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Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms

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Abstract

The impact of ocean acidification and carbonation on microbial community structure was assessed during a large-scale in situ costal pelagic mesocosm study, included as part of the EPOCA 2010 Arctic campaign. The mesocosm experiment included ambient conditions (fjord) and nine mesocosms, with pCO_2 range from ∼145 to ∼1420 µatm. Samples collected at nine time points (t-1, t1, t5, t7, t12, t14, t22, t26 to t28) in seven treatments (ambient fjord (∼145), 2× ∼185, ∼270, ∼685, ∼820, ∼1050 µatm) were analysed for "free-living" and "particle associated" microbial community composition using 16S rRNA amplicon sequencing. This high-throughput sequencing analysis produced ∼20 000 000 16S rRNA V4 reads, which comprised 7000 OTUs. The main variables structuring these communities were, sample origin (fjord or mesocosms) and the filter size fraction (free-living or particle associated). The community was significantly different between the fjord and both the control and elevated CO_2 mesocosms (which were not significant different) after nutrients were added to the mesocosms; suggesting that the addition of nutrients is the primary driver of the change in mesocosm community structure. The relative importance of each structuring variable depended greatly on the time at which the community was sampled in relation to the phytoplankton bloom. The size fraction was the second most important factor for community structure; separating free-living from particle-associated bacteria. When free-living and particle-associated bacteria were analysed separately at different time points, the only taxon pCO_2 was found to significantly affect were the Gammaproteobacteria after nutrient addition. Finally, pCO_2 treatment was found to be significantly correlated (non-linear) with 15 rare taxa, most of which increased in abundance with higher CO_2.

1 Introduction

The acidification of our oceans, caused predominantly by dissolution of anthropogenic carbon dioxide (CO_2) in seawater, has the potential to affect the physiology of marine

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microbes. Therefore, because marine microbes play a major role in global biogeochemical cycles, this increase may have unforeseen consequences on ocean biogeochemistry (Falkowski et al., 2008; Worden and Not, 2008). Experimental manipulation of the partial pressure of carbon dioxide (pCO_2) in marine mesocosms has demonstrated species-specific physiological responses to elevated dissolved CO_2 concentrations, for example, delayed or decreased Coccolithophore calcification (Delille et al., 2005), a significant increase in photosynthetic capacity (Fu et al., 2008), higher CO_2 and N_2 fixation (Hutchins et al., 2007), and a decreased abundance of picoeukaryotes (Newbold et al., 2012). However, the response of bacterial communities to elevated pCO_2 concentrations is less defined, with mixed reports of both significant increases in bacterial protein production (Grossart et al., 2006), and no significant changes in microbial community structure (Tanaka et al., 2008; Allgaier et al., 2008; Newbold et al., 2012). For example, during the 2008 PeECE III mesocosms study, elevated pCO_2 had no significant impact on bacterial abundance, diversity, or activity; however, the community structure of free-living bacteria was significantly altered by the induced phytoplankton bloom (Allgaier et al., 2008; Arnosti et al., 2011; Riebesell et al., 2008).

While these existing studies have observed little impact of elevated pCO_2 on microbial community structure, they were all performed with molecular techniques that offered limited taxonomic resolution (e.g. high-performance liquid chromatography, denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism). To improve that resolution, this study employed high-throughput amplicon sequencing of 16S rRNA to characterize microbial taxonomic community dynamics. High-throughput amplicon sequencing provides an efficient method to obtain a deep molecular overview of microbial community structure, without having to cultivate environmental isolates (Agogué et al., 2011; Gilbert et al., 2009; Hubert et al., 2007; Huse et al., 2008; Margulies et al., 2005; Sogin et al., 2006). In this study, the variation of microbial assemblages was characterised through time, across a gradient of pCO_2, in a large-scale in situ pelagic mesocosm experiment in the coastal Arctic Ocean. In addition, to characterizing the detailed response of the microbial community structure to

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elevated pCO_2, the analysis of the 16S rRNA database provided insight on the effect of isolating the water column in a mesocosm, adding nutrients to the mesocosms to produce a phytoplankton bloom, and to investigate the community structure response to elevated pCO_2.

2 Methods

2.1 Location and carbonate system manipulation

The European Project on Ocean Acidification (EPOCA) supported a large mesocosm experiment in the Arctic which was conducted in the water of Kongsfjorden, Svalbard, Norway (78°56.2′ N; 11°53.6′ E) during the months of June and July 2010. Throughout the experiment diverse environmental parameters were measured to explore the effect of ocean acidification (OA) on multiple biological processes. Briefly, nine mesocosms containing about 45 m^3 of sea water and reaching down to 15 m depth were deployed from the settlement Ny-Ålesund and pCO_2 was manipulated by addition of CO_2-saturated seawater in seven mesocosms; resulting in a initial pCO_2 range from ~185 to ~ 1420 µatm. The two un-manipulated mesocosms were used as controls and had a starting pCO_2 of ~185 µatm. Additionally, samples were taken directly from the fjord (initial pCO_2 ~145 µatm) in which the mesocosms were suspended and from which the mesocosm water originated. These samples were to monitor any natural changes in pCO_2 that may occur in the ambient water during the course of the experiment and were also important to detect deviations in pCO_2 between the fjord and the untreated mesocosms with time. To promote phytoplankton growth, all nine mesocosms were subjected to nutrient additions (nitrate (NO_3), phosphate (PO_4) and silicate (Si)) on day (t) 13, creating pre-nutrient (t-1 to t12) and post-nutrient (t13 to t30) periods (Fig. 1). Detailed information about the experimental set-up, the mesocosms deployment, the carbonate chemistry and the nutrients additions can be found in this issue in Riebesell

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et al. (2012); Czerny et al. (2012a); Czerny et al. (2012b); Bellerby et al. (2012); and finally in Schulz et al. (2012).

2.2 Sampling, filtration and sample selection

A total of 10 l of water was collected using integrated water sampler (Hydrobios, Kiel, Germany) between 0 and 12 m water depth; from the fjord (~145 µatm) and six mesocosms (starting $pCO_2 = 2 \times \sim 185$, ~ 270, ~685, ~820, ~1050 µatm) on t-1, t1, t5, t7, t12, t14, t22, t26 and t28 (Fig. 1). Only six of the mesocosms were chosen for this study due to time, personnel and equipment constraints. The collected water was first pre-filtered on a 20 µm sieve, and sequentially filtered through a 3 µm filter to isolate associate-particle bacterial fraction and through a 0.2 µm filter to isolate the free-living bacterial fraction (Durapore® 47 mm, Millipore). To avoid nucleic acid degradation, processing of the samples from filtration to flash-freezing (in liquid nitrogen) was performed within 30 min of the sampling event filtered and samples were flash frozen respecting this time period, and then stored at −80 °C until DNA/RNA extraction.

2.3 DNA extraction, PCR, and sequencing

Total nucleic acid was extracted from the 0.2 and 3 µm filters using the "Total RNA and DNA purification – NucleoSpin® RNA II RNA/DNA buffer" kits from Macherey-Nagel (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Standard protocol with minor modifications was followed. Changes to the protocol included, cryo vials containing filters being immersed in liquid nitrogen to allow the filters to be kept frozen until disruption, and to facilitate the homogenization of the samples. The filters were crushed with RNase-free plastic pestles and lysozyme was directly added to the filter. DNA quality and quantity was assessed by micro-volume spectrophotometer nanodrop ND-1000 (PeqLab GmbH, Erlangen, Germany) measurements. All samples were kept at −80 °C until further analysis.

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PCR and sequencing were performed following the Illumina HiSeq2000 and MiSeq V4–16S rRNA protocol (Caporaso et al., 2012). Briefly, the V4-V5 region of the 16S rRNA gene was amplified with region-specific primers that included the Illumina (Illumina Inc., CA, USA) paired-end flowcell adapter sequences. The barcode was read using the custom index sequencing primer in an additional cycle (12 bp). Each sample was amplified in triplicate, and was afterwards pooled. Each 25 µl PCR reaction contained 12 µl of MoBio PCR Water (Certified DNA-Free), 10 µl of 5 Prime HotMasterMix, 1 µl of Forward Primer (5 µM initial concentration), 1 µl Golay Barcode Tagged Reverse Primer (5 µM initial concentration), and 1 µl of template DNA. The reactions were heated to 94 °C for 3 min for their initial denaturation, followed by 35 cycles in series of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s. The amplicons were quantified using Quant-it™ Picogreen® (Invitrogen by life technologies™, Ca, USA), and pooled in equal amounts (ng) into a 1.5 ml tube. Once pooled, the entire amplicon pool was cleaned-up with the MO-BIO UltraClean® PCR Clean-Up Kit (MO-BIO Laboratories, INC., CA, USA). Finally, the pooled samples were quantified using a Qubit® fluorometer (Invitrogen by life technologies™, Ca, USA), and the molarity was estimated based on amplicon length. From this estimate, dilutions were made down to 2 µM and the standard Illumina sample preparation for sequencing was followed. Pooled amplicons were sequenced using custom sequencing primers, read 1, read 2, and index. These sequencing primers were designed to be complementary to the V4 amplification primers to avoid sequencing of the primers. Amplicons were sequenced in a paired-end, 100 bp × 100 bp cycle run on the Illumina HiSeq2000, at a concentration of 4 p.m. with a 10 % PhiX spike. An entire control lane devoted to PhiX is also useful when sequencing low base diversity samples, like amplicons, and was included in the present analysis.

2.4 Sequence data analysis

All sequence analyses were performed using Quantitative Insights Into Microbial Ecology v. 1.5.0 (QIIME; Caporaso et al., 2010). QIIME defaults were used for quality

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filtering of raw Illumina data. OTUs were picked against the Greengenes (McDonald et al., 2012) database pre-clustered at 97 % identity, and sequences that did not hit the reference collection were discarded. Representative sequences were aligned to the Greengenes core set with PyNAST (Caporaso et al., 2010). All sequences that failed to align were discarded. A phylogenetic tree was built from the alignment, and taxonomy was assigned to each sequence using the RDP classifier (Wang et al., 2007) retrained on Greengenes. Samples were rarefied to an even depth of 81 181 sequences and only the OTUs that appeared at least twice in any sample in the dataset were included in the further analyses; 106 singleton OTUs were not included in this analysis.

2.5 Statistical analysis

Multivariate analysis of microbial community structure was carried out in CANOCO 4.54 (ter Braak and Šmilauer, 2002), where the count of each OTU (97 % similarity) was used as a measure of abundance. All analyses had samples as scaling focus, and all species data were Hellinger-transformed using the program PrCoord 1.0 (Legendre and Gallagher, 2001; ter Braak and Šmilauer, 2002). Analysis of variance (ANOVA) followed by a Tukey test was done to test for significant differences between treatments (i.e. control vs. fjord, fjord vs. mesocosm, control vs. mesocosm) within each abundant phyla. Detrended correspondence analysis (DCA) of the transformed OTU abundance data showed axes lengths <3.0, suggesting a linear treatment of the data (Ramette, 2007). Redundancy analysis (RDA), with manual forward selection and Monte Carlo permutation tests (999 permutations), was used to evaluate effects of environmental variables (salinity, temperature, pH, chlorophyll *a*, etc.) on the microbial community composition. An indirect gradient analysis (PCoA) was used to plot the distribution of samples in ordination space, with important environmental variables (as indicated by forward selection) overlaid as Supplement. Microbial community structure was also assessed by UniFrac (Lozupone and Knight, 2005) distance using QIIME (Caporaso et al., 2010).

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In order to assess whether or not particular taxa were significantly influenced by pCO_2, a Bonferroni-corrected g-test was done using QIIME in combination with FDR-corrected p-values to remove significance due to chance. All analyses were considered to have a significant difference if $p < 0.05$ after Bonferroni correction.

Heat maps presenting mean abundance count plotted against pCO_2 and time (days) of the three most abundant genus of the OTUs significantly correlated to pCO_2 were created using Ocean data view (Bremen, Germany).

3 Results

The 250 sequenced samples generated ~20 000 000 16S rRNA V4 reads (~2 510 000 sequences per treatment); which clustered at 97 % sequence identity into 6821 OTUs.

3.1 Experimental timeline

Phytoplanktonic bloom evolution was identified using the daily measured chlorophyll *a* (chl *a*) concentration ($\mu g\, l^{-1}$) (Fig. 1). The chl *a* protocol and patterns are presented in Schulz et al. (2012). Briefly, all treatments (fjord included) underwent a natural bloom between t0 and t11, with its highest chl *a* concentration on t6. Subsequently, a second and third strong phytoplankton bloom happened only in the mesocosms following nutrient addition on t13. The second bloom had its highest chl *a* concentration on t19 and the third one, which varied greatly between mesocosms, reached its highest concentration on t27. These 3 blooms were represented as four general phases in phytoplankton chlorophyll phases defined by Schulz et al. (2012): phase 0 occurred from the start of the experiment on t-4 until adjustment of CO_2 was completed on t4; phase 1 started with the end of CO_2 addition on t4 until the nutrient additions on t13; phase 2 included the end of the first bloom on t13 to the end of the second bloom on t22; and phase 3 started from the end of the second bloom on t22 and lasted until the end of the experiment, on t30 (the chl *a* minimum of the third bloom was not recorded) (Fig. 1).

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Detailed fluctuations of chl *a*, nutrient concentrations, pH and pCO_2 are presented in this issue in Schulz et al. (2012) and Bellerby et al. (2012).

3.2 Community structuring variables

The significant structuring variables for the total community during the post-nutrient addition period (t13–t30) of the experiment were (in order of explanatory importance) "fjord vs. mesocosm origin" (i.e. whether the sample was from water contained in a mesocosm or from the open fjord), filter size fraction (i.e. separating free-living (FL) from particle-associated (PA) bacteria), Si concentration, PO_4 concentration, mean primary production 14C-POC (PP), temperature (T), and pH (Fig S1 and Table 1). The microbial community in the FL size fraction (0.2–3 µm) from the fjord and all the mesocosms, was dominated throughout the experiment by Proteobacteria (in order of abundance: Gamma (γ)-, Alpha (α)- and Beta (β)- proteobacteria), however, this phylum began dropping in abundance gradually after t7, coincident with the increase in the abundance of Bacteroidetes (Fig. 2). In the PA size fraction (3–20 µm) Bacteroidetes dominated consistently, while a fourth group comprised of the "Cyanobacteria and eukaryotic chloroplasts" (which included Chlorophyta, Haptophyceae, Rhodophyta and Stramenopiles) were also abundant (Fig. 2). The group classified as "others" in the FL fraction was composed predominately of Cyanobacteria at the beginning of the experiment, and of Actinobacteria towards the end (Fig. S2). In the PA fraction, the "others" group was extremely variable until t7. For example, at t-1 the fjord "others" group was dominated by the Verrucomicrobia while the mesocosms "others" groups was dominated by Actinobacteria; by t5 Firmicutes dominated in most mesocosms, while being almost absent from the fjord. After at t7, the Actinobacteria was the dominant taxa in the "others" group in all treatments for the remainder of the experiment. At the end (t28), some Verrucomicrobia increased in the control, ∼270, and ∼685 µatm mesocosms (Fig S2).

Once the community was analysed with regard to filter size fraction (FL Vs PA), the structuring community variables varied. The fjord had a significantly different

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assemblage from the mesocosms in the FL and PA size fraction before (origin 3 %–4 %) and after (origin 48 %–12 %) mesocosm nutrient addition (Table 2); however, the fjord and mesocosm communities were not significantly different until after t5. The microbial community in the fjord FL fraction was not significantly different from the mesocosms communities in the pre-nutrient addition phase and only the γ-proteobacterial abundance was significantly different ($p < 0.05$) between fjord and mesocosm in the post-nutrient addition phase. The fjord PA size fraction microbial community was significantly different from the mesocosms during both the pre- and post-nutrient addition phases. In particular, the "Cyanobacteria and eukaryotic chloroplasts" group was significantly different between fjord and mesocosms pre- and post-nutrient addition; while the Bacteroidetes, α-proteobacteria and "others" were only significantly different post-nutrient addition (Fig. 3 and Table 3). Furthermore, the significant variables that correlated with community structure changes in the FL size fraction were dimethyl sulphide (DMS–16 %), bacterial production (bp–15 %), density (d–12 %) for the pre-nutrient period (t-4 to t12), and origin (48 %), $p\mathrm{CO_2}$ (10 %), day (10 %) for the post-nutrient period (t13–t30; Table 2). For the "large" size fraction, these variables were oxygen (O_2–7 %), DMS (7 %), nitrate (NO_3–5 %) and origin (4 %) for the pre-nutrient period (t-4 to t12); and Si (27 %) and origin (12 %) for the post-nutrient addition period (t13–t30; Table 2). Therefore, the differences in microbial community structure between the fjord and mesocosms were primarily due to the addition of nutrients to the mesocosms, and not to $p\mathrm{CO_2}$ manipulation, as the control mesocosms were not significantly different from the elevated CO_2 mesocosms post nutrient addition.

3.3 $p\mathrm{CO_2}$ effect on microbial community

Although not identified as a major community structuring variable, the $p\mathrm{CO_2}$ treatment was found to be significantly correlated to the changing relative abundance for 15 rare taxa (% abundance across time and treatment <0.22 %; Table 4). From these 15 rare taxa, for both Fl and PA, 12 increased (even if only slightly), having their maximum abundances in either the medium (~685 and ~820 µatm) or the high (~1050 µatm)

pCO_2 mesocosms. The remaining three decreased, with their highest abundances in the lowest (~185 µatm) pCO_2 mesocosm, or before manipulation started (Fig. 4, Fig. S3 and S4). The three most abundant of these 15 taxa were Methylotenera (β-proteobacteria), Colwellia (γ-proteobacteria) and Fluviicola (Bacteroidetes). Methylotenera and Colwellia abundances were at their highest in, respectively, the ~686 and ~ 824 µatm mesocosms toward the end of the experiment (t22). Fluviicola was present from the beginning of the experiment, but decreased precipitously after CO_2 was added and then recovered in abundance after t10, reaching its highest abundance in the 1050 µatm mesocosm between t12 and t22 (Fig. 4).

4 Discussion

4.1 Mesocosms and structuring effects

In this study a large-scale mesocosm experiment was used to investigate the impacts of OA on the microbial community structure in a coastal, high latitude marine pelagic ecosystem. The experimental design provided the opportunity to test for the effects of 4 different pCO_2 concentrations (~270, ~685, ~ 820, ~1050 µatm) over a six week period, with comparison against two negative controls (~185 µatm). In addition, mesocosm-specific experimental artefacts were monitored for by sampling the fjord microbial community throughout the course of the experiment. The microbial community structure post-nutrient-addition (t13) was significantly correlated with seven variables, the most influential of which was sample origin (fjord or mesocosm). The overall community structure was not significantly different were not significant between mesocosms (including control versus elevated pCO_2) over the course of the experiment. The significant effect of the mesocosm enclosures on microbial community structure could be due to the mesocosms themselves (isolating a microbial community from the surrounding fjord community) or since the effect was not significant before nutrient addition, more likely due to the addition of nutrients into the mesocosms at t13.

Size fraction was the second most important variable in explaining differences in community structure. Before nutrient addition, the free-living size fraction communities were not significantly different between the fjord (ambient), control mesocosms, and the elevated pCO_2 mesocosms. However, post-nutrient addition, γ-proteobacterial abundances were significantly different between fjord and mesocosms, and probably reflect the utilization of metabolites released by decaying phytoplankton in the post-bloom system. Furthermore, the overall abundance of free-living size fraction Bacteroidetes increased in post-blooms conditions, possibly also as a result of the decaying algal bloom. The γ-proteobacteria and Bacteroidetes include many phytodetritus-assimilating organisms (Teske et al., 2011; Abell et al., 2005; Pinhassi et al., 2004). Additionally, increases in Bacteroidetes abundance were observed in the particle-associated size fraction mesocosms post-nutrient addition, as decaying phytoplankton tend to aggregate, creating larger particles. Despite the observation that Bacteroidetes showed bloom-related dynamics, and contradictory to the findings of Zhang et al. (2012), no significant difference in the Bacteroidetes abundance (in either fraction) was found between the control and elevated pCO_2 mesocosms, suggesting that elevated pCO_2 did not impact the relative abundance of Bacteroidetes. However, their abundance in the fjord was significantly lower than in the mesocosms, suggesting that the nutrient addition or influence of the mesocosm enclosure did have an impact.

The particle-associated fraction in the mesocosms also showed differences in the relative abundance of dominant phyla following nutrient addition (t13). It has previously been established that particle-associated assemblages were predominantly connected to phytoplankton development (Riemann et al., 2000; Allgaier et al., 2008). Furthermore, differences in the "Cyanobacteria and eukaryotic chloroplasts" group were measurable before nutrient addition; however these differences are believed to be related to the natural phytoplankton bloom (which occurred in the fjord and mesocosms) that reached its maximum on t7. The "post-nutrient addition" differences were significant between the fjord and mesocosms for almost every abundant phyla throughout the different phytoplankton phases; suggesting that nutrient addition influenced autotrophic and heterotrophic community structure. However, no significant differences were found between the control and the elevated mesocosms, which suggests that pCO_2 elevation was not an important community-structuring variable for the particle associated fraction in this experiment. Si was the third main structuring variable and is potentially related to diatom abundance (de Kluijver et al., 2010). The re-dispersal of Si from decaying diatoms, after a phytoplankton bloom, is carried out by a diverse fast growing bacteria related to cytophagales (from Flavobacteria; Riemann et al., 2000). Indeed, an increase in the abundance of Bacteroidetes, which contains the Flavobacteria, was observed in the post-nutrient addition phase.

However, when separating and analysing the phytoplankton phases independently, for free-living versus particle-associated bacteria, the community structuring variables shifted completely. The most influential variable for the free-living bacteria under pre-nutrient addition was DMS concentration, while origin (Fjord vs. mesocosm) was most-influential under post-nutrient addition conditions. O_2 and Si were the most significant structuring variables for the particulate-associated bacteria, respectively pre- and post-nutrient-addition. Variables associated with phytoplankton bloom dynamics were most important for structuring the community, especially when looking at the taxonomic shifts between fjord, control mesocosms and elevated pCO_2 mesocosms. The differences are greater after t13, when the nutrients were added, inducing two large phytoplankton blooms. These differences were most evident in the particle-associated fraction. Therefore, it is possible to state that nutrients, and therefore the phytoplankton blooms, were the main drivers of microbial community structure in this experiment, which is in agreement with previous studies (Allgaier et al., 2008; de Kluijver et al., 2010; Sperling et al., 2012).

4.2 Elevated pCO_2 effect

The effect of elevated pCO_2 on microbial community structure has also been investigated in previous mesocosms, where no evidence of a major pCO_2 effect on the general bacterial community was found (Zhang et al., 2012; Newbold et al., 2012).

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However, other work suggests that only the community structure of the free-living bacteria is significantly affected by elevated pCO_2 (Allgaier et al., 2008). The extensive database of 16S rRNA sequence obtained in this study provided the high resolution necessary to study subtle but significant changes in community structure hinted at in prior studies. In agreement with Allgaier et al. (2008), the effect of elevated pCO_2 in this experiment was slight and only impacted the free-living bacteria after nutrient addition, which corresponded to post-nutrient addition and post-bloom conditions (after t13) in this study. This increased post-bloom CO_2 effect was previously observed in other mesocosms experiments (Arnosti et al., 2011; de Kluijver et al., 2010); confirming a possible increased CO_2 effect under nutrient (N, P, Si) limitation.

While pH was shown to be a weak driver of microbial community structure in our experiment, the direct impact of pCO_2 was found to be non-significant, except for 15 rare taxa, which did show a response. Therefore, the level of taxonomic resolution afforded by this study suggests that, in this ecosystem, rare organisms may be disproportionately affected by acidification. The most abundant of these 15 rare taxa was Methylotenera (genus) and had its highest mean abundance in the medium pCO_2 mesocosms (~685 µatm). Species from this genus are generally aerobic, ubiquitous bacteria found in a wide range of O_2, salinity, temperature and pH; Methylotenera can colonize multiple pH range (5 to 8.5) but it grows optimally at pH 7.5 (Kalyuzhnaya et al., 2006; Bosh et al., 2009), suggesting that pH may have a strong influence for this taxa. Indeed, the pH close to this value from t5 until the end of the experiment in the mesocosms with a pCO_2 over ~685 µatm. The highest abundance was found from t22 until t28 where the pH was 7.9 and 7.94. A lower pH was found (pHT 7.57–7.80) in the ~1050 µatm mesocosm, but this was not accompanied by an increase in Methylonera abundance, potentially because the pCO_2 concentration itself was toxic to this species at this stage, or this could represent mesocosm variability, suggesting a need for improved replication. Functionally, the species included in this genus have been described as bacteria that require organic compounds containing no carbon–carbon bonds (C_1 compounds) like methylamine and/or methanol as energy sources (Lidstrom, 2006; Kalyuzhnaya et al., 2006, 2010). These organic compounds play an important role in the global carbon cycle. In fact, these compounds were recently identified as responsible for major greenhouse emissions on a scale similar to methane emissions (Chistoserdova et al., 2009). Further investigation of the behaviour of these C_1-compound-degraders in response to elevated CO_2 are, therefore, important for understanding biotic influences on climate dynamics. The second most abundant group of these 15 pCO_2-correlated rare taxa was Colwellia, which includes Arctic seawater bacteria capable of growing within a wide range of T: e.g. −1 to 10 °C (optimal growth 8 °C) for *Colwellia psychrerythraea*; 4 to 25 °C for *Colwellia asteriadis sp.* and 0 to 30 °C for *Colwellia chuckchiensis*. These organisms are also capable of colonising a wide range of pH from 4 to 10 (Yu et al., 2011; Choi et al., 2010; Methé et al., 2005). *C. psychrerythrea* is considered a model organism for psychrophiles. This organism shows multiple molecular adaptations to the cold, like enzymes for cryoprotection, for dissolving high-molecular-weight organic compounds (ex. carbon), for stability in extreme environments (extracellular polymeric substances) and for cold-active processes (Methé et al., 2005; Huston et al., 2004). These features make *Colwellia sp.* key participants in carbon and nutrient cycling in the cold marine environments. Since some methanogenenic enzymes were previously found in *Colwellia sp.* (Methé, et al., 2005) one can speculate that these compounds were found in greater abundance toward the end of the experiment. This would also support the presence of the Methylotenera, which increased in abundant towards the end of the experiment. Finally the genus Fluviicola, the third most abundant OTU correlated with pCO_2, was dominant in the elevated CO_2 mesocosms (∼1058 µatm). Interestingly, Fluviicola was present at the beginning of the experiment but decreased shortly after CO_2 treatment started. The abundance increased under elevated pCO_2, but stayed low in medium pCO_2 mesocosms and absent in the controls, for both size fractions. Little is known about this genus, making speculations about its ecological role difficult.

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5 Conclusions

In summary, multiple parameters were found to significantly influence the structure of the bacterial community in Svalbard mesocosms (2010). The most influential factors were the origin of the sample (fjord or mesocosms) and nutrient addition. Furthermore, the relative importance of size fraction (free-living versus particle-associated), Si, PO_4, primary production, temperature, and pH in structuring the community depended greatly on the time at which the community was sampled in relation to the phytoplankton blooms. The direct impact of pCO_2 was found to be significant for only 15 rare taxa and should be further investigated, as this limited pCO_2 effect could have evolutionary consequences creating a shift in the taxa dominance and/or diversity; profoundly affecting the structure of entire community in a high CO_2 world. However, it should be noted that the pCO_2 conditions in which these organisms dominated were super-elevated compared to predicted outcomes for the surface ocean under current climate change scenarios.

Future work should focus on exploring the functional responses of the community (metagenomics/metatranscriptomics) to evaluate how elevated pCO_2 or OA influence these processes over a longer time period.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/13319/2012/ bgd-9-13319-2012-supplement.pdf.

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Table 1. Redundancy analysis showing the significant structuring variables for the whole bacterial community during the post-nutrient addition period (t13–t30). Significant values are $p < 0.05$.

Variables	%	p	F
Origin	25	0.001	24.84
Fraction	14	0.001	17.77
Si	8	0.001	11.32
PO_4	2	0.01	2.83
Primary production	2	0.026	2.31
Temperature	2	0.042	2.26
pH	1	0.029	2.36

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Table 2. Results from RDA forward selection (with Monte Carlo permutation tests) showing only the significant ($p < 0.05$) structuring variables for free living size fraction (0.2–3 µm) and for the particle associated size fraction (3–20 µm) during the pre-nutrient period from t1 to t12 (a and c, respectively) and post-nutrient period from t13 to t30 (b and d, respectively).

	Variable	%	*p*	*F*
Free living size fraction:				
a)	Dimethyl Sulphide	16	0.001	9.79
	Bacterial production	15	0.001	10.73
	Density	12	0.001	9.65
	NO_2	5	0.001	4.54
	Day	2	0.024	2.14
	Origin	3	0.014	2.53
b)	Origin	48	0.001	35.75
	pCO_2	10	0.001	8.77
	Day	10	0.001	11.43
	CO_2	4	0.001	4
	Mesocosm	2	0.002	3.27
	Turbidity	3	0.001	3.91
	Primary production 14C	1	0.007	2.3
	NH_4	2	0.019	2.06
	Density	1	0.032	1.99
	Temperature	1	0.044	1.72
	PO_4	1	0.033	1.92
Particle associated size fraction:				
c)	O_2	7	0.002	3.81
	Dimethyl sulphide	7	0.005	3.81
	Origin	4	0.016	2.59
	NO_3	5	0.014	2.95
d)	Si	27	0.001	13.36
	Origin	12	0.001	7.11
	PO_4	4	0.039	2.24

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Table 3. Analysis of variance (ANOVA) showing the relationship in between each treatment pre- and post-bloom condition for **(a)** free living and **(b)** particle associated size fraction bacteria of phyla with significant differences. Significant values are $p < 0.05$.

Phylum	Treatment	p
Post-nutrient addition		
Gamma-proteobacteria	fjord-control	0.001
	mesocosm-control	0.140
	mesocosm-fjord	0.038
Pre-nutrient addition		
"Cyanobacteria + euk.chloro"	fjord-control	0.317
	mesocosm-control	0.289
	mesocosm-fjord	0.020
Post-nutrient addition		
Bacteroidetes	fjord-control	0.001
	mesocosm-control	0.864
	mesocosm-fjord	0.002
Alpha-proteobacteria	fjord-control	0.002
	mesocosm-control	0.787
	mesocosm-fjord	0.006
"Cyanobacteria + euk.chloro"	fjord-control	0.000
	mesocosm-control	0.839
	mesocosm-fjord	0.001
"Others"	fjord-control	0.000
	mesocosm-control	0.320
	mesocosm-fjord	0.001

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Table 4. Bonferroni-corrected g-test of significance ($p < 0.05$) demonstrating which and how taxa, for both free living and particle associated size fraction, are significantly correlated with CO_2; where bold highlights mark the OTUs presented in Fig. 4.

OTU	Taxa	Abundance	% total sequences (20 863 517)	General response to elevated pCO_2	p
114 612	Methylotenera (genus)	2907	0.014	Highest in middle pCO_2	0.000
144 699	Oceanospirillaceae (family)	1182	0.006	Increased with pCO_2	0.000
105 727	**Methylotenera (genus)**	**45 915**	**0.220**	**Highest in middle pCO_2**	**0.000**
151 803	Flavobacteriaceae (family)	1841	0.009	Increased with pCO_2	0.000
522 744	Leucothrix (genus)	130	0.001	Decreased with pCO_2	0.000
419 525	Sphingobacteriales (order)	171	0.001	Increased with pCO_2	0.000
94 238	Oxalobacteraceae (family)	322	0.002	Highest in middle pCO_2	0.000
402 252	**Fluviicola (genus)**	**20 950**	**0.100**	**Increased with pCO_2**	**0.001**
592 739	Oleibacter (genus)	2976	0.014	Highest in middle pCO_2/increased	0.001
262 549	HTCC-1288 (genus)	25	0.001	Mixed, highest in high-middle pCO_2	0.001
140 859	Flavobacteriumsuccinicans (species)	344	0.0001	Decrease with pCO_2	0.004
235 556	**Colwellia (genus)**	**32 153**	**0.154**	**Highest in high-middle pCO_2**	**0.008**
591 187	Flavobacteria (class)	231	0.001	Decrease with pCO_2	0.010
243 032	Thioclava (genus)	59	0.0003	Mixed, highest in high pCO_2	0.011
554 148	SC3–41 (family)	571	0.003	Minimum increase	0.027

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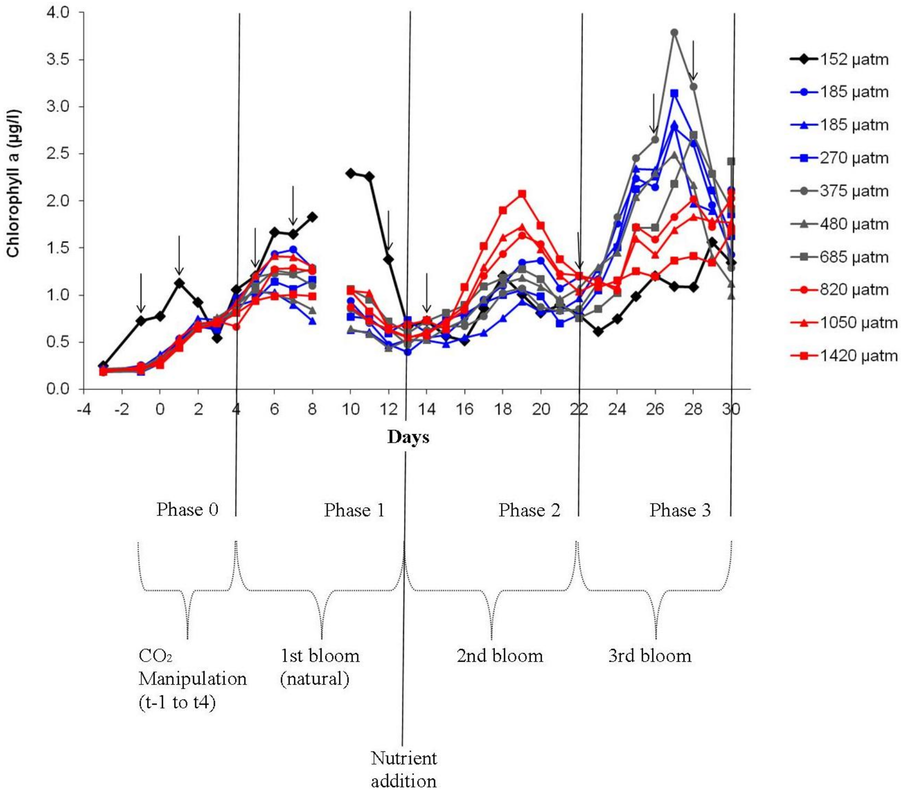

Fig. 1. Chlorophyll *a* ($\mu g\, l^{-1}$) concentrations measurements plotted against days, where arrows marked time points analysed in the present study. Figure derived from Schulz et al. (2012).

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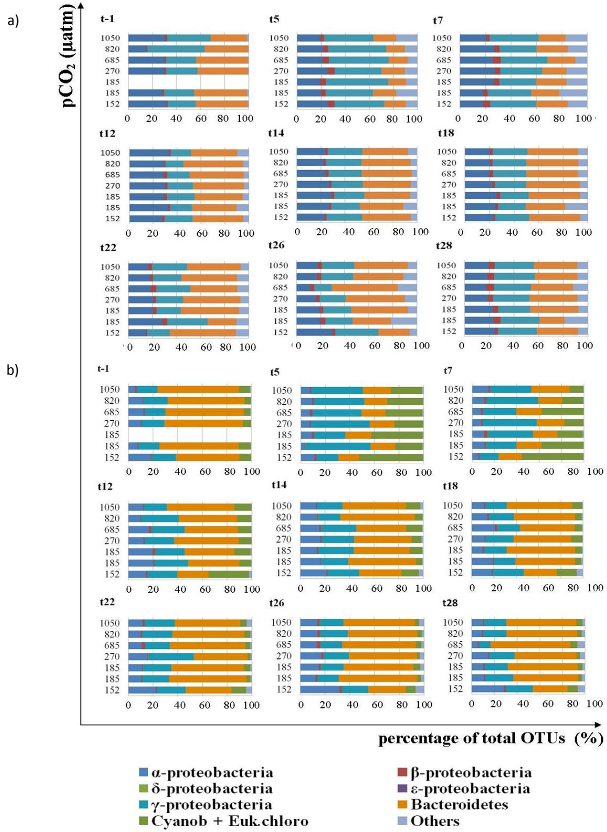

Fig. 2. Microbial community overview of the most abundant phyla in **(a)** the free-living size fraction (0.2–3 µm) and **(b)** the particle-associated size fraction (3–20 µm) during t-1, t5, t7, t12, t14, t18, t22, t26 and t28; x-axis represents percentage of total OTUs and y-axis represents $p\mathrm{CO_2}$ in µatm.

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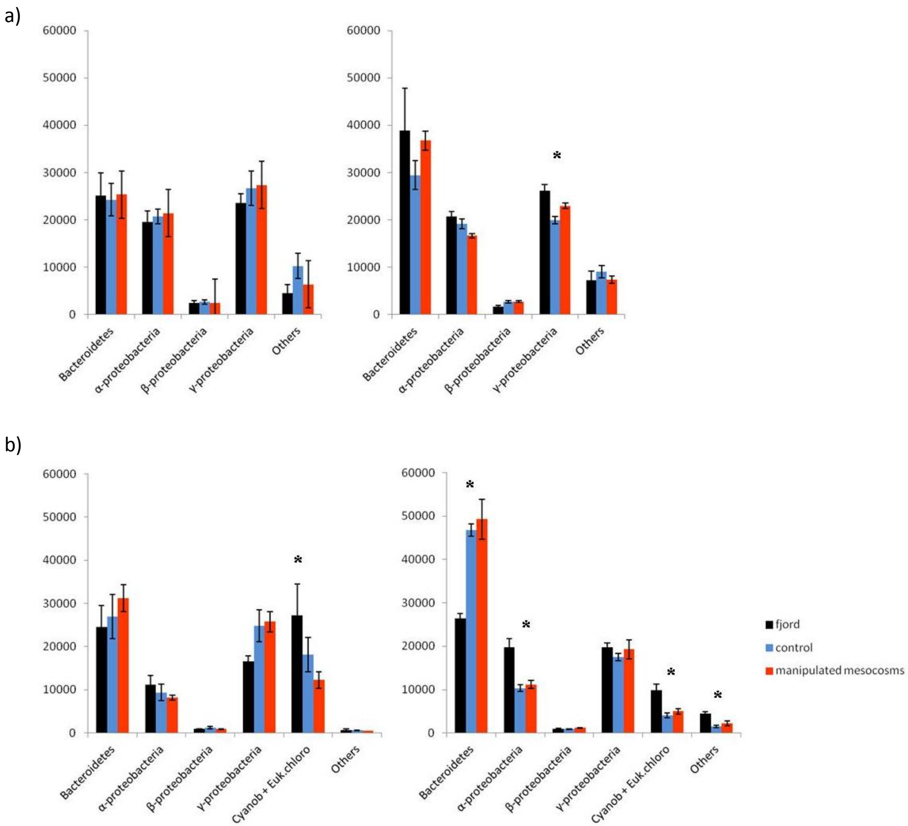

Fig. 3. Mean abundance (±SE) of the main phyla of the bacterial community for the fjord (∼145 µatm), the control (2× ∼185 µatm) and the manipulated mesocosms (∼270, ∼685, ∼820, ∼1050 µatm) of the free living **(a)** and particle associated **(b)** size fraction pre- (left) and post- (right) nutrient addition. Phyla with significant different *p*-values (<0.05) as a function of samples origin are marked with a *.

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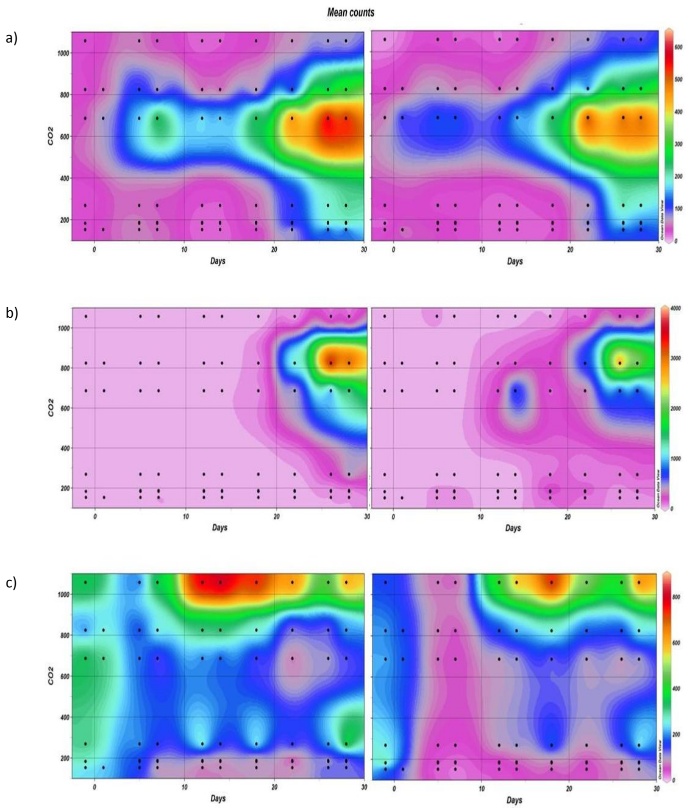

Fig. 4. Heat maps presenting the mean abundance count of the three most abondant taxa that are significantly affected by pCO_2 levels **(a)** Methylotenera (OTU # 105727) **(b)** Colwellia (OTU # 235556) and **(c)** Fluviicola (OTU # 402252) plotted against pCO_2 (µatm, y-axis) and time (days, x-axis). Left and right panel represent, respectively, the free living (0.2–3 µm) and particle-associated size fraction (3–20 µm).

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