



# Effect of elevated CO<sub>2</sub> on the dynamics of particle-attached and free-living bacterioplankton communities in an Arctic fjord

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**Abstract.** In the frame of the European Project on Ocean Acidification (EPOCA), the response of an Arctic pelagic community (<3 mm) to a gradient of seawater  $p\text{CO}_2$  was investigated. For this purpose 9 large-scale in situ mesocosms were deployed in Kongsfjorden, Svalbard (78°56.2' N, 11°53.6' E), in 2010. The present study investigates effects on the communities of particle-attached (PA; >3  $\mu\text{m}$ ) and free-living (FL; <3  $\mu\text{m}$  > 0.2  $\mu\text{m}$ ) bacteria by Automated Ribosomal Intergenic Spacer Analysis (ARISA) in 6 of the mesocosms, ranging from 185 to 1050  $\mu\text{atm}$  initial  $p\text{CO}_2$ , and the surrounding fjord. ARISA was able to resolve, on average, 27 bacterial band classes per sample and allowed for a detailed investigation of the explicit richness and diversity. 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, numbers of ARISA band classes in the PA community were reduced at low and medium CO<sub>2</sub> (~185–685  $\mu\text{atm}$ ) by about 25 %, while they were more or less stable at high CO<sub>2</sub> (~820–1050  $\mu\text{atm}$ ). We hypothesise that enhanced viral lysis and enhanced availability of organic substrates at high CO<sub>2</sub> 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<sub>2</sub> mesocosms, suggesting a positive effect of community richness on this function and on carbon cycling by bacteria.

## 1 Introduction

The increase in anthropogenic carbon dioxide (CO<sub>2</sub>) in the atmosphere causes an enhanced uptake of CO<sub>2</sub> by the oceans (Raven et al., 2005), and, if CO<sub>2</sub> emissions continue at current rates, this 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 expected 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<sub>2</sub> and increased melting of sea ice will 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.

The activity and the resulting ecological and biogeochemical functioning of bacterioplankton are 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; Nikrad et al., 2012). 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 community function also in bacteria (Bell et al., 2005). On the other hand, a negative correlation between community richness and function has been observed, for example in freshwater algae (Naeem and Li, 1997). This can be explained 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 processes have also been described as widespread in marine bacterioplankton and especially among particle-associated communities (Long and Azam, 2001), providing a conceptual basis for the occurrence of “negative complementarity effects” also in marine bacterioplankton. These facts highlight the importance of studying bacterial community development and especially richness under ocean acidification scenarios.

Several laboratory studies indicate that the microbial response to elevated  $p\text{CO}_2$  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). Effects of anthropogenic ocean acidification on different cyanobacterial cultures varied from increased C- and N-fixation in non-heterocystous cyanobacteria (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; Rokkan Iversen and Seuthe, 2011) and other factors like nutrient limitation or interspecific relationships. To investigate potential biogeochemical changes in ecosystems due to ocean acidification, it is necessary to study natural microbial communities, ideally incorporating several trophic levels. Such studies found multiple effects of ocean acidification on the activity and biogeochemical functioning of bacterioplankton. As general outcomes, negative effects 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) while positive effects were 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 variable 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<sub>2</sub> (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). Two of these studies found significant differences between CO<sub>2</sub> treatments in the DGGE 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 CO<sub>2</sub> treatment but closely related to phytoplankton (Allgaier et al., 2008). Another study using T-RFLP to investigate the six most abundant prokaryotes and eukaryotes in the free-living (<2 µm) fraction did not observe significant differences with CO<sub>2</sub> in all but one bacterium (Newbold et al., 2012). All effects of ocean acidification 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 another, 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 pelagic

bacterial communities to lowered seawater pH has, to the best of our knowledge, not been studied so far. Also, little attention has been 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 intergenic 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 that investigated for the first time 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 diversity, with an additional 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, containing all organisms < 3 mm 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<sub>2</sub> supersaturated water to initial *p*CO<sub>2</sub> levels of 270 (together with the controls considered as “low CO<sub>2</sub> treatments”), 375, 480, 685 (considered as “medium CO<sub>2</sub> treatments”), 820, 1050 and 1420 μatm (considered as “high CO<sub>2</sub> treatments”). Two control mesocosms were left unchanged at initial *p*CO<sub>2</sub> of 185 μatm. After 13 days nutrients (NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> 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. Six of the mesocosms (2 × 185, 270, 685, 820 and 1050 μatm initial CO<sub>2</sub>) and the fjord were sampled for this study.

### 2.2 Sampling, filtration and DNA extraction

Samples were collected from six mesocosms of 2 × 185, 270, 685, 820 and 1050 μatm initial CO<sub>2</sub> and from the surrounding fjord water on day -1 (starting conditions before treatment), day 5 (starting conditions after completion of CO<sub>2</sub> 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 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<sup>®</sup>, 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 rediluted in 100 μL of PCR-grade H<sub>2</sub>O, aliquoted and measured in duplicates using a NanoQuant Plate and the plate reader Infinite M200 (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 CCG-3') and the fluorescence-labelled backward primer S-D-Bact-1522-b-S-20 (5'-TGC GGC TGG ATC CCC TCC TT-3') described by Ranjard et al. (2000). Each 25 μL reaction mixture contained 0.56 mM of each primer, 2.5 ng of template DNA, 0.3 mM deoxynucleoside triphosphate mix, 5 μL of TaqMasterPCR Enhancer, 2.5 μL of TaqBuffer advanced with Mg<sup>2+</sup> 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.

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

tetramethylethylenediamine (TEMED) and ammonium persulfate (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<sup>®</sup> 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 minutes. 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 (Applied-Math, Sint-Martens-Latem, Belgium) was used to semiquantitatively 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) were 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<sub>0</sub> 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) 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<sub>2</sub> treatments (~ 175–600 µatm average over experiment) were pooled and tested against high CO<sub>2</sub> treatments (~ 675–860 µatm average over experiment). 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

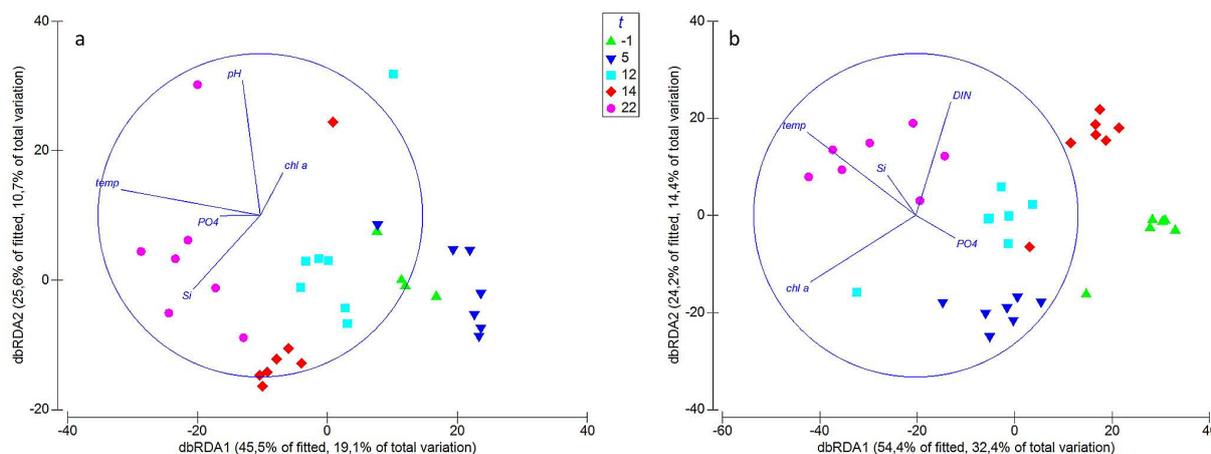
by one-way analysis of variance (ANOVA) in the software package SigmaPlot12.1.

## 3 Results

The number of ARISA band classes observed ranged from 15 to 39, 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), chlorophyll *a* (Chl *a*) and phosphate (PO<sub>4</sub>) exhibited 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 silicate (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 were able to explain about 38 % and 54 % of the variability in the PA and FL communities, respectively.

The potential effect of the CO<sub>2</sub> 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 experiment 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” (CO<sub>2</sub> treatment) in both fractions (Table 2). The influence of mesocosm (CO<sub>2</sub> treatment) was still significant for the PA community however ( $p < 0.05$ ), while this was not the case for the FL community (Table 2).

When investigating the richness in terms of number of ARISA band classes, an influence of  $p$ CO<sub>2</sub> treatment on the PA community was evident in the third phase of the experiment (defined as: Chl *a* minimum until the end of the experiment, t<sub>22</sub>–t<sub>28</sub>, see Schulz et al., 2012). For the PA community, the number of ARISA band classes in low and medium CO<sub>2</sub> mesocosms differed significantly between phase 3 (shortly after breakdown of phytoplankton cell numbers, see Brussaard et al., 2012) and all other phases of the



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

**Table 1.** Distance-based multivariate multiple regression (DistLM; step wise, adjusted  $R^2$ ) on the relation of environmental variables to the community structure (Bray–Curtis) of particle-attached and free-living bacteria. Significant  $P$ -values ( $< 0.05$ ) are given in bold numbers. Prop. is the proportion of variability explained by the respective variable.

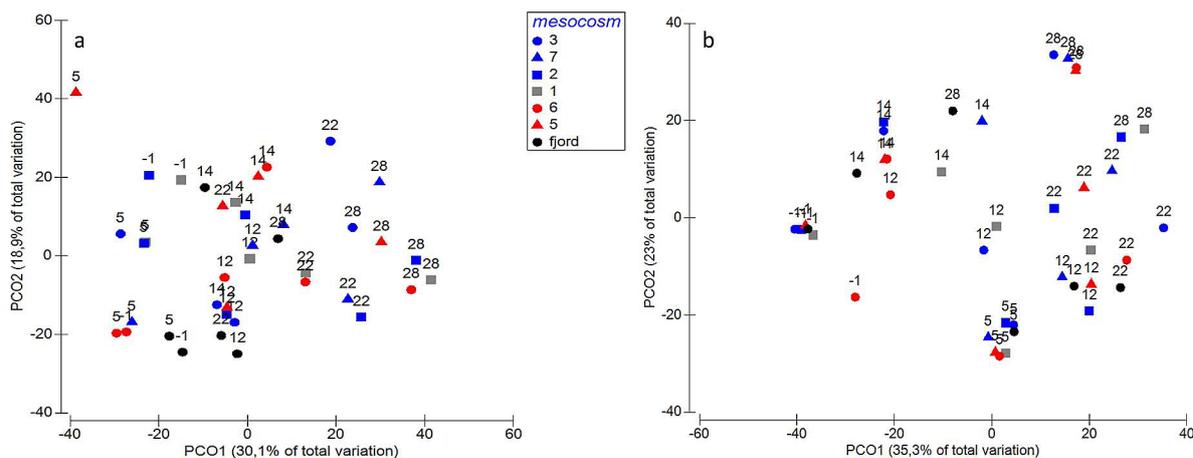
| Variable            | Adj. $R^2$ | $P$          | Prop. | Cumul. (%) |
|---------------------|------------|--------------|-------|------------|
| Particle-associated |            |              |       |            |
| temp                | 0.14       | <b>0.001</b> | 0.17  | 16.80      |
| pH                  | 0.20       | <b>0.004</b> | 0.09  | 25.35      |
| chl <i>a</i>        | 0.25       | <b>0.011</b> | 0.07  | 32.58      |
| PO <sub>4</sub>     | 0.29       | <b>0.029</b> | 0.05  | 38.07      |
| Si                  | 0.31       | 0.102        | 0.04  | 41.99      |
| Free-living         |            |              |       |            |
| chl <i>a</i>        | 0.22       | <b>0.001</b> | 0.25  | 24.50      |
| temp                | 0.38       | <b>0.001</b> | 0.18  | 42.17      |
| Si                  | 0.50       | <b>0.001</b> | 0.12  | 54.23      |
| PO <sub>4</sub>     | 0.51       | 0.098        | 0.03  | 56.89      |
| DIN                 | 0.52       | 0.110        | 0.03  | 59.46      |

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<sub>2</sub> mesocosms 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<sub>2</sub> treatments in phase 3 (Table 3). In the FL community no significant effect of CO<sub>2</sub> treatment on the number of ARISA band classes was detected, but significant differences were observed between the phases at all CO<sub>2</sub> levels (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 decrease in number ( $R^2 = 0.58$ ,  $p < 0.0001$ ) and evenness (Pilu) of 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 experiment 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 community structures. These studies provide an important link between laboratory-based experiments, elucidating the response of single species, and community processes observable in the field (Riebesell et al., 2008). The natural variability of CO<sub>2</sub> in seawater, caused by biological and physical processes, has been proposed to foster a high ability of microbes to adapt to different levels of  $p\text{CO}_2$  (Joint et al., 2010). Also at our study site the very low value of  $\sim 185 \mu\text{atm}$  seawater  $p\text{CO}_2$  at the beginning of our experiment indicates a high temporal variability of CO<sub>2</sub> levels in the Kongsfjorden environment. This stresses the importance to include this CO<sub>2</sub> variability in studies of potential ecosystem responses to ocean acidification. Our study investigates the response of a pelagic bacterial community in an Arctic glacial fjord to a gradient of  $p\text{CO}_2$  values by Automated Ribosomal Intergenic Spacer Analysis (ARISA). Previous studies in Kongsfjorden found a maximum of 11 DGGE bands (Denaturing Gradient Gel Electrophoresis) in summer (Zeng et al., 2009) and up to 24 in a mesocosm study in August (Töpper et al., 2010). The ARISA method was able to resolve on average 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



**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<sub>2</sub> addition (~ 175–250 μatm average over experiment), grey: medium CO<sub>2</sub> addition (~ 600 μatm average over experiment) and red: high CO<sub>2</sub> addition (~ 675–860 μatm average over experiment). Numbers in the plots denote experiment day.

**Table 2.** Permutational multivariate analysis of variance (PERMANOVA) on ARISA banding pattern (Bray–Curtis) 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        | <i>F</i> | <i>P</i> | Sq.root |
|---------------------|------|-----------|----------|----------|---------|
| Particle-associated |      |           |          |          |         |
| Day                 | 4    | 15 180.00 | 4.88     | 0.001    | 21.97   |
| Mesocosm            | 6    | 7800.40   | 1.67     | 0.023    | 10.77   |
| Residuals           | 21   | 16 340.00 |          |          | 27.89   |
| Free-living         |      |           |          |          |         |
| Day                 | 4    | 28 448.00 | 14.92    | 0.001    | 31.35   |
| Mesocosm            | 6    | 4046.90   | 1.41     | 0.063    | 6.40    |
| Residuals           | 23   | 10 965.00 |          |          | 21.84   |

of DNA (2.5 ng) for each analysis allowed us to compare the samples according to presence or absence of bands as well as to estimate the relative abundances of the individual bands according to their fluorescence intensity. While representation of actual species richness by ARISA bands is controversial (Crosby and Criddle, 2003; Brown et al., 2005; Kovacs et al., 2010), 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 with minor 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 commu-

nity structures, 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<sub>2</sub> 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 are 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 phytoplankton.

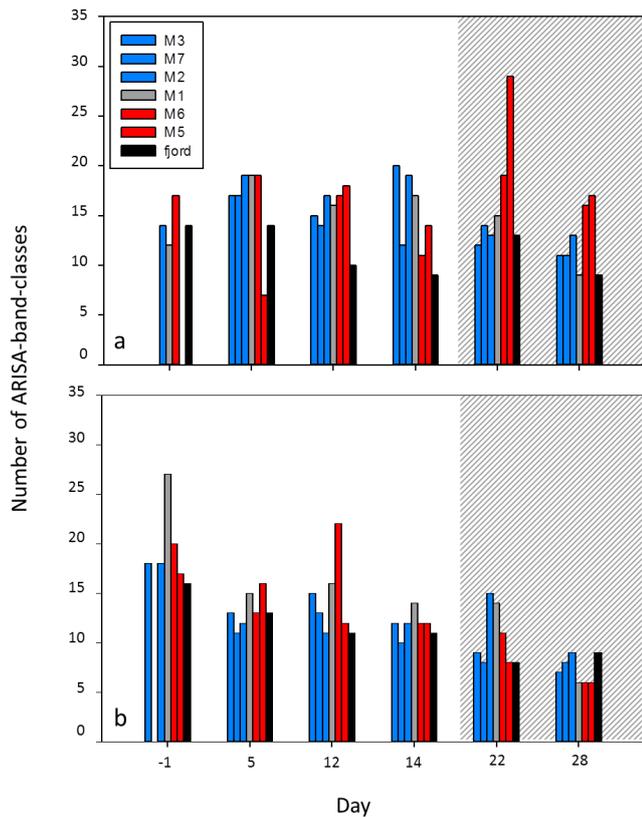
**Table 3.** One-way analysis of variance (ANOVA) testing for differences in the number of ARISA band classes before and during a phytoplankton bloom\* (phases 0–2) vs. after the bloom (phase 3) as well as in low and medium CO<sub>2</sub> treatment levels (~175–600 μatm average over experiment) vs. high CO<sub>2</sub> levels (~675–860 μatm average over experiment). Significant *P*-values (< 0.05) are given in bold numbers. M7 was not sampled at day –1.

| Data                           | Groups                | <i>P</i>     | Interpretation  |
|--------------------------------|-----------------------|--------------|---|
| Particle-associated            |                       |              |   |
| Low and medium CO <sub>2</sub> | bloom phase           | <b>0.003</b> | bloom phases differ only in low and medium CO <sub>2</sub> treatments |
| High CO <sub>2</sub>           | bloom phase           | 0.216        |   |
| Before and during bloom        | CO <sub>2</sub> level | 0.309        | the treatments differ only after the bloom significantly              |
| After bloom                    | CO <sub>2</sub> level | <b>0.031</b> |   |
| Free-living                    |                       |              |   |
| Low and medium CO <sub>2</sub> | bloom phase           | <b>0.007</b> | both treatment levels show differences between bloom phases           |
| High CO <sub>2</sub>           | bloom phase           | <b>0.005</b> |   |
| Before and during bloom        | CO <sub>2</sub> level | 0.434        | no effect of treatment in any bloom phase                             |
| After bloom                    | CO <sub>2</sub> level | 0.365        |   |

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

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 *p*CO<sub>2</sub> 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<sub>2</sub> 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 abundance (Brussaard et al., 2012) exhibited 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). Also, bacterial enzyme activity (Piontek et al., 2012) showed sudden changes in trends at this point in the experiment. The breakdown of picophytoplankton cell numbers was also followed by a rise in dissolved and particulate primary production in the mesocosms, which was significantly higher in high CO<sub>2</sub> 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<sub>2</sub> mesocosms (~675–860 μatm *p*CO<sub>2</sub> average over experiment) than in low and medium CO<sub>2</sub> treatments (~175–600 μatm *p*CO<sub>2</sub> average over experiment). At low and medium CO<sub>2</sub>, the number of ARISA band classes was reduced by about 25 % in the post-bloom phase. In contrast, in high CO<sub>2</sub> mesocosms it was more or less stable. Ac-

cordingly, a significant difference in community richness of PA bacteria between low and medium CO<sub>2</sub> treatments versus high CO<sub>2</sub> treatments was observed during phase 3 of the experiment. This suggests *p*CO<sub>2</sub> values projected to arise by the end of the century (Barry et al., 2010) have the potential to significantly affect PA bacterial richness. 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). However, it seems not likely that bacteria were to a large extent recruited from the free-living fraction to particles, because FL richness is reduced similarly in all treatments, while PA richness is enhanced only in high CO<sub>2</sub> mesocosms (compare Fig. 3a and b). In accordance with a study by Newbold et al. (2012), 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. It is important to note that 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 lysis in high CO<sub>2</sub> 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



**Fig. 3.** Development of ARISA band classes in the (a) particle-associated community and (b) free-living community during the experiment. The colours encode treatment level. Blue: low CO<sub>2</sub> = M3, 7 and 2 (~175–250 μatm average over experiment); grey: medium CO<sub>2</sub> = M1 (~600 μatm average over experiment); red: high CO<sub>2</sub> = M6 and M5 (~675–860 μatm average over experiment). The shaded area indicates phase 3 of the experiment (shortly after breakdown of picophytoplankton cell numbers).

species dominating the community after the breakdown of the picophytoplankton bloom, thus allowing for a higher bacterial diversity in high CO<sub>2</sub> treatments. This is in accordance with lower total bacterial cell numbers and higher viral cell numbers in high CO<sub>2</sub> 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 viral lysis and grazing susceptibility of the bacterial community in high CO<sub>2</sub> treatments. A treatment dependent influence of other grazers seems not likely, as no changes were observed in protozooplankton with CO<sub>2</sub> in the mesocosms (Aberle et al., 2012). The higher DOC and POC production under high CO<sub>2</sub> (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 impor-

tant 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 *p*CO<sub>2</sub>, 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 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<sub>2</sub> 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; Thingstad et al., 2008). The activity of two bacterial extracellular enzymes (*β*-glucosidase, leucinaminopeptidase), 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<sub>2</sub> 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.

The water masses in Kongsfjorden can be regarded as relatively similar to those 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 homogeneous 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<sub>2</sub> 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 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 Conclusions

Both the particle-associated (PA) and the free-living (FL) bacterioplankton communities exhibited a strong temporal development, which was correlated mainly to temperature and Chl *a* values. For the PA community (Bray–Curtis), a considerable proportion (9%) of community variability was associated with 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 earlier studies 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<sub>2</sub> (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, the number of ARISA band classes reduced at low and medium CO<sub>2</sub> (~175–600 μatm average over experiment) by about 25%, while it was more stable at high CO<sub>2</sub> (~675–860 μatm average over experiment). We hypothesise enhanced viral lysis at high CO<sub>2</sub> prevented specialists for post-bloom situations in the PA bacterial community from outcompeting other community members in high CO<sub>2</sub> treatments. 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<sub>2</sub> treatments, suggesting higher richness in the PA community allowed for a more complete use of available resources (“complementarity mechanism”), despite lower total bacterial cell numbers.

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