesell. Interactive effects of rising temperature and nutrient availability on organic matter build-up, composition, and degradability.

In addition, I have contributed to the following publications and manuscripts in the framework of the AQUASHIFT-project:

- Ulrich Sommer, Nicole Aberle-Malzahn, Anja Engel, Thomas Hansen, Kathrin Lengfellner, Marcel Sandow, **Julia Wohlers**, Eckart Zöllner, Ulf Riebesell (2007). An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of Baltic Sea phyto- and zooplankton. *Oecologia* 150:655-667

- Judith Piontek, Nicole Händel, Gerald Langer, **Julia Wohlers**, Ulf Riebesell, Anja Engel (2009). Effects of rising temperature on the formation and microbial degradation of marine diatom aggregates. *Aquat. Microb. Ecol.* 54:305-318

- Ursula Gaedke, Miriam Ruhenstroth-Bauer, Ina Wiegand, Katrin Tirok, Nicole Aberle-Malzahn, Petra Breithaupt, Kathrin Lengfellner, **Julia Wohlers**, Ulrich Sommer. Spring phytoplankton dynamics depend on temperature, cloudiness, grazing and overwintering biomasses – a process oriented modelling study based on mesocosm experiments. (accepted for publication in *Global Change Biol.*)

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Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertation, abgesehen von der Beratung durch meinen akademischen Lehrer, nach Inhalt und Form meine eigene Arbeit ist und ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Des Weiteren versichere ich, dass die vorliegende Dissertation weder im Ganzen noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat und unter Einhaltung der Regeln guter wissenschaftlicher Praxis entstanden ist.

Kiel, den 10. Juni 2009 Julia Wohlers