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Effect of elevated CO_2 on the dynamics of particle attached and free living bacterioplankton communities in an Arctic fjord

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Abstract

The increase in atmospheric carbon dioxide (CO_2) results in acidification of the oceans, expected to lead to the fastest drop in ocean pH in the last 300 million years, if anthropogenic emissions are continued at present rate. Due to higher solubility of gases in cold waters and increased exposure to the atmosphere by decreasing ice cover, the Arctic Ocean will be among the areas most strongly affected by ocean acidification. Yet, the response of the plankton community of high latitudes to ocean acidification has not been studied so far. This work is part of the Arctic campaign of the European Project on Ocean Acidification (EPOCA) in 2010, employing 9 in situ mesocosms of about 45 000 l each to simulate ocean acidification in Kongsfjorden, Svalbard (78° 56.2′ N 11° 53.6′ E). In the present study, we investigated effects of elevated CO_2 on the composition and richness of particle attached (PA; > 3 µm) and free living (FL; < 3 µm > 0.2 µm) bacterial communities by Automated Ribosomal Intergenic Spacer Analysis (ARISA) in 6 of the mesocosms and the surrounding fjord, ranging from 185 to 1050 initial µatm pCO_2. ARISA was able to resolve about 20–30 bacterial band-classes per sample and allowed for a detailed investigation of the explicit richness. Both, the PA and the FL bacterioplankton community exhibited a strong temporal development, which was driven mainly by temperature and phytoplankton development. In response to the breakdown of a picophytoplankton bloom (phase 3 of the experiment), number of ARISA-band classes in the PA-community were reduced at low and medium CO_2 (~ 180–600 µatm) by about 25 %, while it was more or less stable at high CO_2 (~ 650–800 µatm). We hypothesise that enhanced viral lysis and enhanced availability of organic substrates at high CO_2 resulted in a more diverse PA-bacterial community in the post-bloom phase. Despite lower cell numbers and extracellular enzyme activities in the post-bloom phase, bacterial protein production was enhanced in high CO_2-treatments, suggesting a positive effect of community richness on this function and on carbon cycling by bacteria.

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1 Introduction

The increase in anthropogenic carbon dioxide (CO_2) in the atmosphere causes an enhanced uptake of CO_2 by the oceans (Raven et al., 2005) and, if CO_2 emissions continue at current rates, is expected to lead to the fastest drop in ocean pH in the last 300 million years (Caldera and Wickett, 2003). The Arctic Ocean is projected to be among the areas experiencing the most extensive changes due to ocean acidification (Steinacher et al., 2009). The cold water has a high solubility for CO_2 and increased melting of sea ice is projected to expand the area of surface water directly exposed to atmospheric influences within the current century. The related freshwater input will also reduce the buffering capacity of the seawater. The sum of these processes is projected by the NCAR global coupled carbon cycle-climate model to result in an acceleration of ocean acidification by 20 % and a drop of 0.45 pH-units at the end of the present century, thus turning the Arctic Ocean from a region of, compared to global average, high pH into a relatively low pH region (Steinacher et al., 2009). Yet, potential consequences for Arctic marine ecosystems and their biogeochemical feedbacks are largely unknown to date.

Heterotrophic bacterioplankton are a central part of marine ecosystems and can strongly influence their biogeochemical functioning. They use dissolved organic carbon (DOC) to assimilate it into biomass or to generate energy by respiration (e.g. Williams, 2000). The assimilated carbon can be transferred to higher trophic levels by bactivory, while the respired carbon is released as CO_2 (e.g. Pomeroy et al., 2007). Also in Kongsfjorden both processes are exhibited, depending on the state of the ecosystem. When autotrophic production is limited, e.g. by light or nutrients, bacteria mainly remineralise organic carbon to CO_2 while in conditions more favourable for primary production more of the carbon is transferred to higher trophic levels by bactivory (Iversen and Seuthe, 2011). Furthermore, heterotrophic bacteria play an important role in the degradation of particulate organic carbon (POC) preventing it from sinking to the deep sea and thereby being separated from the climate system for long time periods (Smith et al.,

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1992). Especially these particle associated bacteria have been shown to be metabolically highly active (e.g. Simon et al., 2002; Grossart et al., 2007), often contributing a higher proportion to community function than their abundance relative to free living bacteria suggests. Another influential role of bacterioplankton on marine ecosystems is competition with phytoplankton for inorganic nutrients. Thingstad et al. (2008) showed that heterotrophic bacterioplankton is able to out-compete phytoplankton for mineral nutrients in Arctic pelagic ecosystems, when carbon limitation is reduced, resulting in an inhibition of organic carbon accumulation in the system. In these ways, pelagic bacterial activity has an influential role on organic carbon cycling from autotrophic production through recycling of organic carbon to release of CO_2 to the climate system.

The activity, and therefore the ecological and biogeochemical functioning, of bacterioplankton is determined by the influence of environmental factors on the metabolic activity of the bacterial community and by the composition of the community. Repeatable community patterns of marine bacteria in response to environmental factors suggest that bacterial communities can exhibit low functional redundancy under natural conditions (Fuhrman et al., 2006). It has also been shown that different bacterial groups take up different low molecular weight-compounds (Alonso-Sáez and Gasol, 2007). In this way a changed community could feedback on metabolic diversity or change the dominant functions of the bacterial community in the long term. In this context the richness, i.e. number of different species, in the bacterial community is important. A high richness can enhance community function in two ways (Loreau and Hector, 2001): firstly, by taking advantage of a larger proportion of the available resources by higher metabolic diversity or positive interactions between differing species ("complementarity mechanism") and secondly, by a higher probability to harbour highly active species in the community that have a large impact on the function of interest ("selection mechanism"). These mechanisms can also work in parallel and richness has been shown to enhance bacterial community function (Bell et al., 2005). On the other hand a negative correlation between community richness and function has been observed on some occasions, for example in freshwater algae (Naeem and Li, 1997). This can be explained

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by the "negative selection effect" (e.g. Jiang et al., 2008), when competitively strong species or groups, do not contribute a large part to the community function of interest. An important competitive advantage, which is not necessarily related to metabolic activity of the organism, could for example be strategies of resistance or avoidance of grazing and viral lysis, as these are important factors shaping the bacterial community (Strom, 2008). If predation-resistant species are affected by ocean acidification, this could be a major factor resulting in changes of bacterial community composition and function. Another form of negative relation between richness and function has been observed in rhizosphere bacteria (Becker et al., 2012). Here in a process called "negative complementarity effect", the different bacteria poisoned each other to gain competitive advantage in this enclosed environment, resulting in an overall reduction of community function. Antagonistic process have also been described as widespread in bacterioplankton and especially among those associated to marine particles (Long and Azam, 2001), providing a conceptual basis for the occurrence of "negative complementarity effects" also in marine bacterioplankton. These facts highlight the importance to study bacterial community development and especially richness under ocean acidification scenarios.

Several laboratory studies indicate that the microbial response to elevated pCO_2 may be species specific. A study investigating the impact of very low pH-values in the range of 5–6.5 on 11 individual marine bacterial species from a culture collection found varying threshold values resulting in a 50 % growth rate reduction in different species (Takeuchi et al., 1997). Studies investigating the effect of anthropogenic ocean acidification on different cyanobacterial cultures also observed responses varying from increased C- and N-fixation in non-heterocystous cyanobacteria under elevated CO_2-levels (e.g. Kranz et al., 2009) to reduced cell division rates and N-fixation in a species of heterocystous cyanobacteria (Czerny et al., 2009). While very valuable to understand direct impacts on single species, these results cannot be transferred directly to natural communities. In natural environments the dynamics of bacterioplankton depend to a large degree also on interaction with phytoplankton (e.g. Allgaier et al., 2008;

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Iversen and Seuthe, 2011) and other factors like nutrient limitation or interspecific relationships. To investigate potential biogeochemical changes in ecosystems to ocean acidification it is necessary to study natural microbial communities, ideally incorporating several trophic levels. Several studies investigated whole marine microbial communities by using recently sampled natural communities in short-term laboratory incubation experiments or applying in situ mesocosms running for several weeks. From such experiments, negative effects of ocean acidification were reported on bacterial nitrification rates across the world ocean, consequently reducing the availability of nitrate and considerably changing nitrogen cycling (Beman et al., 2011). Another pronounced effect on biogeochemistry mediated by bacterioplankton was observed on the activity of extracellular enzymes (Grossart et al., 2006; Tanaka et al., 2008; Piontek et al., 2010; Yamada and Suzumura, 2010), potentially resulting in faster degradation of organic matter and a reduced carbon export from the surface to the deep ocean. Effects on other bacterioplankton activities and overall performance have been found to be more contrasting between experiments. While for one in situ mesocosm experiment at the Bergen large scale facility, higher bacterial protein production (BPP), higher growth rates for particle associated and free living bacteria as well as higher bacterial biomass but not abundance have been reported under high CO_2 (Grossart et al., 2006; Paulino et al., 2008; de Kluijver et al., 2010), in two similar experiments at the same site, no significant changes in bacterial abundance or activity could be observed (Allgaier et al., 2008; Newbold et al., 2012). Apart from some minor differences in experimental design, these differences in the response of bacterioplankton activity were possibly due to differences in initial limitation of the bacterial community, as noted by Riebesell et al. (2008). The bacterial communities in these studies were investigated by considerably different analytical approaches. Two of the studies used denaturing gradient gel electrophoresis (DGGE) on particle associated (> 5 µm) and free living (> 0.2 µm, < 5 µm) bacteria (Allgaier et al., 2008) or on total bacteria (> 0.45 µm; Arnosti et al., 2011), respectively. The third study analysed the six most abundant bacterial and eukaryotic peaks found in the picoplankton (0.2–2 µm) which had been determined by using terminal restriction

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fragment length polymorphism (T-RFLP) fingerprinting (Newbold et al., 2012). Both DGGE-studies found significant differences between CO_2-treatments in the banding-pattern of free living (< 5 µm) and total bacterial community (> 0.45 µm), respectively (Allgaier et al., 2008; Arnosti et al., 2011). The composition of the particle attached community was independent of treatment but closely related to phytoplankton (Allgaier et al., 2008). The T-RFLP-study did not observe significant differences with CO_2 in all but one bacterium of the six investigated peaks (Newbold et al., 2012). All these effects on bacterioplankton were observed almost exclusively in the post-bloom phase of a phytoplankton bloom. Studies thus indicate that bacterioplankton is affected by ocean acidification in one way or the other, but that responses are not uniform.

Despite the fact that Arctic seawater will experience the fastest and most intense changes due to ocean acidification (Steinacher et al., 2009) the response of high Arctic bacterial communities to lowered seawater pH has, to the best of our knowledge, not been studied so far. Also little attention was paid so far to the basic factor of bacterial community richness in ocean acidification experiments. It has been shown that resolution of bacterial community fingerprinting can be improved by investigating the inter-genic spacer (IGS)-region between 16S-and 23S-genes on the ribosomal operon (Barry et al., 1991). This region is targeted by Automated Ribosomal Intergenic Spacer Analysis (ARISA) and resolution of this method was found to exceed not only that of DGGE but also of Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Danovaro et al., 2006). The high resolution of ARISA may give the ability to detect small but none the less ecologically relevant changes in the bacterial community structure. Especially community richness can be assessed more elaborately when inferred from number of ARISA-band-classes.

The present study is part of a large scale in situ mesocosm experiment for the first time investigating the influence of ocean acidification on a natural microbial community in the high Arctic at the Svalbard Archipelago. The aims of this study are to investigate the influence of ocean acidification on the community composition, with an additional

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focus on richness, of particle attached and free living bacteria by high resolution genetic fingerprinting (ARISA).

2 Material and methods

2.1 Experimental setup

A mesocosm study was conducted in the framework of the European Project on Ocean Acidification (EPOCA) in the Arctic in 2010. Details on mesocosm setup, manipulations and performance can be found in Riebesell et al. (2012). Briefly, nine floating in situ-mesocosms reaching to 15 m water depth were setup to enclose roughly 45 000 l of seawater, each, at in situ temperature and close to in situ light levels in a high Arctic fjord system (Kongsfjorden, Svalbard) at 78° 56.2′ N 11° 53.6′ E. The water in seven mesocosms was amended with CO_2 supersaturated water to initial pCO_2 levels of 270 (together with the controls considered as "low CO_2-treatment"), 375, 480, 685 (considered as "medium CO_2-treatments"), 820, 1050 and 1420 µatm (considered as "high CO_2-treatments"). Two control mesocosms were left unchanged at initial pCO_2 of 185 µatm. After 13 days nutrients (NO_3^-, PO_4^{3-} and Si to final concentrations of about 5.56, 0.39 and 1.47 µM, respectively) were added to all mesocosms to initiate a phytoplankton bloom.

2.2 Sampling, filtration and DNA extraction

Samples were collected from six mesocosms of 2 × 185, 270, 685, 820 and 1050 µatm initial CO_2 and from the surrounding fjord water. Samples were investigated from day −1 (starting conditions before treatment), day 5 (starting conditions after completion of CO_2 perturbation), day 13 (before nutrient addition), day 14 (after nutrient addition), day 22 and day 28 (both in the post-bloom phase). Integrated water samplers (IWS; Hydrobios, Kiel, Germany) with a volume of 5 l were used to sample the water column

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between 0 and 12 m water depth. The 5 l water samples were transferred to plastic carboys and kept in the dark until filtration for DNA.

One to five l of seawater were filtered through 10 µm- and 3 µm-pore size filters (TCTP and TSTP, 47 mm, Millipore) for pre-filtration and collection of particle attached bacteria, respectively. To collect free living bacteria the < 3 µm filtrate was filtered on 0.22 µm – pore size filters (Durapore®, 47 mm, Millipore). Filters were transferred to cryo-vials, flash frozen in liquid nitrogen and stored at −80 °C until processing. DNA was extracted using the NucleoSpin RNAII kit (Macherey-Nagel, Düren, Germany) following the standard manufacturer's instructions with minor modifications in the lysis step: prior to addition of lysozyme filters were shock frozen in liquid nitrogen for about 10 s. and crushed using a sterile pestle. The DNA-elution step was repeated once to achieve higher DNA concentrations. DNA was re-diluted in 100 µl of PCR-grade-H_2O, aliquoted and measured in duplicates using a NanoQuant Plate and the plate reader infiniteM200 (both Tecan Deutschland GmbH, Crailsheim, Germany).

2.3 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Particle associated and free living bacterial communities in the mesocosms and the fjord were investigated by ARISA. The intergenic spacer (IGS) of the ribosomal DNA gene was amplified in an Eppendorf MasterCycler (Eppendorf, Hamburg, Germany) using the forward primer L-D-Bact-132-a-A-18 (5′-CCG GGT TTC CCC ATT CGG-3′) and the fluorescence labelled backward primer S-D-Bact-1522-b-S-20 (5′-TGC GGC TGG ATC CCC TCC TT-3′). Each 25 µl reaction mixture contained 0.56 mM of each primer, 2.5 ng of template DNA, 0.3 mM deoxynucleoside triphospate mix, 5 µl of Taq-MasterPCR Enhancer, 2.5 µl of TaqBuffer advanced with Mg^{2+} and 1.4 U of TaqDNA Polymerase (all reagents by 5 PRIME, VWR International, Darmstadt, Germany). The PCR comprised an initial step of denaturation at 95 °C for 3 min, 30 cycles of 95 °C for 1 min, 50 °C for 1 min and 68 °C for 1 min and a final cooling to 5 °C until abortion of the run.

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For length separation, 41 cm polyacrylamide gels were used. The acrylamide (5.5 % ready to use matrix by Li-Cor Biosciences) polymerized for 2 h after the addition of Tetramethylethylendiamine (TEMED) and Ammoniumpersulfate (APS) at final concentrations of 0.057 % and 0.571 %, respectively. The PCR-products were amended with Blue Stop Solution (Li-Cor, Bad Homburg, Germany) at a 1 : 1 ratio and were, together with the size standard IRDye® 700, 50 bp–1500 bp (Li-Cor, Bad Homburg, Germany), denatured at 95° C in a MasterCycler (Eppendorf, Hamburg) and subsequently cooled down on ice for 10 min. Each of the 64 pockets of the comb was loaded with 0.25 µl of sample or every 10 pockets with 0.5 µl of standard. The gels were run for 14 h in 1× TBE-buffer (Li-Cor, Bad Homburg, Germany) at a temperature of 45 °C and 1500 V in a Li-Cor DNA analyzer 4300. The software package BioNumerics (AppliedMath, Sint-Martens-Latem, Belgium) was used to semi-quantitatively analyse the gel-images.

2.4 ARISA-band-classes and statistical analysis

Subject to analysis were bands between 300 and 1500 bp length. The bands were binned in size classes (termed "ARISA-band-classes") to correct for minor length variations of IGS-regions between lineages with almost identical 16S rDNA-sequences. Depending on the length of the detected fragment, bins of 3 bp were used for fragments up to 700 bp in length, bins of 5 bp for fragments between 700 and 1000 bp and bins of 10 bp for fragments larger than 1000 bp (Brown et al., 2005; Kovacs et al., 2010). In this way the total number of bands was reduced by about 1.2 % and 2.6 % in particle associated and free living data, respectively. For multivariate statistical analyses the software package PRIMER v.6 and the add-on PERMANOVA+ (both PRIMER-E, Plymouth, UK) was used. The analyses were performed on Bray-Curtis-matrices, generated from square root transformed ARISA-band-class data for each sample. To test for the H_0 of no community assemblage differences between mesocosms or sampling days, permutational multivariate analysis of variance (PERMANOVA, 999 permutations) was applied after Anderson (2001) and the results were visualised by principal coordinate analyses (PCO). Distance based multivariate multiple regression (DistLM)

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was used to calculate correlations of community composition to environmental factors and distance based redundancy analysis (dbRDA) to visualise these correlations.

To investigate differences in the number of ARISA-band-classes, samples were split into two groups of treatment-levels: low- and medium CO_2-treatments (~ 180–600 µatm) were pooled and tested against high CO_2-treatments (~ 650–800 µatm). Phases in the experiment were agreed upon by the participating scientists based on manipulations and Chl *a*-values (Schulz et al., 2012). For our analysis we pooled phases 0–2 (before and during the prominent peak in picophytoplankton cell numbers, see Brussaard et al., 2012) and tested them against phase 3 (after the breakdown of picophytoplankton cell numbers). Differences between the groups were tested by one-way analysis of variance (ANOVA) in the software package SigmaPlot12.1.

3 Results

The number of ARISA-band classes observed ranged from 20 to 30, when combining particle attached (PA) and free living (FL) communities. The development of the bacterioplankton community was influenced by several environmental factors. Among the investigated environmental variables, temperature, pH (see Bellerby et al., 2012), Chl *a* and PO_4exhibited a significant effect on the community composition of PA-bacteria, as revealed by distance-based multivariate multiple regression (DistLM, Table 1). Temperature was the most influential factor explaining 17 % of the variation in the PA-community (DistLM, Table 1) and the community structure largely developed parallel to it in all mesocosms (Fig. 1a). Also for the FL-bacterial community Chl *a* and temperature had the largest effect together with Si, while other inorganic nutrients and pH had no significant effect (Table 1). Chl *a* and temperature were both very important for the community development and explained 25 and 18 % of the variation, respectively (DistLM, Table 1). Therefore, the gradients predominantly explaining the development of the FL-community over the course of the experiment were more evenly split between Chl *a* and temperature (Fig. 1b) than for the PA-community. Overall, the significant factors

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were able to explain about 38 % and 54 % of the variability in the PA-and FL-community, respectively.

The potential effect of the treatment on the bacterial community composition was tested by principal coordinate analyses (PCO) of Bray-Curtis-Similarities over the whole duration of the experiment. This did not reveal any obvious grouping of samples according to mesocosm or treatment-level in both, the PA-fraction (Fig. 2a) and the FL-fraction (Fig. 2b) of the bacterial community. However a clear grouping of the community structure of both bacterioplankton fractions with the experimental day was observed (Figs. 1 and 2). These results are confirmed by PERMANOVA tests showing that the factor "day" was a much larger source of variation (square root of variation attributable) than "mesocosm" in both fractions (Table 2). For the PA-community the influence of mesocosm was still significant however ($p < 0.05$), while for the FL-community it was not (Table 2).

When investigating the richness in terms of number of ARISA-band-classes, an influence of pCO_2-treatment on the PA-community was evident in the third phase of the experiment (defined as: Chl minimum until the end of the experiment, t22–t28, see Schulz et al., 2012). For the PA-community, the number of ARISA-band-classes in low and medium CO_2-mesocosms differed significantly between phase 3 (shortly after breakdown of phytoplankton cell numbers, see Brussaard et al., 2012) and all other phases of the experiment (Table 3). This difference was due to a drop in the number of ARISA-band-classes during phase 3 (shaded area, Fig. 3a) by about 25 %. The high CO_2-treatments on the other hand did not differ significantly in number of ARISA-band-classes over the course of the experiment (Table 3, Fig. 3a) and were therefore significantly different from low and medium CO_2-treatments in phase 3 (Table 3). In the FL-community no significant effect of CO_2-treatment on the number of ARISA-band-classes was detected, but in all CO_2-levels significant differences were observed between the phases (Table 3). The number of ARISA-band-classes in the FL-community in all mesocosms developed similar to that of the surrounding fjord water (Fig. 3b). It showed a general linear reduction in number ($R^2 = 0.58$, $p < 0.0001$)

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and evenness (Pilou) of their fluorescence intensity ($R^2 = 0.59$, $p < 0.0001$) over the course of the experiment.

4 Discussion

This study is part of the large scale in situ mesocosm study of the European Project on Ocean Acidification (EPOCA) conducted in the Arctic in 2010. In situ mesocosm studies are a very useful tool to investigate the response of organisms to ocean acidification close to natural environmental conditions and in complex natural community structures. These studies provide an important link of laboratory-based experiments, elucidating the response of single species, to community processes observable in the field (Riebesell et al., 2008). The natural variability of CO_2in seawater, caused by biological and physical processes, has been proposed to foster a high ability of microbes to adapt to different levels of pCO_2 (Joint et al., 2010). Also at our study site the very low value of ~ 185 µatm seawater pCO_2 at the beginning of our experiment indicates a high temporal variability of CO_2-levels in the Kongsfjorden environment. This stresses the importance to include this CO_2-variability in studies of potential ecosystem responses to ocean acidification. This study investigates the response of the pelagic bacterial community to a gradient of pCO_2-values in large scale in situ mesocosms in the Arctic by Automated Ribosomal Intergenic Spacer Analysis (ARISA). A previous study found a maximum of 11 DGGE-bands (Denaturing Gradient Gel Electrophoresis) in summer in the Kongsfjorden ecosystem (Zeng et al., 2009). The ARISA-method was able to resolve about twice as many band classes as DGGE in this environment, despite the fact that the applied binning slightly reduced band number per sample (see methods section). The use of a standardised amount of DNA (2.5 ng) for each analysis not only allowed to compare the samples according to presence or absence of bands but also to estimate their relative abundances according to the fluorescence intensity of the individual bands. While representation of actual species richness by ARISA-bands is controversial (Crosby and Criddle, 2003; Brown et al., 2005; Kovacs et al., 2010),

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the most common difficulties due to fragment length variability can be overcome by the binning applied in this study (Brown et al., 2005; Kovacs et al., 2010) and relative band-richness can be investigated without expecting too much bias.

The analysis of Bray-Curtis-Similarity-matrices revealed that the bacterial communities in all mesocosms showed a similar temporal development over the course of the experiment. This was the case for both, the PA- and the FL-community-structure, despite the fact that the community structures of the two fractions were significantly different from each other (PERMANOVA, $p < 0.001$). This development was to a large degree determined by the two collinear factors temperature and Chl *a*, which showed equal or similar temporal trends in all mesocosms and explained about 24 % and 42 % of the variability in the PA- and FL-community, respectively. Hence, the FL-community was explained to a large degree by these variables, while the PA-community was also strongly influenced by other factors. The correlation to pH (9 %) was even larger than to Chl *a* (7 %) and the PA-community showed a significant response to CO_2-treatment in multivariate analyses (PERMANOVA, $p = 0.023$). The correlation of the FL-community composition to Si is probably coincidental, as both develop more or less temporally linear over large parts of the experiment. The assumption that there is no functional relation between Si and FL-bacteria is supported by the fact that addition of Si together with other nutrients at day 13 (Schulz et al., 2012) had no significant effect on FL-bacterial community composition or richness. Overall the investigated abiotic parameters and Chl *a* explained only 38 % of the variability in the PA-community and 54 % in the FL-community. This suggests a large influence of other, probably biotic, factors especially on the PA-community.

It was previously described, that dynamics of bacterioplankton and especially of the PA-fraction is often strongly related to the development of phytoplankton-blooms (e.g. Riemann et al., 2000; Pinhassi et al., 2004; Rinta-Kanto et al., 2012) and we assume that this is represented in our data by the correlation to Chl *a*. Also in an ocean acidification experiment in a temperate fjord in Bergen, Norway, Allgaier et al. (2008) found the dynamics of the PA-community to be predominantly related to the development of

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phytoplankton. Their data also suggest, in accordance to findings of a similar study by Arnosti et al. (2011), that the response of the bacterial community to elevated pCO_2-levels was most pronounced after the breakdown of a phytoplankton bloom in their experiments. It is therefore reasonable to distinguish the response of the bacterial community to CO_2-treatments in the phytoplankton bloom phase from the post-bloom phase. While there were several peaks in Chl *a* during the experiment (Schulz et al., 2012), bacterial activity (Piontek et al., 2012) and abundance (Brussaard et al., 2012) exhibited sudden and treatment-related changes only from the beginning of phase 3 of the experiment, directly after the breakdown of a single peak in picophytoplankton cell numbers (≤ 3 µm; see Brussaard et al., 2012), which was not clearly reflected in Chl *a*-values (Schulz et al., 2012). The breakdown of picophytoplankton cell numbers was followed by a rise in DOC and POC production in the mesocosms, which was significantly higher in high CO_2-treatments (Engel et al., 2012), suggesting higher availability of organic material for heterotrophic bacteria. We therefore focused on comparing the communities before and after this picophytoplankton bloom. The richness of the PA-community, in terms of ARISA-band-classes, responded differently to the breakdown of picophytoplankton cell numbers in high CO_2-treatments (~ 650–800 µatm pCO_2 than in low- and medium-treatments (~ 180–600 µatm pCO_2. At low and medium CO_2 the number of ARISA-band-classes was reduced by about 25 % in the post-bloom phase. In contrast, in high CO_2-treatments it was more or less stable. Accordingly, a significant difference in community richness between low and medium CO_2-treatments versus high CO_2-treatments was observed during phase 3 of the experiment, suggesting significant effects of pCO_2-values projected to arise by the end of the century (Barry et al., 2010), on the richness of PA-bacteria. These changes in PA bacterial diversity were not clearly reflected in data of total bacteria (Zhang et al., 2012), because the richness of FL-bacteria reduced at the same time (Fig. 3b). It seems, however, not likely that bacteria were to a large extend recruited from free living to particles, because FL-richness is reduced similarly in all treatments, while PA-richness is enhanced only in high CO_2-treatments (compare Fig. 3a, b). In accordance with a study by Newbold et al. (2012)

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no significant effect of ocean acidification was found on the FL bacterial community in our experiment. Another study by Allgaier et al. (2008) found an influence on the community composition of FL rather than PA-bacteria in a temperate fjord in Norway, but their FL-community was defined < 5 µm, suggesting that a considerable part of the community defined as free living by these authors would have been retained on our 3 µm-filters collecting the PA-community. Thus we expect that effects on the bacterial community do not necessarily have to be of a different nature in the high Arctic than in the temperate fjord. For our experiment a stronger top-down control by viral grazing in high CO_2-treatments is proposed (Brussaard et al., 2012) and we hypothesise that this together with picophytoplankton dynamics (Brussaard et al., 2012) strongly influenced the composition of the PA bacterial community. It seems likely that viruses lysed predominantly those bacterial species dominating the community after the breakdown of the picophytoplankton bloom, thus allowing for a higher bacterial diversity in high CO_2-treatments. This is in accordance with lower total bacterial cell numbers and higher viral cell numbers in high CO_2-mesocosms in phase 3 of the experiment (Brussaard et al., 2012). In addition, enhanced competitive relationships in a more diverse community (Jiang et al., 2008; Becker et al., 2012) and adverse effects on bacterial cells could also have contributed to a higher susceptibility of the bacterial community in high CO_2-treatments to viral lysis or grazing. A treatment dependent influence of other grazers seems not likely, as no changes were observed in protozooplankton with CO_2 in the mesocosms (Abele et al., 2012). The higher DOC and POC production under high CO_2 (Engel et al., 2012) may not only have added dynamics to the community structure by posing a substrate pulse, but may also have increased the availability of substrate related niches for marine bacteria. This has also been found to be an important factor shaping marine bacterial communities (Teeling et al., 2012).

The differences in total bacterial cell numbers (Brussaard et al., 2012) complicate the interpretation of the reaction of net bacterial activity to elevated pCO_2 and the influence of bacterial richness on the community function is hard to evaluate. Based on the widely accepted model of the "complementary mechanism" and the "selection

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mechanism" (Loreau and Hector, 2001) we would expect higher community productivity during times with higher community richness. In fact bacterial protein production (BPP) was higher in high CO_2-treatments during phase 3 (Piontek et al., 2012), despite lower total bacterial cell numbers (Brussaard et al., 2012). This suggests that the more diverse community efficiently competes with phytoplankton for inorganic nutrients during this phase, probably contributing to the limitation of planktonic primary production in the system (Engel et al., 2012). The net activity of two bacterial extracellular enzymes (β-glucosidase, leucin-aminopeptidase) on the other hand was decreased in treatments showing high diversity in the PA-community, most probably reflecting lower bacterial cell numbers, as enzymatic rates per cell for both enzymes were not affected (Piontek et al., 2012). Overall our data suggest that, despite the fact that bacterial strains can survive varying pH-conditions (Joint et al., 2010), the bacterioplankton community composition can still be changed considerably by elevated seawater CO_2 via grazing impact or other secondary effects. Although not easily observable on short time scales, these changes in bacterial community have a high potential to result in changes in type or intensity of bacterial activity, for example via changes in resource partitioning.

The water masses in Kongsfjorden can be regarded as relatively similar to that in the West-Spitsbergen-Current (WSC), due to relatively high exchange rates with water at the mouth of the fjord (Svendsen et al., 2002). Nevertheless the overall conditions are surely influenced by the relative enclosed fjord situation, e.g. the runoff of melt water. While the bacterial community was found to be spatially relatively homogenous in Kongsfjorden (Zeng et al., 2009), it was shown to differ significantly from that of a neighbouring fjord with less water exchange (Piquet et al., 2010) indicating spatial variability on a larger scale. A high spatial variability is also present in the factors limiting bacterial growth in the coastal waters of Svalbard (Vadstein, 2011) and this has been proposed to influence the reaction of the bacterial community to CO_2-perturbation (Riebesell et al., 2008). Therefore caution has to be taken when judging the general validity of our results for the response of the bacterial community to ocean acidification in Arctic waters. In addition, the bacterial community structure in Kongsfjorden can vary

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considerably seasonally (Piquet et al., 2010). Nevertheless, the fact that other mesocosm studies in a Norwegian fjord found similar results, suggests an emerging pattern of secondary effects on PA-bacteria under ocean acidification, while FL-bacteria stay rather unchanged. However, in our study the richness and evenness in the FL-community showed a constant decrease in all mesocosms and the fjord over the duration of our experiment. Especially community evenness has been previously identified as a key factor for functional stability of a community in response to stress (Wittebolle et al., 2009). As a consequence the FL-community and its functionality might be more vulnerable to ocean acidification later in the year, suggesting further investigation.

5 Conclusion

Both, the particle associated (PA) and the free living (FL) bacterioplankton community exhibited a strong temporal development, which was correlated mainly to temperature and Chl *a* values. For the PA-community, a considerable proportion (9 %) of community variability was associated to seawater pH, while it did not significantly affect FL-community structure. The resolution of ARISA was about twice as high, with respect to number of bacterial band-classes, as that of DGGE in an early study in Kongsfjorden and allowed for a detailed investigation of the explicit richness. In contrast to the FL-community, we observed a significant influence of elevated CO_2 (levels expected at the end of the century) on the number of ARISA-band-classes in the PA-community in response to the breakdown of a phytoplankton bloom. At this time, number of ARISA-band-classes reduced at low and medium CO_2 (~ 180–600 µatm) by about 25 %, while it was more stable at high CO_2 (~ 650–800 µatm). We hypothesise enhanced viral lysis at high CO_2 prevented specialists for post-bloom situations in the PA-bacterial community to outcompete other community members. In addition enhanced availability of organic substrates probably resulted in a more diverse structure of ecological niches for heterotrophic bacteria. Bacterial protein production in this phase (phase 3) was enhanced in high CO_2-treatments, suggesting higher richness in the PA-community

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allowed for a more complete use of available resources ("complementarity mechanism"), despite lower total bacterial cell numbers.

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Table 1. Distance based multivariate multiple regression (DistLM; step wise, adjusted R^2) on the relation of environmental variables to the community structure of particle attached and free living bacteria. Significant P-values (< 0.5) are given in bold numbers.

Variable	Adj R^2	P	Prop.	Cumul. (%)
Particle associated				
temp	0.14	**0.001**	0.17	16.80
pH	0.20	**0.004**	0.09	25.35
chl *a*	0.25	**0.011**	0.07	32.58
PO_4	0.29	**0.029**	0.05	38.07
Si	0.31	0.102	0.04	41.99
Free living				
chl *a*	0.22	**0.001**	0.25	24.50
temp	0.38	**0.001**	0.18	42.17
Si	0.50	**0.001**	0.12	54.23
PO_4	0.51	0.098	0.03	56.89
DIN	0.52	0.110	0.03	59.46

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Table 2. Permutational multivariate analysis of variance (PERMANOVA) on ARISA banding pattern of particle associated and free living bacteria resolving differences between experimental days and mesocosms. *F* is a multivariate analogue to Fisher's F-ratio (Anderson, 2001).

Source of variation	d.f.	SS	*F*	*P*	Sq.root
Particle associated					
Day	4	15180.00	4.88	0.001	21.97
Mesocosm	6	7800.40	1.67	0.023	10.77
Residuals	21	16340.00			27.89
Free living					
Day	4	28448.00	14.92	0.001	31.35
Mesocosm	6	4046.90	1.41	0.063	6.40
Residuals	23	10965.00			21.84

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Table 3. One-Way Analysis of Variance (ANOVA) testing for differences in the number of ARISA-band-classes before and during a phytoplankton-bloom[1] (phases 0–2) vs. after the bloom (phase 3) as well as in low- and medium CO_2-treatment-levels (~ 180–600 µatm) vs. high CO_2-levels (~ 650–800 µatm). Significant *P*-values (< 0.5) are given in bold numbers. M7 was not sampled at day −1.

Data	Groups	*P*	Interpretation
Particle associated			
low and medium CO_2	bloom phase	**0.003**	bloom phases differ only in low and
high CO_2	bloom phase	0.216	medium CO_2-treatments
before and during bloom	CO_2 level	0.309	the treatments differ only after the
after bloom	CO_2 level	**0.031**	bloom significantly
Free living			
low and medium CO_2	bloom phase	**0.007**	both treatment levels show differences
high CO_2	bloom phase	**0.005**	between bloom phases
before and during bloom	CO_2 level	0.434	no effect of treatment
after bloom	CO_2 level	0.365	in any bloom phase

[1]The bloom situation was determined by the development of picophytoplankton cell numbers (see Brussaard et al., 2012). The pre- and bloom phase correspond to phase 0–2 and the post-bloom phase to phase 3 of the experiment.

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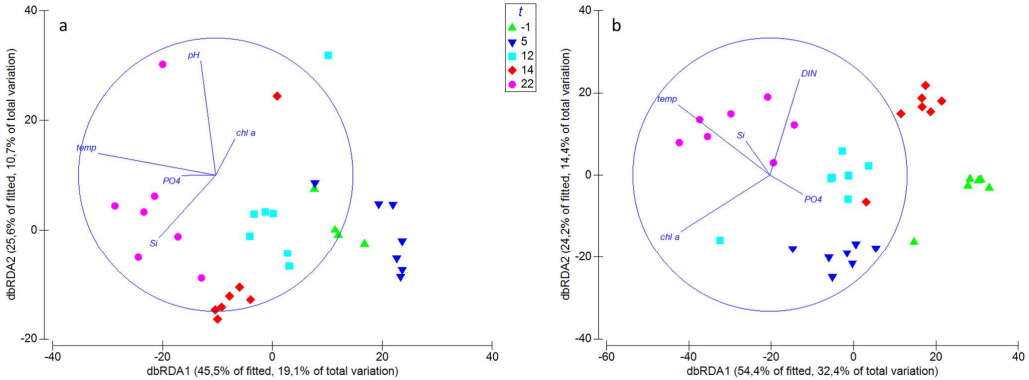

Fig. 1. Distance based Redundancy Analyses (dbRDA) for **(a)** particle associated bacteria and **(b)** free living bacteria. Symbols distinguish experiment days (*t*).

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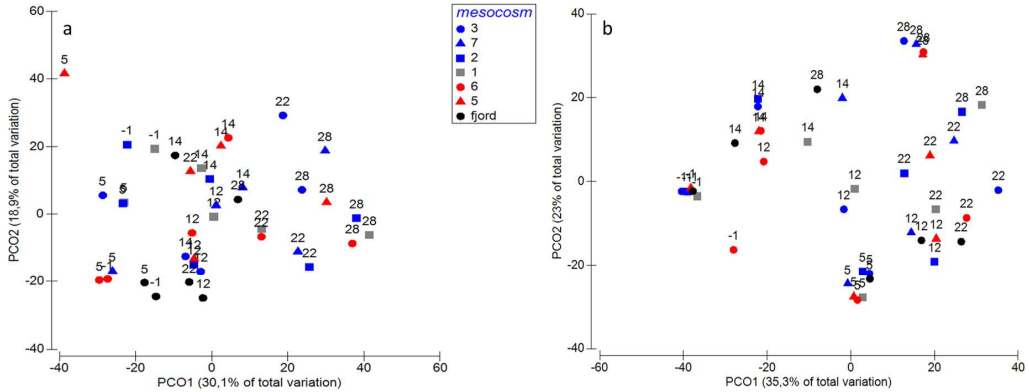

Fig. 2. Principal Coordinate Analyses (PCO) on a Bray-Curtis similarity matrix of **(a)** particle associated community and **(b)** free living community. Colours and symbols show mesocosm and treatment level – blue: low CO_2 addition (~ 170–250 µatm), grey: medium CO_2 addition (~ 590 µatm) and red: high CO_2 addition (~ 720–800 µatm). Numbers in the plots denote experiment day.

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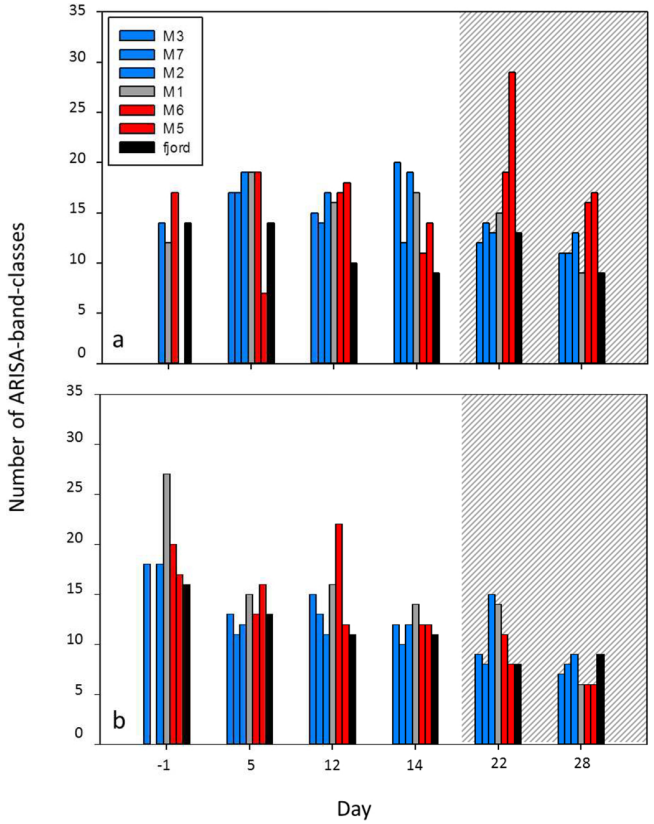

Fig. 3. Development of ARISA-band-classes in the **(a)** PA-community and **(b)** FL-community during the experiment. The colours encode treatment level. Blue: low CO_2 = M3, 7 and 2 (~ 180–250 µatm); grey: medium CO_2 = M1 (~ 600 µatm); red: high CO_2 = M6 and M5 (~ 650–800 µatm). The shaded area indicates phase 3 of the experiment (shortly after breakdown of picophytoplankton cell numbers).

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