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Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO₂ levels: a mesocosm study

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Received: 13 November 2007 – Accepted: 19 December 2007 – Published: 29 January 2008

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Abstract

The predicted rise in anthropogenic CO₂ emissions will increase CO₂ concentrations and decrease seawater pH in the upper ocean. Recent studies have revealed effects of pCO₂ induced changes in seawater chemistry on a variety of marine life forms, in particular calcifying organisms. To test whether the predicted increase in pCO₂ will directly or indirectly (via changes in phytoplankton dynamics) affect abundance, activities, and community composition of heterotrophic bacteria during phytoplankton bloom development, we have aerated mesocosms with CO₂ to obtain triplicates with three different partial pressures of CO₂ (pCO₂): 350 μatm (1×CO₂), 700 μatm (2×CO₂) and 1050 μatm (3×CO₂). The development of a phytoplankton bloom was initiated by the addition of nitrate and phosphate. In accordance to an elevated carbon to nitrogen drawdown at increasing pCO₂, bacterial production (BPP) of free-living and attached bacteria as well as cell-specific BPP (csBPP) of attached bacteria were related to the C:N ratio of suspended matter. These relationships significantly differed among treatments. However, bacterial abundance and activities were not statistically different among treatments. Solely community structure of free-living bacteria changed with pCO₂ whereas that of attached bacteria seemed to be independent of pCO₂ but tightly coupled to phytoplankton bloom development. Our findings imply that changes in pCO₂, although reflected by changes in community structure of free-living bacteria, do not directly affect bacterial activity. Furthermore, bacterial activity and dynamics of heterotrophic bacteria, especially of attached bacteria, were tightly linked to phytoplankton development and, hence, may also potentially depend on changes in pCO₂.

1 Introduction

Photosynthetically derived organic carbon is one of the major carbon and energy sources for heterotrophic bacteria in the pelagic ocean (Azam et al., 1983). In general, 10% of the primary production is released as dissolved organic matter (DOM, Carlson

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et al., 1998). During phytoplankton bloom development the organic carbon is made available for bacteria through different pathways. Healthy and dying algal cells can release large amounts of DOM into their surrounding (Sharp, 1977; Mykkestad, 1995). Massive production of extracellular algal polymers often occurs under nutrient depletion, as the synthesis and exudation of polymers often increase under these conditions (e.g. Obernosterer and Herndl, 1995; Biddanda and Benner, 1997; Søndergaard et al., 2000). Changes in phytoplankton exudation during algal growth lead to varying concentrations and chemical signatures of the released DOM pool which greatly affect activities and community composition of heterotrophic bacteria (e.g. Biersmith and Benner, 1998; Grossart et al., 2005). On the other hand, phytoplanktonic DOM can be released via viral lysis (Brussaard and Riegemann, 1998; Suttle et al., 1990) and autolysis of cells. Although lysis may occur permanently during algal blooms, both processes are mainly associated with algal senescence. Thus, lysis may for short periods of time represent a much larger input of DOM to the system than excretion. Additionally, in the open ocean a substantial fraction of the phytoplankton biomass (>90%) may be grazed by zooplankton (Longhurst, 1983). This percentage, however, can greatly vary among different ecosystems and is usually much lower in continental shelf and upwelling regions (Kiørboe, 1993). In general, viral lysis and zooplankton grazing can be considered as major causes of phytoplankton bloom decline during which high concentrations of DOM can be released in a short period of time. Thus, the difference between continuous excretion of DOM and cell lysis with massive pulses of DOM release may be of significant ecological relevance. For example, a steady but relatively low release of DOM may select for bacteria with slow growth whereas massive phytoplankton lysis may select for bacteria which quickly respond to short but large DOM pulses. Excretion and leaching from intact phytoplankton as well as release due to viral lysis and zooplankton grazing have been recognized as the main pathways of DOM from phytoplankton to bacteria (Jumars et al., 1989). Changes in bacterial action on DOM of algal origin greatly impact nutrient and energy fluxes including various pathways: microbial loop, sinking, grazing food chain, storage, and fixation (Azam, 1998)

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and, hence, are of global importance for oceanic carbon cycling.

The role of heterotrophic bacteria in decomposing phytoplankton-derived particulate organic matter (POM) and phytodetrital aggregates has been studied in great detail. Senescent planktonic algae and aggregates are rapidly colonized by bacteria (e.g. Smith et al., 1995; Simon et al., 2002) which have a repertoire of hydrolytic enzymes (Hoppe et al., 1993; Martinez et al., 1996) for efficient POM solubilisation to DOM (Smith et al., 1992; Grossart and Ploug, 2001). For example, silicon dissolution is greatly enhanced by the proteolytic activities of bacteria attached to diatoms resulting in a significant reduction of the particulate organic carbon (POC) sinking flux (Bidle and Azam, 1999). The freshly produced DOM is then preferentially taken up by bacteria and may contribute substantially to bacterial production.

Even though DOM of algal origin can be rapidly used by bacteria, a seasonal accumulation of DOM in the oceanic photic zone is commonly observed (Williams, 1995) and may indicate a semi-labile nature of the released dissolved organic carbon (DOC; Søndergaard et al., 2000). A substantial fraction (25–35%) of DOC released from phytoplankton can even resist microbial degradation for years (Fry et al., 1996). In addition, accumulation of refractory and semi-labile DOC in the ocean may also be of bacterial origin (Ogawa et al., 2001). Alternatively to low bioavailability, temporary accumulation of POC and DOC has been explained by a “malfunctioning microbial loop”, e.g. when nutrient availability limits bacterial growth and viral lysis as well as grazing the bacterial biomass (Thingstad et al., 1997; Williams, 1995). However, DOM released by phytoplankton does not only serve as bacterial substrate but may also inhibit bacterial activities (Cole, 1982) and may affect formation of particles, such as transparent exopolymer particles (TEP) and aggregates (Grossart et al., 2006a). Most notably, TEP have been identified as an important agent for aggregation (Passow, 2002). Various studies have shown that TEP are produced by planktonic algae, but also by bacteria and from dissolved precursor material (Zhou et al., 1998; Passow, 2002; Engel et al., 2004). Those processes, however, greatly depend on the physiological state of the algae (Grossart et al., 2006a).

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Many recent studies on phytoplankton carbon acquisition mechanisms indicate large effects on physiology and composition due to on-going changes in aquatic pCO₂ (e.g. Burkhart et al., 2001; Tortell et al., 2002). The expected change in pCO₂ will result in a pH drop by ca. 0.35 units by the year 2100 (Wolf-Gladrow et al., 1999) and may even drop by 0.7 units over the next two centuries (Caldeira and Wickett, 2003). This may result in a significant reduction of biogenic calcification, in particular of coccolithophores (Riebesell et al., 2000), a higher loss of POC (Engel et al., 2005), and changes in particle flux to deeper waters (Armstrong et al., 2002; Klaas and Archer, 2002). Due to changes in phytoplanktonic production of extracellular organic matter concentration of TEP may increase with increasing pCO₂ (Engel et al., 2004). Thus, changes in pCO₂ may also affect the C:N ratio of the algae and, hence, degradability of phytoplankton derived DOC and POC in the ocean. In a previous mesocosm study (Grossart et al., 2006b) we have shown a measurable but indirect effect of changes in pCO₂ on bacterial abundance and activities, which was mainly linked to algal and presumably particle dynamics.

Hence, the main purpose of the present study was to evaluate whether the expected future changes in pCO₂ will change abundance, activities, and community structure of heterotrophic bacteria during the build up and decline of a phytoplankton bloom. We wanted to test whether heterotrophic bacteria are directly affected by changes in pCO₂ or more indirectly react to pCO₂ induced changes in phytoplankton bloom development (as has been proposed by our earlier paper; Grossart et al., 2006b).

2 Materials and methods

2.1 Experimental set up and sampling

The mesocosm study was performed between 15 May and 9 June 2005 at the Esperegrend Marine Biological Station (at Raunefjorden, 60.2° N, 5.1° E) of the University of Bergen, Norway. Nine polyethylene enclosures (~27 m³, 9.5 m water depth) were

moored to a raft equipped with a small floating laboratory. The enclosures were simultaneously filled with unfiltered, nutrient-poor, post-bloom fjord water from 13.5 m depth. To avoid disturbances by faecal matter of seabirds and to maintain headspaces at target $p\text{CO}_2$, the enclosures were covered by gas-tight tents made of ETFE foil, which allowed for 95% light transmission of the complete spectrum of sunlight. The mesocosms were aerated with CO_2 to obtain triplicates of three different levels, $350 \mu\text{atm}$ ($1 \times \text{CO}_2$), $700 \mu\text{atm}$ ($2 \times \text{CO}_2$) and $1050 \mu\text{atm}$ ($3 \times \text{CO}_2$) (for details see Riebesell et al., 2007, and Schulz et al., 2007). Continuous flushing of the tents with air adjusted to target CO_2 concentrations ensured that starting values were kept in the headspace throughout the experiment. The addition and subsequent mixing of 800 litres of freshwater into the upper 5.5 m of the mesocosms, resulted in water column stratification with a salinity gradient of 1.5 psu between the surface mixed layer ($S=30.5$ psu) and the underlying water column. A homogenous distribution of dissolved compounds was achieved by continuous mixing of the upper layer by peristaltic pumps (flow rate 450 L h^{-1}). The development of a phytoplankton bloom was initiated by the addition of nitrate and phosphate (initial concentrations of $14 \mu\text{mol L}^{-1} \text{NO}_3$ and $0.7 \mu\text{mol L}^{-1} \text{PO}_4$). The experiment started at a post-bloom Si(OH)_4 level of $3.2 \mu\text{mol L}^{-1}$. Development and decline of the phytoplankton bloom was monitored daily over a 24 day period. Depth-integrated water samples were taken daily at 10 a.m. by means of a 5 m long, 8 cm diameter tube which was lowered into the mesocosms, closed at the top, pulled up onto the raft and emptied into sampling bottles. Bottles were stored until further processing in a cold room adjusted to the in situ temperature of the fjord. For comparison water samples were also taken from the adjacent fjord at the same depth.

Throughout the whole experiment a multitude of environmental parameters, such as NO_3 , PO_4 , Si(OH)_4 , particulate organic carbon (POC), C:N ratio, transparent exopolymer particles (TEP), algal abundance, chlorophyll *a* (Chl-*a*), dissolved organic carbon (DOC) (see Schulz et al., 2007), primary production (PrProd) (Egge et al., 2007), and viral abundance (Larsen et al., 2007) were continuously monitored. Particulate and dissolved dimethylsulfoniopropionate (DMSP_p and DMSP_d , respectively), and dimethyl-

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sulphide (DMS) were measured by Vogt et al. (2007).

2.2 Bacterial numbers

For enumeration of free and particle-associated bacteria 1 or 5 mL of seawater were filtered onto black 0.2 and 5.0 μm pore size Nuclepore membranes, respectively. The filters were stained with SYBR Gold (Invitrogen) and stored frozen at -20°C until counting. Bacteria were counted by epifluorescence microscopy (DR-MB, Leica, Germany) at 1000x magnification. The number of free bacteria was calculated by subtracting the number of particle-associated bacteria (5.0 μm filters) from that of total bacteria (0.2 μm filters). Comparison with flow cytometry (Paulino et al., 2007) indicated that our numbers were overestimated due to the presence of big viruses within a size range of 100–200 nm which could not be reliably distinguished from bacteria by epifluorescence microscopy. Therefore, the abundance of free-living bacteria has been corrected for the abundance of big viruses.

2.3 Bacterial production

Rates of bacterial protein production (BPP) were determined by incorporation of ^{14}C -leucine (^{14}C -Leu, Simon and Azam, 1989). Triplicates and a formalin-killed control were incubated with ^{14}C -Leu ($1.15 \times 10^{10} \text{ Bq mmol}^{-1}$, Amersham, England) at a final concentration of 50 nmol L^{-1} , which ensured saturation of uptake systems of both free and particle-associated bacteria. Incubation was performed in the dark at in situ temperature ($9\text{--}11.5^{\circ}\text{C}$) for 1 h. After fixation with 2% formalin, samples were filtered onto 5.0 μm (attached) and 0.2 μm (total isotope incorporation) nitrocellulose filters (Sartorius, Germany) and extracted with ice-cold 5% trichloroacetic acid (TCA) for 5 min. Thereafter, filters were rinsed twice with ice-cold 5% TCA, once with ethanol (96% v/v), and dissolved with ethylacetate for measurement by liquid scintillation counting. Standard deviation of triplicate measurements was usually $<15\%$. BPP of free bacteria was calculated by subtraction of attached BPP from total BPP. The amount of incorporated

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^{14}C -Leu was converted into BPP by using an intracellular isotope dilution factor of 2. A conversion factor of 0.86 was used to convert the protein produced into carbon (Simon and Azam, 1989). Cell-specific BPP was calculated for both bacterial fractions by taking the respective cell numbers into account.

5 2.4 DNA extraction and PCR amplification of 16S rRNA gene fragments

Particle-associated and free-living bacteria were separated by sequential filtration of the water samples throughout 5.0 and 0.2 μm Nuclepore polycarbonate filters, respectively. Particle-associated bacteria were retained by filtering 150 ml of sample onto a 5.0 μm Nuclepore membrane, whereas free-living bacteria were collected by filtering 100 ml of the 5.0 μm filtrate onto a 0.2 μm Nuclepore membrane. Filters were transferred into sterile Eppendorf tubes and kept frozen at -20°C until DNA extraction. Extraction of genomic DNA was performed, using a standard protocol with phenol/chloroform/isoamylalcohol, SDS, polyvinylpyrrolidone, and zirconium beads as described previously (Allgaier and Grossart, 2006).

For denaturing gradient gel electrophoresis (DGGE) a 550 bp fragment of the 16S rRNA gene was amplified using the primer pair 341f and 907r (5' – CCT ACG GGA GGC AGC AG – 3' and 5' – CCG TCA ATT CMT TTG AGT TT – 3', Muyzer et al., 1998). At the 5'-end of the primer 341f an additional 40 bp GC-rich nucleotide sequence (GC-clamp) was added to stabilize migration of the DNA fragment in the DGGE (Muyzer et al., 1993). The PCR reaction mixture contained: 2–5 μl template DNA, each primer at a concentration of 200 nM, each deoxyribonucleoside triphosphate at a concentration of 250 μM , 2 mM MgCl_2 , 5 μl of 10 \times PCR reaction buffer, and 0.5 U of BIOTAQ Red DNA Polymerase (Bioline) in a total volume of 50 μl . PCR amplification was performed with a Gradient Cycler PT-200 (MJ Research) using the following conditions: initial denaturation at 95°C (3 min), followed by 30 cycles of denaturation at 95°C (1 min), annealing at 55°C (1 min), and extension at 72°C (2 min). A final extension at 72°C for 10 min completed the reaction.

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2.5 Denaturing gradient gel electrophoresis (DGGE) and sequencing

DGGE was performed as described previously (Allgaier and Grossart, 2006b) in a 7% (v/v) polyacrylamide gel with a denaturing gradient from 40 to 70% of urea and formamide. Prior loading of PCR products onto DGGE gels, DNA was quantified on agarose gels using a quantitative DNA ladder (Low DNA Mass Ladder, Invitrogen) and similar amounts of DNA were loaded onto the DGGE gels. DGGE gels were run in 1×TAE electrophoresis buffer (40 mM Tris-HCl (pH 8.3), 20 mM acetic acid, 1 mM EDTA) for 20 h at a constant voltage of 100 V and a constant temperature of 60°C. The gels were stained with 1×SYBR Gold (Invitrogen) and documented using an AlphaImager 2200 Transilluminator (Biozym).

For sequencing, several DGGE bands were excised and incubated over night at 35°C in elution buffer (0.5 M Ammonia acetate, 1 mM EDTA). Eluted DNA fragments were precipitated using 0.4 vol. 7.5 M ammonia acetate and 0.7 vol. isopropanol and purified with 70% ethanol. DNA fragments were sequenced as described previously (Allgaier and Grossart, 2006a) using primers 341f and 907r.

2.6 Analyses of DGGE profiles

DGGE banding patterns were analyzed by using the Software packages GelCompar II version 3.5 (Applied Maths) and PRIMER 5, version 5.2.9 (PRIMER-E Ltd.). Within GelCompar II first a band based binary presence/absence table was calculated applying Dice similarity coefficient. This presence/absence table was imported into the software PRIMER 5 and used for hierarchical clustering analyses and analyses of similarity (ANOSIM). To avoid distortions originating from non-normal distribution of the species data of the DGGE gels we used a Bray-Curtis similarity matrix for cluster analyses and ANOSIM rather than the original data matrix. For cluster analyses the *complete linkage* algorithm was used provided by the software package. This algorithm calculates distances between clusters (Clarke and Gorley, 2001).

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2.7 Phylogenetic analysis

Phylogenetic analyses of the partial 16S rRNA gene sequences were done using the ARB software package (<http://www.arb-home.de>). The retrieved sequences were imported into an ARB database of 52 000 reference sequences including the closest related sequences determined by BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). Sequences were first aligned automatically by the integrated alignment module within the ARB package and subsequently corrected manually. For stability of the phylogenetic tree a backbone tree was calculated comprising only sequences of ≥ 1400 nucleotides. Sequences ≤ 1400 nucleotides were added afterwards to the tree according to maximum parsimony criteria. Consistence of branching patterns was checked applying the three phylogenetic reconstruction methods: neighbor-joining, maximum parsimony, and maximum likelihood to the appropriate set of sequences. The final tree was calculated using the maximum likelihood algorithm.

2.8 Nucleotide sequence accession numbers

Partial sequences of 16S rRNA gene fragments obtained in this study have been deposited in GenBank with the following accession numbers: EU179278–EU179311.

2.9 Statistical analysis

Statistical analyses were done by repeated measures ANOVA for analyzing the treatment effect using the software SPSS 9.0. Furthermore, we used the “linear regression” module of the SPSS 9.0 software. All regressions were tested for dependency on pCO₂ by applying the CHOW test with univariate GLM. Dependencies of bacterial parameters from other environmental parameters were tested by “stepwise” multiple regression analysis. Significance was given at values < 0.05 .

To test the significance of differences between the DGGE banding patterns ANOSIM (Clarke and Green, 1988) was applied. ANOSIM generates a test statistic (R) which

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is an indication of the degree of separation between groups. A score of 1 indicates complete separation whereas a score of 0 indicates no separation. DGGE banding patterns were also analyzed by non-metric multidimensional scaling (NMS) ordinations using the software package PC-ORD, Version 4.0 (MJM Software Design). Similar to ANOSIM a binary presence/absence table was used for all NMS analyses. The advantage of NMS over other multivariate statistical methods (e.g. canonical correspondence analysis, CCA) is that this method uses rank order information of a similarity matrix of the samples rather than the original data matrix. Thus, NMS avoids distortions originating from the non-normal distribution of the species data of the DGGE gels (McCune and Grace, 2002).

3 Results

3.1 Bacterial abundance

Abundances of either free-living or attached bacteria were almost identically among all mesocosms irrespective of their pCO₂ (Fig. 1a and b). Except for high abundances at the beginning of the experiment, free-living bacteria reached a pronounced maximum of ca. $5\text{--}6 \times 10^6$ cells mL⁻¹ between days 12–16 (Fig. 1a) during the decline of the algal bloom. Abundances of free-living bacteria dropped to a low of ca. $1\text{--}3 \times 10^6$ cells mL⁻¹ between days 18–20 and thereafter increased to ca. $3\text{--}6 \times 10^6$ cells mL⁻¹ at the end of the experiment when dinoflagellate and cyanobacterial abundances increased. Numbers of attached bacteria were lower and showed a different temporal pattern (Fig. 1b). Numbers of attached bacteria were relatively high at the beginning of the experiment but declined until day 6. Attached bacteria reached a maximum of $0.28\text{--}0.42 \times 10^6$ cells mL⁻¹ between days 10–14, slightly earlier than free-living bacteria. Thereafter, they slowly decreased to $\sim 0.20\text{--}0.23 \times 10^6$ cells mL⁻¹ at the end of the experiment.

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3.2 Bacterial protein production (BPP)

BPP of both free-living and attached bacteria followed primary production (Egge et al., 2007) with a lapse of ca. 2 days (Fig. 2a and b). BPP of free-living bacteria increased from day 6 on and varied between 1.5 and $3.2 \mu\text{g C L}^{-1} \text{h}^{-1}$ on day 10 and further increased until day 14 to $2.8\text{--}3.9 \mu\text{g C L}^{-1} \text{h}^{-1}$ (Fig. 2a). BPP of free-living bacteria was low (ca. $0.4\text{--}1.4 \mu\text{g C L}^{-1} \text{h}^{-1}$) between days 18–22 and only slightly increased at the end of the experiment. BPP of attached bacteria increased slightly earlier (between days 6–8) but much steeper ($3.1\text{--}4.8 \mu\text{g C L}^{-1} \text{h}^{-1}$) than that of free-living bacteria (Fig. 2b). A second peak occurred on day 14 in parallel to that of free-living bacteria and reached $2.9\text{--}4.6 \mu\text{g C L}^{-1} \text{h}^{-1}$. Similarly to free-living bacteria, BPP of attached bacteria was rather low ($0.9\text{--}2.2 \mu\text{g C L}^{-1} \text{h}^{-1}$) between days 18–22 and slightly increased at the end of the experiment.

3.3 Cell-specific bacterial protein production (csBPP)

CsBPP of both free-living and attached bacteria showed a similar temporal pattern but greatly differed in magnitude (Fig. 3a and b). A pronounced maximum of csBPP of free-living bacteria occurred on days 6–8 ($1.1\text{--}2.4 \text{fg C cell}^{-1} \text{h}^{-1}$, Fig. 3a) almost in parallel to the first peak in primary production indicating a strong coupling between phytoplankton growth and bacterial activity. However, csBPP of free-living bacteria was rather low ($0.6\text{--}0.8 \text{fg C cell}^{-1} \text{h}^{-1}$) during and shortly after the second peak in primary production. Thereafter, csBPP of free-living bacteria continuously decreased towards the end of the experiment. CsBPP of attached bacteria increased in parallel to primary production and reached a first peak ($16\text{--}36 \text{fg C cell}^{-1} \text{h}^{-1}$) on days 6–8. In parallel to csBPP of free-living bacteria, csBPP of attached bacteria was also lower ($7\text{--}14 \text{fg C cell}^{-1} \text{h}^{-1}$) during and shortly after the second peak in primary production. CsBPP of attached bacteria further dropped (to $3\text{--}10 \text{fg C cell}^{-1} \text{h}^{-1}$) between days 18–24 when primary production and phytoplankton abundance was rather low.

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3.4 Statistical analysis

None of the parameters tested by repeated measures ANOVA revealed a statistically significant dependency on pCO₂ (data not shown). Linear regression analysis between measured bacterial parameters and environmental variables, however, revealed several highly significant relationships (Table 1). Abundance as well as BPP of both free-living and particle-associated bacteria negatively correlated to NO₃, PO₄, and Si(OH)₄ indicating that bacterial abundance and BPP were closely linked to the temporal course of phytoplankton development. The positive relationships between NO₃ and csBPP of both free-living and attached bacteria, however, may indicate a direct dependency of bacterial activity on NO₃ availability.

BPP and csBPP of both free-living and attached bacteria were positively linked to almost all algal parameters (chlorophyll *a* (Chl-*a*), abundances of *Emiliania huxleyi* (Ehux) and diatoms (Diatoms), primary production (PrProd), dissolved and particulate dimethylsulfoniopropionate (DMSP_p and DMSP_d, respectively), and dimethylsulfide (DMS) but negatively linked to abundances of cyanobacteria (Cyano), total viruses (Virus), virus in the size range of 100–200 nm (Big Virus), and surprisingly to DOC. POC and C:N ratio of the suspended matter were positively correlated to BPP but not to csBPP of free-living and attached bacteria suggesting that increased POC and C:N ratios do not affect the activity of individual cells but set an activity level for total bacteria. CsBPP of attached bacteria was negatively correlated to TEP. Bacterial numbers (both free-living and attached), however, were positively related to POC, C:N, TEP, DOC, and DMSP_{p+d} (and DMS) indicating that the presence of potential bacterial substrates lead to increases in bacterial abundance.

To reduce the number of significant correlations between measured bacterial and environmental variables we have used a stepwise multiple regression analysis (Table 2). For particulates (Table 2a) the analysis revealed that BPP of free-living and attached bacteria was tightly linked to DMSP_p. Additionally, BPP of attached bacteria was negatively correlated with TEP and positively with C:N ratio of particulates. CsBPP of free-

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living was exclusively positively linked to Ehux whereas csBPP of attached bacteria was negatively related to the abundance of Cyano and TEP and positively to DMSP_p. Abundance of free-living bacteria was positively related to POC, Cyano, DMSP_p, and Ehux whereas abundance of attached bacteria was positively correlated with TEP and POC. The contrary effect of TEP on csBPP and abundance of attached bacteria indicates that TEP -due to its sticking properties- may be efficient in scavenging bacteria from the surrounding water but a poor bacterial substrate.

For dissolved organic matter and primary production (Table 2b) the analysis revealed that BPP of free-living and attached bacteria was positively linked to DMS, DMSP_d, and PrProd but in a varying order. PrProd most strongly correlated with csBPP of free-living and attached bacteria. CsBPP of free-living bacteria was also positively related to DMS whereas csBPP of attached bacteria was negatively related to DOC. Abundance of both free-living and attached bacteria, however, was closely linked to DMSP_d, DMS, and DOC but in a varying order.

The stepwise multiple regression analysis for virus revealed a negative relationship between the abundance of big virus and most bacterial parameters (BPP and abundance of free-living and attached bacteria and csBPP of free-living bacteria). Interestingly, csBPP of free-living and attached bacteria were negatively but abundances of both free-living and attached bacteria positively correlated to total virus. To get a better estimate on virus production we have estimated viral production by $n_t - n_{t-1}$ (whereby n_t = viral abundance at a given time point and n_{t-1} = viral abundance 1 day before sampling time point) and specific viral production by $(n_t - n_{t-1}) / (n_t + n_{t-1})$. Our estimates revealed that solely numbers of free-living and attached bacteria were positively correlated with viral production and even better with specific viral production. In contrast, production of big virus negatively correlated with csBPP of free-living and attached bacteria but not with their abundance. This suggests that the role of virus in controlling bacterial dynamics can be contrary. Besides bacteriophages Larsen et al. (2007) found several phytoplankton viruses in the present experiment. Preferential lysis of algal cells may result in release of phytoplanktonic DOM and, hence, serves as

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an important DOM source for bacteria.

To further test for dependency on $p\text{CO}_2$ we have performed the CHOW test with univariate GLM (Table 3). Solely, linear regressions between BPP of free-living bacteria, BPP of attached bacteria or csBPP of attached bacteria and the C:N ratio of suspended matter revealed a significant effect of $p\text{CO}_2$.

3.5 Molecular characterization of free-living and particle-associated bacterial communities

Structural diversity of bacterial communities was investigated by DGGE analyses of partial 16S rRNA gene fragments. DGGE analyses were performed for free-living and particle-associated bacteria separately to obtain higher phylogenetic resolution and to receive closer information on potential responses of bacterial communities to differences in $p\text{CO}_2$ concentrations. Investigation of bacterial community structure was conducted for mesocosms M2 ($3\times\text{CO}_2$), M5 ($2\times\text{CO}_2$), and M8 ($1\times\text{CO}_2$), each representing one of the three different $p\text{CO}_2$ treatments. Samples were taken in intervals of 4 to 6 days from the beginning (day zero, T0) to the end (day 24, T24) of the experiment. In all mesocosms both free-living and particle-associated bacteria showed a relatively high phylogenetic diversity in respect to absolute numbers of DGGE bands. Numbers of DGGE bands varied between 11–21 (mean 15 ± 2.7) and between 12–25 (mean 17 ± 3.3) for free-living and particle-associated bacteria, respectively. Even though free-living and particle-associated bacteria showed similar numbers of DGGE bands, distinct differences occurred in their respective banding patterns (Fig. 4). However, as determined by ANOSIM of the DGGE banding patterns differences between free-living and particle-associated bacteria communities were significant only within mesocosm M5 ($p\leq 0.001$). Differences between the two bacterial fractions in mesocosms M2 and M8 were not statistically significant ($p\geq 0.05$).

As indicated by cluster analyses of DGGE banding patterns and ANOSIM community composition of free-living bacteria significantly differed between mesocosms (Fig. 4, Table 4). In contrast, cluster analyses of particle-associated bacteria did not reveal

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any clustering between samples of the three mesocosms (Fig. 4) and no significant differences between mesocosms were observed by ANOSIM (Table 4).

Cluster analyses of particle-associated bacteria, however, indicate temporal clusters which can be directly related to phytoplankton bloom development (Fig. 4). Three major clusters (Cluster 1–3) were identified, representing a) initiation (T0) and end of the experiment (T24), b) phytoplankton bloom increase (T6) and decline (T18), and c) maximum of the phytoplankton bloom (T10 and T14), respectively. DGGE banding patterns of both free-living and particle-associated bacteria from the fjord formed distinct clusters which were clearly separated from those in the mesocosms (Fig. 4).

NMS analyses of DGGE banding patterns support the results of our ANOSIM analyses and reveal rather distinct populations of free-living bacteria (Fig. 5a) but highly intermixed populations of particle-associated bacteria (Fig. 5b) at different pCO₂ levels. In both bacterial fractions the samples from the fjord formed a narrow cluster which, especially for particle-associated bacteria, was different from those in the mesocosms. Samples M2-T6 and M5-T18 of the free-living bacteria were exceptional in their position in the NMS ordination plot which may have been the result of their very weak banding pattern.

3.6 Phylogenetic analyses of sequenced DGGE bands

Sequencing and phylogenetic characterization of selected DGGE bands indicated the occurrence of *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Actinobacteria* in the three investigated mesocosms. We also detected several sequences originating from chloroplasts of *Emiliana huxley*, uncultured diatoms, and members of the phylum *Prasinophyta* (Fig. 6). Except for the single actinobacterial sequence belonging to freshwater *Actinobacteria*, all other sequences were phylogenetically affiliated to marine bacterial clusters. No distinct differences in bacterial community composition were observed on the phylogenetic level between the three mesocosms in respect to different pCO₂ concentration and phytoplankton bloom development. We also did not observe any distinct phylogenetic differences between free-living and particle-

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associated bacteria. Sequences of free-living and particle-associated bacteria were distributed equally throughout the constructed phylogenetic tree without indicating formation of fraction-specific clusters (Fig. 6).

4 Discussion

4.1 Dependency of microbial dynamics on $p\text{CO}_2$

In a previous mesocosm study using a similar experimental set up Grossart et al. (2006b) found that growth rate and BPP of heterotrophic bacteria were highest at highest $p\text{CO}_2$ whereas bacterial abundance remained similar for most time of the experiment. Although mean ectoenzymatic activities (protease, α - and β -glucosidase) were also increased at higher $p\text{CO}_2$, $p\text{CO}_2$ dependency was statistically significant only for protease activity. In contrast to our present study, algal dynamics in the former study was strongly dependent on $p\text{CO}_2$ suggesting that changes in bacterial activities were mainly linked to $p\text{CO}_2$ induced changes in phytoplankton growth and community composition. In the present study, however, neither bacterial abundance nor BPP were different among $p\text{CO}_2$ treatments. Solely, linear regressions between BPP of free-living bacteria, BPP of attached bacteria or csBPP of attached bacteria and C:N ratio of suspended matter were significantly different between $p\text{CO}_2$ levels (Table 3). This result seems to be surprising since neither C:N ratios of suspended matter nor bacterial parameters were significantly dependent on $p\text{CO}_2$. The C:N ratios of the suspended particulate organic matter in the epilimnion may have been largely dominated by living algal biomass leading to a rather constant C:N ratio in all treatments. A higher loss of organic carbon from the upper layer of the stratified mesocosms at higher $p\text{CO}_2$ has been previously shown (Engel, 2002; Engel et al., 2004) and has been assigned to higher concentrations of TEP at higher $p\text{CO}_2$. Higher abundances of TEP provide larger surfaces for bacterial attachment which may result in higher bacterial activities (Grossart et al., 2007), and promote aggregation and loss of C-rich particulate organic

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matter (Passow, 2002). In our study, the stoichiometry of carbon to nitrogen drawdown (C:N) increased from a value of 6.0 under present $p\text{CO}_2$ to 8.1 at high $p\text{CO}_2$ (Riebesell et al., 2007) which may indicate $p\text{CO}_2$ -dependent changes in carbon and/or nitrogen consumption by heterotrophic bacteria, especially of attached bacteria.

Our stepwise multiple regression analysis revealed that TEP positively correlated with abundance of attached bacteria but negatively with their csBPP. Due to their sticking properties TEP can efficiently scavenge bacteria but may lower csBPP of attached bacteria because of their low nitrogen and phosphorus content (Passow, 2002). In addition, a $p\text{CO}_2$ dependent algal carbon consumption may result in increased phytoplankton exudation (e.g. Obernosterer and Herndl, 1995; Biddanda and Benner, 1997) with high proportions of carbon rich DOM which is less accessible to microbial degradation (Søndergaard et al., 2000; Thingstad et al., 1997). Since draw down of nutrients and phytoplankton community composition were similar in all mesocosms (Riebesell et al., 2007), we conclude that in addition to nutrient regime and phytoplankton community composition (Conan et al., 2007) carbon availability can greatly affect production and composition of new DOM. If so, an increasing $p\text{CO}_2$ in the upper ocean must have profound implications for microbial utilization of the oceanic DOM pool. In our experiment, DOC and BPP as well as csBPP of both free-living and attached bacteria were negatively correlated to each other and indicate that increasing concentrations of DOC throughout the bloom do not necessarily stimulate bacteria secondary production. As indicated by Tanaka et al. (2007) after the peak of the phytoplankton bloom the released labile DOC may have been rich in carbon such as glucose whereas at the same time P and N were depleted. Hence, heterotrophic bacteria were not capable to rapidly degrade the present DOC even not its labile fraction. However, accumulation of DOC in our experiment did not significantly increase at higher $p\text{CO}_2$ presumably because TEP formation from dissolved precursors and subsequent sedimentation may have been high (see above). Our results indicate that there is a slight but rather indirect effect of changes in $p\text{CO}_2$ on bacterial activities and community structure that is mainly related to phytoplankton carbon consumption, DOC exudation, as well as TEP formation and

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subsequent sedimentation (also see Tanaka et al., 2007).

4.2 Coupling of heterotrophic bacteria to phytoplankton bloom development

Abundance and BPP of both free-living and attached bacteria were tightly coupled to phytoplankton bloom development and did not show any significant differences between the treatments. The relatively high bacterial abundance at the beginning of the experiment, in particular of free-living bacteria, may have been caused by addition of freshwater to the upper water layers of each mesocosm, disruption of jelly fish during mesocosm filling, and bubbling. When excluding the initial phase of the mesocosm experiment, abundance of free-living bacteria increased from day 6 on and peaked during the decline of the algal bloom whereas the second increase in numbers of free-living bacteria occurred in parallel to an increase in dinoflagellates and unicellular cyanobacteria towards the end of the experiment. In contrast, abundance of attached bacteria showed only one peak which was slightly earlier than the first peak of free-living bacteria and in parallel to phytoplankton development (Schulz et al., 2007). Thereafter, abundance of attached bacteria slowly decreased until the end of the experiment. Numbers of attached bacteria were much lower than those of free-living bacteria. In a previous study (Grossart et al., 2006b) we also found that maxima in abundance of free-living bacteria surpassed that of attached bacteria by ca. 10-fold. However, in that study phytoplankton abundance and composition was different among the different pCO₂ treatments and abundance of free-living bacteria increased much earlier than that of attached bacteria. In the present study numbers of free-living bacteria dramatically dropped several days before the phytoplankton bloom had reached its maximum. At the same time viral numbers and estimated production greatly increased suggesting viral lysis of free-living bacteria. In contrast, attached bacteria continuously increased during the second half of the bloom and even during its brake down. Differences in coupling between abundances of both bacterial fractions and phytoplankton bloom development may be also explained by the observed differences in phytoplankton composition and growth dynamics of the two studies. In a laboratory

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study (Grossart et al., 2006a) we even found differences in coupling of heterotrophic bacteria and phytoplankton when using two closely related diatom species or even the same species but at different growth stages. Not only absolute numbers of free-living and attached bacteria but also the relation between both bacterial fractions showed a high temporal variability. Since there were no pronounced differences in phytoplankton and bacterial dynamics between the different treatments of the present study, we conclude that coupling between phytoplankton and heterotrophic bacteria was similar for all mesocosms.

In contrast to abundance, BPP of free-living and attached bacteria were in the same range and highest during the peak and the decline of the phytoplankton bloom. Whereas BPP of attached bacteria dramatically increased at the peak of the bloom and remained high during the breakdown of the bloom, BPP of free-living bacteria strongly increased after the phytoplankton bloom and reached its maximum when phytoplankton biomass was low but POC, PON, and POP still high (Schulz et al., 2007). High exoenzymatic activities of attached bacteria on senescent diatom cells leading to a subsequent increase in dissolved DOM have been recently found (Grossart et al., 2005). Other studies (e.g. Smith et al., 1992, 1995) show that high exoenzymatic activities on marine snow lead to an increasing release of DOM into the surrounding water which stimulates growth of free-living bacteria. Since dynamics of free-living bacteria followed that of attached bacteria a similar scenario seems to be likely for the present study. CsBPP of both bacterial fractions, however, reached its maximum at the high of the algal bloom indicating a tight coupling between bacteria activity and phytoplankton development, especially when P and N were depleted in the ambient water (Tanaka et al., 2007). The much higher csBPP of attached than of free-living bacteria may indicate a closer and more efficient coupling of phytoplankton-associated bacteria presumably due to a lower spatial distance between algae and bacteria and a higher availability of nutrients. In addition, our statistics (Table 3a and b) shows that indeed both bacterial fractions were tightly coupled to algal parameters but in a different manner. Surprisingly, BPP of free-living bacteria was negatively correlated with diatoms,

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whereas csBPP of attached bacteria was negatively correlated with Cyano and TEP. In contrast, both csBPP of free-living and attached bacteria positively correlated with Ehux. Hence, the presence of different algal species leads to differences in the linkage between free-living and attached bacteria to phytoplankton bloom development. This notion points to differences in the functional role of free-living vs. attached bacteria for oceanic nutrient and energy cycling (Simon et al., 2002).

Another important point is that free-living and attached bacteria at certain times may have experienced great differences in the availability of limiting nutrients, such as phosphorus and nitrogen (Tanaka et al., 2007). It has been suggested by Thingstad et al. (2005) that specific bacteria, such as *Vibrio splendidus*, obtain a competitive advantage for mineral nutrients by using a non-limiting carbon source to increase their size, without thereby increasing their cellular quota of the limiting element. According to these authors the benefit would be threefold: 1) increased affinity, 2) decreased predation pressure, and 3) storage of energy and carbon for potential later use under C-limited conditions. The specific environment required for such a beneficial strategy would be access to a pool of assimilable organic C in excess of that required for growth. Similar conditions are given for bacteria clustering in the vicinity or being attached to phytoplankton cells and organic matter aggregates where a substantial release of C rich organic matter due to phytoplankton exudation or by bacterial ectoenzymatic activities has been observed (Kjørboe and Jackson 2001; Grossart et al., 2006a, 2007). The much higher cell-specific bacterial activities, the much larger cell size, and the temporal decoupling of bacterial dynamics of free-living and attached bacteria during the present and a past mesocosm phytoplankton bloom (Grossart et al., 2006b) imply that particle-associated bacteria indeed haunt a similar strategy. An increase in organic matter and nutrient supply once a bacterium stays in the vicinity or has attached to a larger particle has been previously calculated by Kjørboe and Jackson (2001). It may not only explain higher cell-specific ectoenzymatic activities of bacteria upon attachment (Grossart et al., 2007) but also the much higher cell-specific BPP rates and cell sizes of attached than of free-living bacteria as has been observed in this study. Whereby in our study

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mainly members of the *Roseobacter* clade and of γ -*Proteobacteria* seem to follow the above mentioned strategy (see also below).

4.3 Cycling of DMSP_{d+p} and DMS

Abundance and BPP of both free-living and attached bacteria correlated well with concentration of DMSP_d and especially DMSP_p (Table 1, 2a and b). This also points to a tight coupling between bacteria and phytoplankton dynamics. DMSP_p is produced in some phytoplankton groups (Keller et al., 1989) as an osmolyte (e.g. Dickson and Kirst, 1986), cryoprotectant (Karsten et al., 1996), as an antioxidant (Sunda et al., 2002) or overflow mechanism (Stefels, 2000). Most DMSP_p is transferred to the dissolved phase (DMSP_d) upon demise of phytoplanktonic cells through grazing, autolysis, and viral lysis (Stefels et al., 2007). There, DMSP_d is available for bacterial degradation and may serve as an important substrate and energy source for marine bacteria (Kiene and Linn, 2000). Furthermore, marine bacteria can satisfy almost all their sulphur demand through DMSP consumption (e.g. Kiene et al., 2000). By using a combination of microautoradiography and CARD-FISH Vila-Costa et al. (2007b) showed that members of *Alphaproteobacteria* (*Roseobacter* and SAR11) and *Gammaproteobacteria* accounted for most of the bacterial DMSP-S assimilating cells during a seasonal cycle in the Mediterranean. These two groups are the major bacterial groups found in the present study (see below). The most common pathway of DMSP consumption, however, is the demethylation/demethiolation pathway, which drives DMSP away from DMS production.

A Lagrangian study of a coccolithophore bloom in the North Sea revealed that microzooplankton grazing can account for the majority of DMSP_p degradation (Archer et al., 2003). In addition, viruses have been implicated in the collapse of blooms of *E. huxleyi* (Wilson et al., 2002), which was also the case in our study (Larsen et al., 2007). In a more recent study, however, Evans et al. (2007) found that for *E. huxleyi* microzooplankton grazing is more important for DMS production than viral lysis – most likely because virally infected cells have lower lyases activities. Unfortunately, we

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do not have enough data on microzooplankton grazing to study its relevance for DMS production.

In the North Sea, Zubkov et al. (2001) found that free-living bacteria of the genus *Roseobacter* are actively involved into the pelagic sulphur cycling via degradation of DMSP_d. In our study, this genus comprised a major fraction of *Alphaproteobacteria*, both free-living and attached and BPP and abundance of both bacterial fractions correlated well with DMSP_d and even better with DMSP_p. This suggests that these bacteria either free-living or attached are involved in DMSP cycling.

CsBPP of both bacterial fractions was most strongly linked to primary production and only csBPP of free-living bacteria was also positively coupled to DMS. DMS is a climatically active trace gas (Charlson et al., 1987) and is primarily formed in seawater by interactions of the microbial food web (Simo et al., 2002). DMS concentrations in seawater are the result of a complex web of production and loss processes (Simo et al., 2002). In particular, bacterial processes are central in controlling dissolved DMS concentrations (Vila-Costa et al., 2007a; Howard et al., 2006; Kiene et al., 2000). DMS is formed when DMSP is cleaved by a group of isozymes, which are produced in the microbial community by phytoplankton species such as *E. huxleyi*, *Phaeocystis* species and some dinoflagellates (Steinke et al., 1998, 2002; Stefels et al., 1995) and by some bacteria (Ledyard and Dacey, 1994; Todd et al., 2007). Kiene et al. (2000) suggests that the bacterial yield of DMS from DMSP consumption depends upon bacterial sulphur demand and DMSP concentration. Besides photolysis bacterial degradation is the dominant loss process for DMS in the ocean (e.g. Kiene and Bates, 1990). Whereas most marine bacteria can assimilate DMSP (generalists) it has been suggested that DMS degraders are specialists, comprising approximately 33% of the bacterial community (Vila-Costa et al., 2007a).

The linkage of csBPP of free-living bacteria to DMS concentration in our study (Table 2B) implies that free-living bacteria participated in DMS consumption. Vila-Costa et al. (2007a) found that not only bacteria of the *Methylophaga* group are prolific DMS consumers, but that also members of the *Roseobacter* group are able to assimilate

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DMS to a certain extent. In our study, members of the *Roseobacter* group formed several dominant DGGE bands whereas members of the *Methylophaga* group were not found (see below).

4.4 Community composition and phylogeny

5 Although differences in DGGE banding patterns of free-living and attached bacteria were minor, temporal succession of both bacterial fractions was different. Whereas free-living bacteria formed distinct clusters among the different treatments throughout the whole study period, temporal dynamics of attached bacteria closely followed that of phytoplankton development. This was not reflected by our 16S rRNA gene
10 based phylogeny of the excised DGGE bands which revealed that the majority of free-living as well as attached bacteria belonged to similar groups of the *Alpha*- and *Gammaproteobacteria*, and *Bacteroidetes*. *Alphaproteobacteria*, mainly of the *Roseobacter* group, *Gammaproteobacteria*, in particular of the *Pseudoaltermonas* group, and specific *Bacteroidetes* have been frequently found to be associated to various phytoplankton species of different phylogenetic groups in laboratory cultures and in the sea (Grossart et al., 2005; Garces et al., 2007, and references therein). Whereas
15 Grossart et al. (2005) found significant differences between free-living and attached bacterial communities in laboratory diatom cultures, differences between the two bacterial fractions in the present study were less obvious. Both *Roseobacter* and *Pseudoaltermonads* are highly motile and it has been shown that some *Roseobacter* species express high chemotaxis in the presence of organic matter such as marine broth and DMSP (Kjørboe et al., 2002). Furthermore, quorum sensing via acylated homoserine lactones (AHLs) seems to be a common feature of *Roseobacter* species (Gram et al., 2002; Martens et al., 2007). In addition to their blooming during phytoplankton blooms,
20 *Roseobacter* and presumably *Pseudoaltermonas* and *Bacteroidetes* seem to be well adapted to rapidly respond to phytoplankton development. It may be difficult to distinguish between free-living and attached bacterial communities since many chemotactic species show frequent attachment and subsequent detachment (Kjørboe et al., 2002).

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Our results suggest that bacterial community analysis, solely based on the 16S rRNA gene, can provide valuable results on the interaction between heterotrophic bacteria and phytoplankton development and, hence, the functioning of the microbial food web.

5 Summary

5 Microbial dynamics in our mesocosm plankton communities was tightly linked to phytoplankton development. Changes in pCO₂ led to significant changes in community structure of free-living but not of attached bacteria which were more tightly linked to phytoplankton dynamics. Statistical analysis revealed a pCO₂ dependency for linear regressions between C:N ratio of suspended matter and BPP of free-living and attached bacteria as well as csBPP of attached bacteria indicating a different relation between bacterial activity and substrate quality at increased pCO₂ levels. However, since bacterial communities, in particular that of attached bacteria, directly depended on phytoplankton dynamics and since algal development was similar in all mesocosms, most of the measured bacterial parameters seem to be independent of pCO₂. On the other hand, our results provide some indication that pCO₂ induced changes in phytoplankton carbon fixation and community succession will have an impact on microbial energy consumption and carbon as well as sulphur cycling due to the rather tight coupling between phytoplankton and microbial dynamics in the pelagic ocean. In particular, chemotactic and attached bacteria may greatly benefit from a tight coupling to phytoplankton cells and organic aggregates since by doing so they may have a competitive advantage not only for organic carbon but also for mineral nutrients. Slight changes in organic matter cycling due to differences in pCO₂ may be hard to detect in short-term mesocosm experiments but may accumulate in the long-term. Hence, we suggest that further studies on pCO₂ induced changes on microbial activities should take a longer time scale into account.

Acknowledgements. We would like to thank all participants of the PeECE III mesocosm experiment (<http://peece.ifm-geomar.de>) for their valuable technical assistance, data acquisition,

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and many fruitful discussions. The staff at the Marine Biological Station, University of Bergen, in particular T. Sørli and A. Aadnesen, and the Bergen Marine Research infrastructure (RI) are gratefully acknowledged for support in mesocosm logistics. M. Steinke and S. Turner are thanked for assistance in DMS/DMSP measurements and numerous discussions. K. Pohlmann is warmly acknowledged for help in statistical analyses and her comments on this manuscript. This work was supported by the Leibniz-Foundation and a grant of the German National Academic Foundation given to M. Allgaier.

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Table 1. Linear regression analysis between measured bacterial parameters and environmental variables. BPP free/attached (bacterial production of free and attached bacteria, respectively), cs BPP free/attached (cell-specific BPP of free and attached bacteria, respectively), Bacteria free/attached (abundance of free-living and attached bacteria, respectively), NO₃, PO₄, Si(OH)₄, POC (particulate organic carbon), C:N (C:N ratio of particulate matter), TEP (transparent exopolymer particles), Chl-*a* (chlorophyll *a*), Ehux (coccolithophore *Emiliania huxleyi*), Diatoms (diatoms primarily *Skeletonema costatum* and *Nitzschia longissima*), Cyano (cyanobacteria), PrProd (primary production), Virus (total virus), Big Virus (distinct group of big virus with a size of up to 200 nm), DOC (dissolved organic matter), DMSP (dimethylsulfonio-propionate), DMS (dimethylsulfide), ns (not significant).

Parameter	BPP free T-, p-value	BPP attached T-, p-value	csBPP free T-, p-value	csBPP attached T-, p-value	Bacteria free T-, p-value	Bacteria attached T-, p-value
NO ₃	-2.309, 0.024	ns	3.348, 0.001	8.915, <0.001	-12.778, <0.001	-8.647, <0.001
PO ₄	-6.239, <0.001	-4.958, <0.001	-2.785, 0.007	ns	-8.243, <0.001	-10.898, <0.001
Si(OH) ₄	-4.165, <0.001	ns	ns	6.124, <0.001	-14.181, <0.001	-13.761, <0.001
POC	10.869, <0.001	5.342, <0.001	ns	ns	12.117, <0.001	12.074, <0.001
C:N	2.768, 0.007	2.104, 0.038	ns	ns	3.149, 0.002	5.447, <0.001
TEP	5.97, <0.001	2.098, 0.039	ns	-4.885, <0.001	10.256, <0.001	13.365, <0.001
Chl- <i>a</i>	8.513, <0.001	9.395, <0.001	6.446, <0.001	3.495, 0.001	ns	3.665, <0.001
Ehux	4.881, <0.001	7.280, <0.001	7.459, <0.001	5.659, <0.001	ns	ns
Diatoms	3.922, <0.001	6.656, <0.001	6.756, <0.001	5.481, <0.001	ns	ns
Cyano	-3.403, 0.001	-5.902, <0.001	-6.066, <0.001	-6.555, <0.001	2.546, 0.013	ns
PrProd	4.212, <0.001	9.256, <0.001	13.146, <0.001	9.168, <0.001	ns	ns
Virus	-3.622, <0.001	-6.525, <0.001	-7.139, <0.001	-7.873, <0.001	2.38, 0.022	ns
Big Virus	-9.041, <0.001	-12.581, <0.001	-9.139, <0.001	-3.993, <0.001	-2.730, 0.008	-4.553, <0.001
DOC	-2.208, 0.046	-3.906, <0.001	-4.083, <0.001	-6.360, <0.001	2.052, 0.044	2.319, 0.036
DMSP _d	6.856, <0.001	3.993, <0.001	ns	ns	7.558, <0.001	5.915, <0.001
DMSP _p	16.984, <0.001	9.591, <0.001	4.742, <0.001	ns	7.208, <0.001	8.157, <0.001
DMS	11.361, <0.001	8.547, <0.001	5.131, <0.001	ns	4.885, <0.001	6.616, <0.001

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Table 2. Results of stepwise multiple regression analysis. Parameters linked to **(a)** particulates (POC, C:N, TEP, DMSP_D, Chl-*a*, Ehux, Diatoms, Cyano), **(b)** dissolved organic matter and primary production (DOC, DMS, DMSP_D, PrProd), and **(c)** virus (Virus, Big Virus). For abbreviations see legend Table 1.

	BPP free	BPP attached	csBPP free	csBPP attached	Bacteria free	Bacteria attached
(a)	F=272.118, <0.001 parameter,T, p-value DMSP _D , 16.496, <0.001	F=48.232, <0.001 parameter,T, p-value DMSP _D , 11.453, <0.001 TEP, -5.172, <0.001 C:N, 2.075, <0.041	F=52.006, <0.001 parameter,T, p-value Ehux, 7.211, <0.001	F=37.004, <0.001 parameter,T, p-value Cyano, -3.539, 0.001 TEP, -6.522, <0.001 DMSP _D , 3.532, 0.001	F=89.150, <0.001 parameter,T, p-value POC, 3.436, 0.001 Cyano, 5.362, <0.001 DMSP _D , 4.962, <0.001 Ehux, 3.551, 0.001	F=109.739, <0.001 parameter,T, p-value TEP, 5.644, <0.001 POC, 3.442, 0.001
(b)	F=81.083, <0.001 parameter,T, p-value DMS, 8.219, <0.001 DMSP _D , 5.739, <0.001 PrProd, 4.091, <0.001	F=79.114, <0.001 parameter,T, p-value PrProd, 9.344, <0.001 DMA, 5.896, <0.001 DMSP _D , 3.451, 0.001	F=85.915, <0.001 parameter,T, p-value PrProd, 10.957, <0.001 DMS, 3.177, 0.002	F=46.075, <0.001 parameter,T, p-value PrProd, 5.725, <0.001 DOC, -3.864, <0.001	F=29.375, <0.001 parameter,T, p-value DMSP _D , 4.463, <0.001 DMS, 4.826, <0.001 DOC, 4.381, 0.001	F=41.772, <0.001 parameter,T, p-value DMS, 8.217, <0.001 DOC, 5.972, <0.001 DMSP _D , 2.292, 0.025
(c)	F=81.734, <0.001 parameter,T, p-value Big Virus, -9.041, <0.001	F=158.281, <0.001 parameter,T, p-value Big Virus, -12.581, <0.001	F=47.048, <0.001 parameter,T, p-value Big Virus, -5.261, <0.001 Virus, -2.434, 0.017	F=61.984, <0.001 parameter,T, p-value Virus, -7.873, <0.001	F=31.473, <0.001 parameter,T, p-value Big Virus, -7.365, <0.001 Virus, 7.158, <0.001	F=44.143, <0.001 parameter,T, p-value Big Virus, -9.270, <0.001 Virus, 7.408, <0.001

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Bacteria dynamics in mesocosms with increased pCO₂

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Table 3. CHOW test with univariate GLM to test for dependencies on pCO₂. For abbreviations see legend Table 1.

Parameter	BPP free	BPP attached	csBPP free	csBPP attached
C:N	intercept p=0.004 F=5.923 slope p=0.004 F=5.819 df=2	intercept p=0.003 F=6.432 slope p=0.003 F=6.407 df=2	ns	intercept p=0.027 F=3.762 slope p=0.034 F=3.526 df=2

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Table 4. Comparison of DGGE banding patterns of free-living and particle-associated bacteria of mesocosms M2 (3×CO₂), M5 (2×CO₂), and M8 (1×CO₂) by using ANOSIM statistics. Significance values with $p \leq 0.05$ are given in grey.

free-living bacteria	Sample Statistic (R)	Significance level (p)
M2 vs. M5	0.487	0.002
M2 vs. M8	0.395	0.002
M5 vs. M8	0.525	0.002
particle-associated bacteria		
M2 vs. M5	−0.089	0.85
M2 vs. M8	0.016	0.417
M5 vs. M8	0.074	0.224

The global tests revealed sample statistics of 0.468 for free-living bacteria and 0.452 for particle-associated bacteria with significance values of p 0.001 and p 0.453, respectively.

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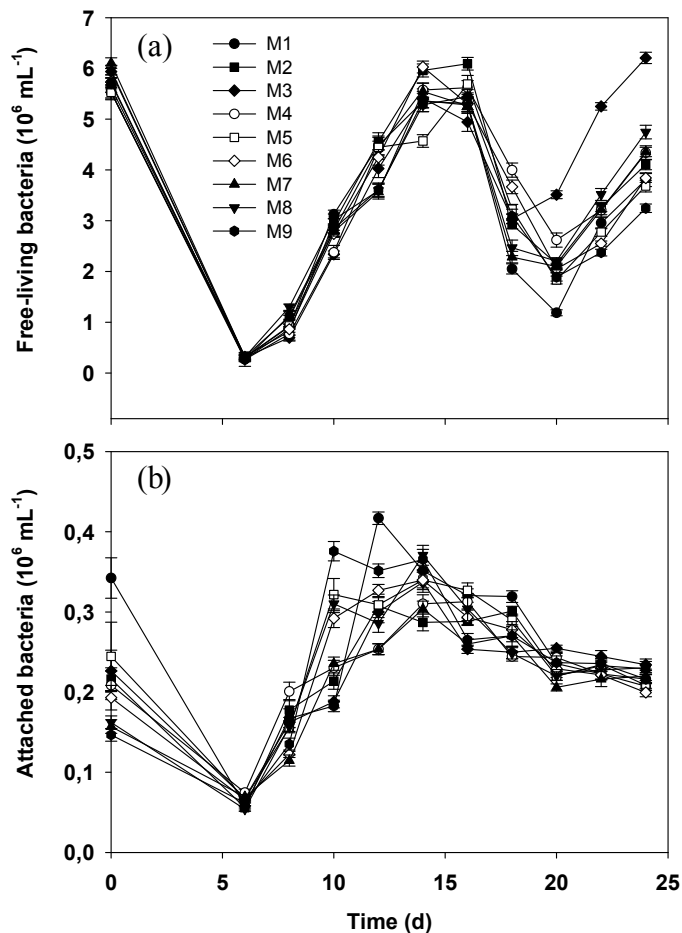


Fig. 1. Abundance of free-living ($\leq 5.0 \mu\text{m} \geq 0.2 \mu\text{m}$) and **B**) attached bacteria ($\geq 5.0 \mu\text{m}$). M1-M3 mesocosms with $3 \times \text{CO}_2$, M4-M6 mesocosms with $2 \times \text{CO}_2$, and M7-M9 mesocosms with $1 \times \text{CO}_2$.

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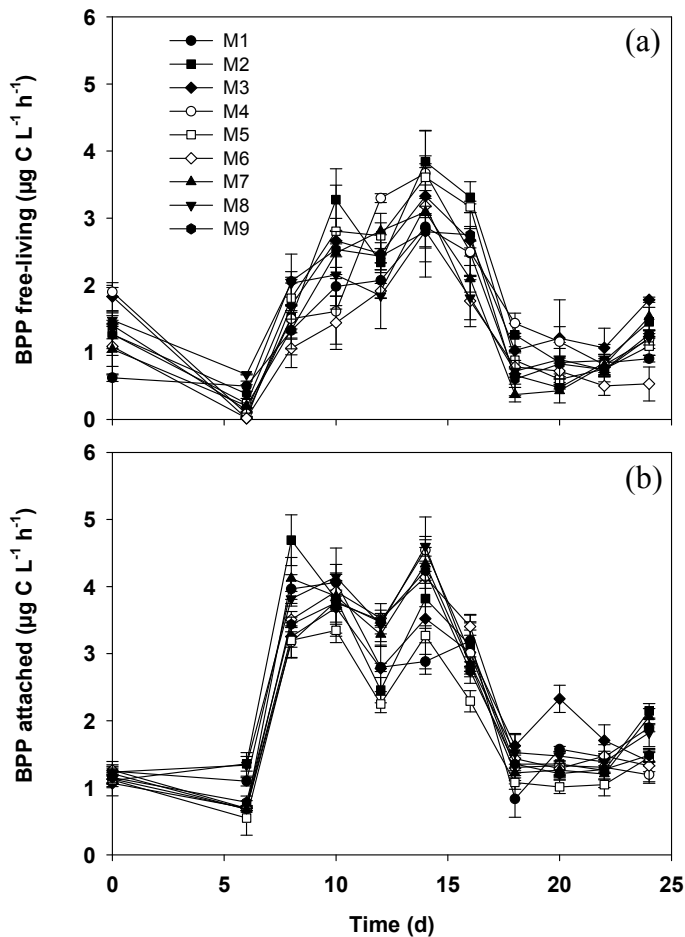


Fig. 2. Bacterial protein production (BPP) of (a) free-living ($\leq 5.0 \mu\text{m} \geq 0.2 \mu\text{m}$) and (b) attached bacteria ($\geq 5.0 \mu\text{m}$). M1-M3 mesocosms with $3 \times \text{CO}_2$, M4-M6 mesocosms with $2 \times \text{CO}_2$, and M7-M9 mesocosms with $1 \times \text{CO}_2$.

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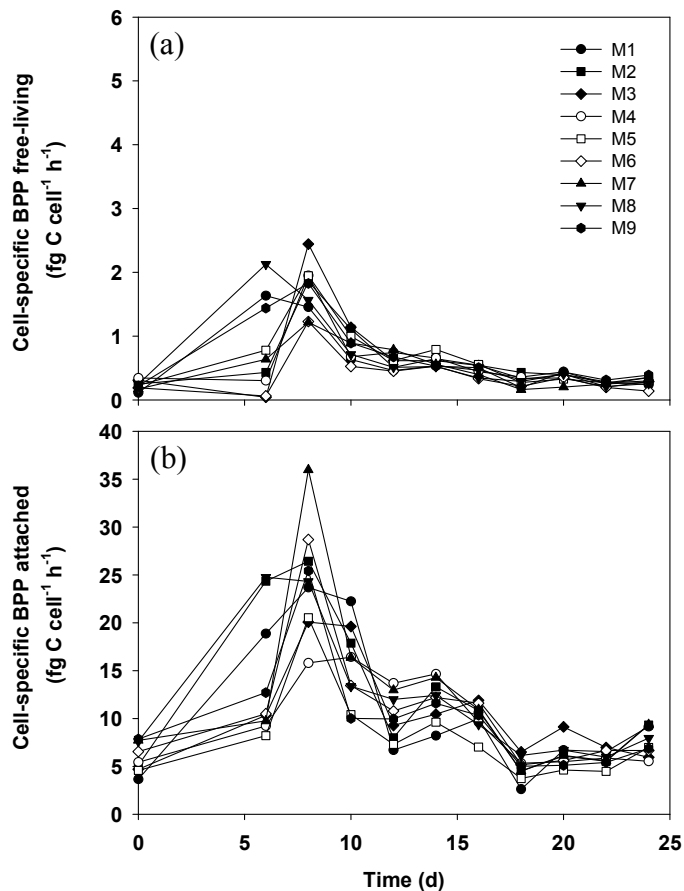


Fig. 3. Cell-specific BPP of (a) free-living ($\leq 5.0 \mu\text{m} \geq 0.2 \mu\text{m}$) and (b) attached bacteria ($\geq 5.0 \mu\text{m}$). M1-M3 mesocosms with $3 \times \text{CO}_2$, M4-M6 mesocosms with $2 \times \text{CO}_2$, and M7-M9 mesocosms with $1 \times \text{CO}_2$.

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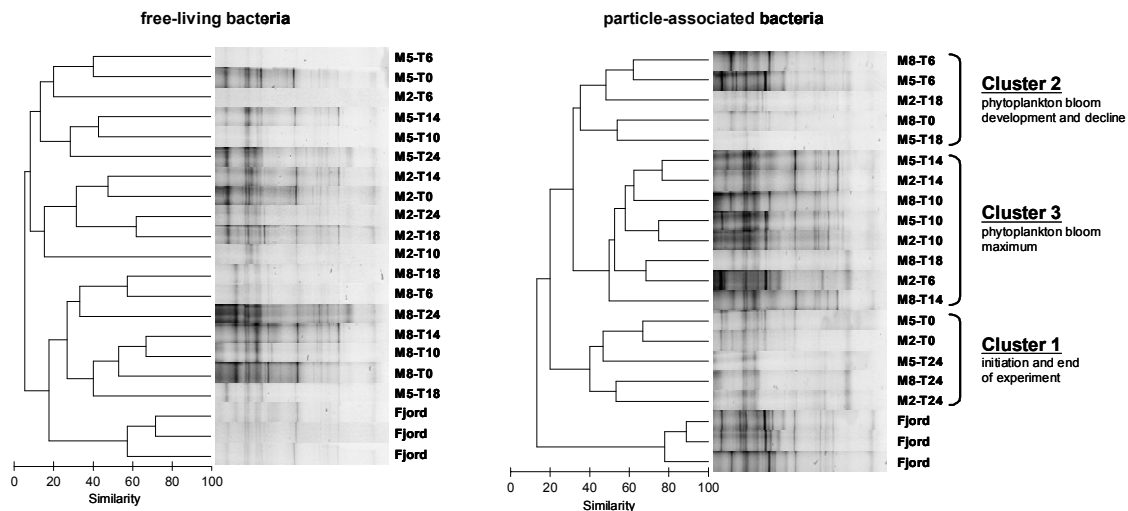


Fig. 4. Cluster analyses including the corresponding DGGE profiles of PCR-amplified 16S rRNA gene fragments of free-living and particle-associated bacterial communities of the mesocosm M2 ($3\times\text{CO}_2$), M5 ($2\times\text{CO}_2$), and M8 ($1\times\text{CO}_2$). Sample identification numbers indicate mesocosm and date of each sample. Samples designated with “Fjord” are water samples originating from the Fjord outside of the mesocosms which were used as external standard on each DGGE gel for comparison of the DGGE profiles across different gels.

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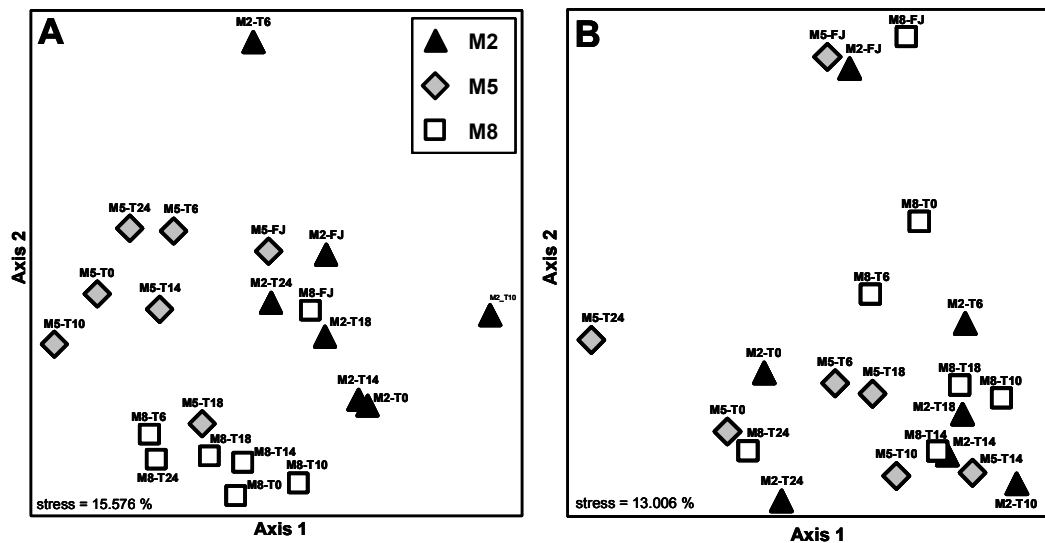


Fig. 5. NMS ordination plots of DGGE banding patterns of (a) free-living ($\leq 5.0 \mu\text{m}$) and (b) particle-associated bacteria ($\geq 5.0 \mu\text{m}$).

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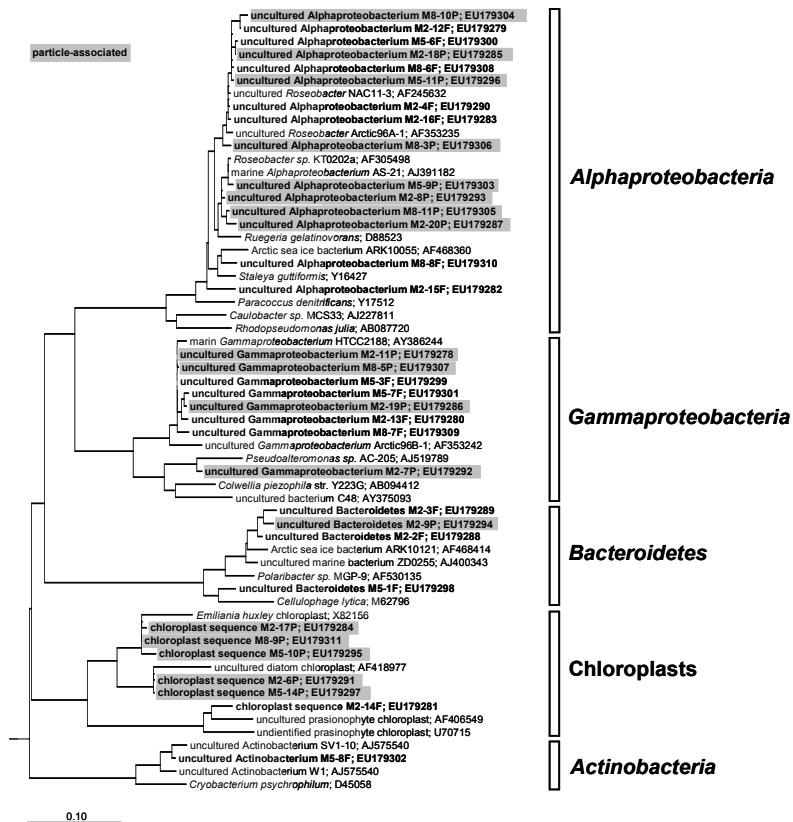


Fig. 6. Maximum Likelihood tree of 16S rRNA gene sequences of free-living and particle-associated bacteria derived from DGGE bands of the three mesocosms M2 ($3\times\text{CO}_2$), M5 ($2\times\text{CO}_2$), and M8 ($1\times\text{CO}_2$). Sequences of this study are shown in bold letters. Sequences of particle-associated bacteria are additionally marked by grey boxes. GenBank accession numbers are given. The sequence identification number indicates from which mesocosm the sequences are derived (e.g. M2-X, M5-X, or M8-X). The scale bar corresponds to 10% base substitutions.