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Response of bacterioplankton activity in an Arctic fjord system to elevated pCO_2: results from a mesocosm perturbation study

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Abstract. The effect of elevated seawater carbon dioxide (CO_2) on the activity of a natural bacterioplankton community in an Arctic fjord system was investigated by a mesocosm perturbation study in the frame of the European Project on Ocean Acidification (EPOCA). A pCO_2 range of 175–1085 µatm was set up in nine mesocosms deployed in the Kongsfjorden (Svalbard). The activity of natural extracellular enzyme assemblages increased in response to acidification. Rates of β-glucosidase and leucine-aminopeptidase increased along the gradient of mesocosm pCO_2. A decrease in seawater pH of 0.5 units almost doubled rates of both enzymes.

Heterotrophic bacterial activity was closely coupled to phytoplankton productivity in this experiment. The bacterioplankton community responded to rising chlorophyll *a* concentrations after a lag phase of only a few days with increasing protein production and extracellular enzyme activity. Time-integrated primary production and bacterial protein production were positively correlated, strongly suggesting that higher amounts of phytoplankton-derived organic matter were assimilated by heterotrophic bacteria at increased primary production. Primary production increased under high pCO_2 in this study, and it can be suggested that the efficient heterotrophic carbon utilisation had the potential to counteract the enhanced autotrophic CO_2 fixation. However, our results also show that beneficial pCO_2-related effects on bacterial activity can be mitigated by the top-down control of bacterial abundances in natural microbial communities.

1 Introduction

After early investigations suggesting that heterotrophic bacterial remineralisation is low in perennially cold environments (Pomeroy and Deibel, 1986), a multitude of field observations and experimental studies over the last two decades revealed a fully active microbial loop in the polar oceans (Rivkin et al., 1996; Rich et al., 1997; Yager et al., 2001).

The bottom-up regulation of bacterial activity in Arctic microbial food webs is mainly achieved by interactions with temperature and dissolved organic matter (DOM) (Pomeroy and Wiebe, 2001; Kirchman et al., 2005, 2009a). Psychrophilic and psychrotolerant heterotrophic bacteria in polar marine environments are physiologically adapted to low temperatures. Nevertheless, they often act far below their temperature optimum, so that cold ambient temperature has a high potential to reduce their metabolic activity and growth, thereby decreasing the rate of bacterial DOM degradation (e.g., Simon et al., 1999; Huston et al., 2004). More recently, the availability of labile and semilabile DOM was proposed as a major constraint on bacterial activity in the Arctic Ocean (Kirchman et al., 2009a). Thus, Arctic marine systems are not only characterised by low temperature, but also by a deficiency of carbon and energy resources as a second regulating factor of heterotrophic microbial growth (Kirchman et al., 2009a).

There is growing evidence that impacts of climate change are amplified in the Arctic environment, making the Arctic Ocean particularly sensitive to warming and acidification. A temperature increase of 0.4 °C in the Arctic over the past 150 yr corresponds to a rate two to three times higher than the global average, amplified by a decreasing surface albedo at enhanced ice and snow melting (Overpeck et al., 1997).

The additional freshwater input from ice melting reduces the alkalinity of seawater and, hence, the buffering capacity of the Arctic Ocean to acidification (Steinacher et al., 2009). Investigating the relevance of seawater pH for the structure and functioning of marine microbial communities gained little attention in marine research before ocean acidification induced by anthropogenic emissions became evident. However, substantial natural variability of pH occurs on short time scales and in marine microhabitats like particle aggregates and biofilms (Ploug et al., 1997; Dexter and Chandrasekaran, 2000), and might co-determine biological activity. Biochemical studies demonstrated that fundamental processes of the marine microbial metabolism like numerous enzymatic reactions and the respiratory electron transfer are strongly dependent on pH conditions (e.g., Groudieva et al., 2004; Kolber, 2007). Therefore, ongoing systematic changes in seawater pH and pCO_2 imply effects on the regulation of both autotrophic and heterotrophic microbial activity in natural communities, suggesting consequences of ocean acidification for food web processes and organic matter turnover.

Our study was part of the joint EPOCA mesocosm experiment conducted in Kongsfjorden (Svalbard), 2010. The Kongsfjorden system in West Spitsbergen is an open fjord system without sill and, therefore, largely affected by mixing processes on the adjacent shelf. On the shelf northbound transported Atlantic water is mixed with Arctic water and freshwater derived from glacier melt and precipitation. The mixing of these water sources varies seasonally and interannually, resulting in a warmer and more saline regime under strong influence of Atlantic water and in colder and fresher conditions during a state of Arctic dominance (Hop et al., 2006). Heterotrophic bacteria contribute substantially to the biomass of the microbial community and play an important role in the structure and function of the microbial food web in all seasons (Rokkan Iversen and Seuthe, 2011; Seuthe et al., 2011). Intense grazing by heterotrophic dinoflagellates and ciliates leads to cascading effects on carbon transfer through the microbial food web during the vernal bloom in April. In the following nutrient-limited post-bloom period the microbial food web enters a regenerative state that is characterised by an efficient recycling of produced carbon by the microbial loop (Rokkan Iversen and Seuthe, 2011; Seuthe et al., 2011). Hence, metabolic activity of heterotrophic bacterioplankton can be expected to be a major constraint on fluxes of labile organic carbon and nutrients in this high-latitude ecosystem. Nine mesocosms were deployed in the Kongsfjorden during June and July 2010, to investigate the CO_2 sensitivity of Arctic pelagic organisms and resulting consequences for the production and turnover of organic matter. More specifically, we tested in the framework of this experiment (i) whether bacterial protein production and rates of extracellular enzymes in the natural Kongsfjorden plankton community change under elevated pCO_2 and (ii) how changes in pCO_2 can induce systematic effects on heterotrophic bacterial activity in this perennially cold marine environment.

2 Material and methods

2.1 Mesocosm set up and sampling

A mesocosm study was conducted on Spitsbergen (Svalbard) between 30 May and 7 July 2010, in the framework of the European Project on Acidification (EPOCA). Kiel Off-Shore Mesocosms for Future Ocean Simulations (KOSMOS) were deployed in the Kongsfjorden, (78°56.2′ N, 11°53.6′ E), an Arctic glacial fjord system located on the west coast of Spitsbergen that is influenced by both Atlantic and Arctic water masses (Hop et al., 2002). A detailed description of the mesocosm set up is given by Riebesell et al. (2012) and Schulz et al. (2012). Briefly, nine mesocosms, each consisting of a 15 m long polyurethane bag, were deployed close to the shoreline. Each mesocosm enclosed about 45 m^3 of seawater. A pCO_2 gradient that ranged from 250–1085 µatm was adjusted by the stepwise addition of CO_2-enriched seawater during days −1 to 4. Two mesocosms at in situ pCO_2 of 175–180 µatm served as controls. The low in situ pCO_2 reflected the post-bloom stage of a *Phaeocystis*-dominated bloom event (F. Buchholz, personal communication, 2010). In the first phase of the experiment (days 4–13), the development of the mesocosm plankton communities was observed without any further manipulation under inorganic nutrient-deplete conditions of 0.1 µmol L^{-1} nitrate, 0.5–0.7 µmol L^{-1} ammonium and 0.06–0.09 µmol L^{-1} phosphate. Nine days after the adjustment of target pCO_2-levels (on day 13), 5 µmol L^{-1} nitrate, 0.3 µmol L^{-1} phosphate and 2.5 µmol L^{-1} silicate were added. A detailed description and analysis of nutrient consumption and phytoplankton growth is given by Schulz et al. (2012).

Depth-integrated samples of the water column enclosed by the mesocosm bags were taken by the use of a motor-operated water sampler (Hydrobios, Kiel, Germany) that permanently and evenly collected water while being lowered from the surface to a 12 m depth (Riebesell et al., 2012). Depth-integrated samples of all mesocosms and the fjord were analysed for extracellular enzyme activity and bacterial protein production daily or every second day.

2.2 Rates of extracellular enzymes

Activity of extracellular enzymes released into seawater and attached to outer cell surfaces was determined by the use of fluorogenic substrate analogues (Hoppe, 1983). Accordingly, rates of β-glucosidase, leucine (leu)-aminopeptidase and alkaline phosphatase were assessed from the hydrolysis of 4-methylumbelliferyl-β-glucopyranoside, L-leucyl-4-methylcoumarinylamid-hydrochlorid and 4-methylumbelliferyl-phosphate, respectively. The substrate analogues were added to whole seawater samples at final concentrations of 1, 5, 10, 20, 50, 80, 100 and 200 µmol L^{-1} to determine enzyme kinetics. The fluorescence emitted by 4-methylumbelliferone (MUF) and

7-amino-4-methyl-coumarine (AMC) after enzymatic cleavage of the substrate analogues was detected at 355 nm excitation and 460 nm emission wavelength. Relative fluorescence units were converted into concentrations of MUF and AMC, respectively, after calibration with standard solutions of 2–100 nmol L^{-1}. The fluorescence of MUF and AMC is pH dependent. Therefore, calibration factors were determined at five different pH values ranging from 7.6 to 8.4. MUF shows maximum fluorescence at a pH 10 and the calibration factor converting fluorescence into MUF concentration was almost two times higher at pH 7.6 than at than at pH 8.4. Assays using AMC as fluorescent marker are less affected by pH differences due to the low basicity of the amino group. Accordingly, calibration factors determined for the applied pH range of 7.6 to 8.4 were not significantly different.

Enzymatic rates were calculated from the increase in MUF and AMC concentration, respectively, over time. An initial fluorescence measurement was conducted immediately after the addition of the substrate analogue followed by 3–4 measurements within 24 h of incubation in the dark at 2 °C. On days 12, 14, 20 and 24 of the mesocosm study additional incubations at 5.5–6.5 °C were conducted. Instead of using single end point measurements, the slope of the linear regression between incubation time and the concentration of the fluorescent marker was applied for rate calculations. Two analytical replicates per sample had on average a standard deviation of 9 %. Boiled mesocosm and fjord water served as blank and did not show any increase in fluorescence over time. Experimental data were fitted to a kinetic model using the Michaelis-Menten equation to determine the maximum velocity ($V_{\max}$) and the half-saturation constant K_m of the enzymatic reactions.

2.3 Bacterial protein production

Bacterial protein production (BPP) was estimated from the uptake of ^{14}C-leucine that was added to depth-integrated mesocosm and fjord samples at a final concentration of 40 nmol L^{-1}. Two series of duplicate incubations were conducted in the dark in a temperature-controlled walk-in room at 2 °C ($BPP_{2\,°C}$) and in situ in 1 m water depth, respectively. Tests revealed a linear increase of leucine uptake over at least 50 h, so that extended incubation times of up to 24 h could be applied at low bacterial activity. Incubations were terminated by the addition of trichloracetic acid (TCA) at a final concentration of 5 %. Both incubations at 2 °C and in situ included one control per mesocosm and fjord that was immediately poisoned after the addition of the radiotracer with TCA (5 % final concentration). All samples were processed by the micorcentrifuge method (Smith and Azam, 1992). Briefly, samples were centrifuged at $14\,000 \times g$ to gain a cell pellet that was washed twice with 5 % TCA. Incorporation into the TCA-insoluble fraction was measured by liquid scintillation counting after resuspension of the cell pellet in scintillation cocktail. Duplicate incubations had an analytical error ≤ 10 % for both incubation modes. Leucine incorporation was converted into BPP applying a conversion factor of 1.5 kg C mol^{-1} leucine, assuming no intracellular isotope dilution (Simon and Azam, 1989).

2.4 Cell-specific activity

A comprehensive analysis of microbial growth dynamics in this mesocosm experiment, including abundances of picophytoplankton, nanophytoplankton, bacteria and viruses, is given by Brussaard et al. (2012). In order to optimise calculations of cell-specific rates for extracellular enzymes and BPP, we additionally determined bacterial cell numbers in the mesocosm subsamples that were used for our assays. Bacterial abundances were determined by flow cytometry (FACSCalibur, Becton Dickinson) after nucleic acid staining with SybrGreen I (Invitrogen). Bacterial cell numbers were estimated after visual inspection and manual gating of the bacterial subpopulation in the cytogram of side scatter vs. green fluorescence. Yellow-green fluorescent latex beads (Polyscience) and TruCount beads (Becton Dickinson) were used to normalise the counted events to volume (Gasol and del Giorgio, 2000). Bacterial cell numbers were converted into bacterial biomass assuming a carbon content of 10 fg C $cell^{-1}$ (Caron et al., 1995).

Cell-specific rates of extracellular enzymes and BPP were determined by dividing the rate scaled to volume by total bacterial abundance. Specific growth rates (μ) were calculated according to

$$\mu = \mathrm{BPP}/B \quad (1)$$

where BPP is bacterial protein production (ng C L^{-1} d^{-1}) and B bacterial biomass (ng C L^{-1}) (Kirchman, 2001).

2.5 ^{14}C primary production

Primary production was determined by the use of the radiotracer $NaH^{14}CO_3$ (Steemann Nielsen, 1952; Gargas, 1975). Briefly, samples spiked with 8 µCi L^{-1} $NaH^{14}CO_3$ were incubated in situ at 1 m depth for 24 h. After incubation samples were filtered onto 0.4 µm polycarbonate filters. Particulate primary production was determined from the particulate matter collected on the filters. Subsamples of the filtrate were used to determine exudation. Both filters and filtrate were acidified to remove inorganic carbon prior to analysis by liquid scintillation counting. A detailed method description is given by Engel et al. (2012).

2.6 Supplemental experiments

2.6.1 Enrichment assay

An enrichment assay was conducted to investigate effects of labile carbon and inorganic nitrogen input on bacterial activity and growth. For this purpose, a sample integrated

over 12 m depth was collected close to the mesocosm site in Kongsfjorden during the days of mesocosm deployment on 2 June 2010. Three replicates of 20 mL seawater were supplemented separately with 20 µmol L^{-1} glucose and 10 µmol L^{-1} ammonium (final concentrations). Furthermore, triplicate samples were amended with glucose and ammonium in combination to test whether ammonium addition can stimulate the assimilation of labile carbon. Samples without any addition served as control. Rates of extracellular enzyme activity, BPP, and bacterial cell numbers were determined according to the methods described above in the initial fjord sample and in all incubations after 4 days in the dark at in situ temperature of 2 °C.

2.6.2 Acidification assay

The biochemical sensitivity of the natural extracellular enzyme assemblages to decreasing seawater pH was investigated by an acidification assay. Depth-integrated samples collected in the fjord and one non-acidified control mesocosm on day 25 of the study (2 July 2010) were acidified by the addition of 225–750 µL 0.1 N hydrochloric acid to 200 mL of sample. Samples were allowed to equilibrate at 2 °C in the dark for 3 h before pH was measured with a combined pH-temperature electrode (WTW, Sentix 41; standard DIN/NBS buffers) and the initial sampling was conducted. Non-manipulated samples of the fjord and the mesocosm served as control incubations at in situ pH. Measurements for β-glucosidase and leu-aminopeptidase activity were accomplished at 4 time points within 24 h after the initial time point.

2.7 Data analysis

To test the effect of temperature on BPP and extracellular enzyme activity, Q_{10} factors were calculated as

$$Q_{10} = (r_2/r_1)^{10/(T_2-T_1)} \quad (2)$$

where r_2 and r_1 are rates at high (T_2) and low (T_1) temperature, respectively. T_1 and T_2 were 2 °C and 5.5–6.5 °C, respectively, for enzyme assays (see also Sect. 2.2). BPP at elevated temperature was derived from in situ incubations (see also Sect. 2.3). It is likely that these incubations were exposed to slight temperature changes during incubation time. Accordingly, temperature sensitivity of BPP is estimated from

$$Q^*_{10} = (r_{\text{in situ}}/r_{2\,^\circ\text{C}})^{10/(T_{\text{in situ}}-2\,^\circ\text{C})} \quad (3)$$

where $T_{\text{in situ}}$ ranged from 3.2 °C for incubations on day 14 to 7.7 °C on day 22, and $T_{\text{in situ}}$ and $r_{\text{in situ}}$ integrate the temperature variability of a day-night cycle.

The hydrolysis potential for polysaccharides and proteins (µmol L^{-1}) was calculated by integrating $V_{\max}$ of β-glucosidase and leu-aminopeptidase (µmol L^{-1} d^{-1}), respectively, over time according to

$$\sum_{t=m}^{n} r_t \quad (4)$$

where m and n are the initial and final time point, respectively, and r_t the rate of an individual day. Since rate measurements for extracellular enzymes were conducted every second day of the experiment rates for the missing days were interpolated by

$$r_t = \frac{(r_{t-1} + r_{t+1})}{2} \quad (5)$$

where r_{t-1} is the measured rate of the previous day and r_{t+1} the measured rate of the day after.

The difference between time-integrated enzymatic rates of the 7 acidified mesocosms and the mean of the two control mesocosms was calculated and is referred to as Δ *hydrolysis potential*. Hence, positive values for Δ *hydrolysis* represent an enhanced hydrolytic potential in acidified mesocosms, while negative values reveal a lower hydrolytic potential relative to the control mesocosms. Calculating daily values for Δ *hydrolysis* explores the temporal variation of differences in the hydrolytic potential between acidified and non-acidified mesocosms (Fig. 6).

Differences were tested for significance by means of t-test, paired t-test and ANOVA. Wilcoxon signed rank test and ANOVA on ranks were applied, if tests for normality or equal variance failed. Linear and nonlinear regression analyses were conducted using the software Sigma Plot 12.1 (Systat). Significance was accepted for $p \leq 0.05$.

3 Results

3.1 Enrichment assays

The substrate supply to the bacterioplankton community in the Kongsfjorden was tested for limiting resources by an enrichment assay at the beginning of the mesocosm study. The amendment of glucose alone and in combination with ammonium led to a significant increase in BPP, bacterial abundance and β-glucosidase activity in comparison to the non-amended control within 4 days of incubation (one-way ANOVA, $p < 0.05$; Fig. 1, Table 1). Sole ammonium enrichment did not result in significant changes (one-way ANOVA, $p > 0.05$; Fig. 1, Table 1). These results show that growth of the bacterioplankton community in the fjord was limited by the deficiency of labile carbon or energy when the mesocosm study started. Cell-specific $V_{\max}$ of leu-aminopeptidase remained constant over incubation time and showed similar values of 32.7–36.9 amol $cell^{-1}$ h^{-1} in non-amended controls and in amended incubations after four days (Fig. 1). This suggests that independent of carbon or energy limitation also the demand for organic nitrogen was high and could not be compensated by the supply of an inorganic nitrogen source. The initial β-glucosidase

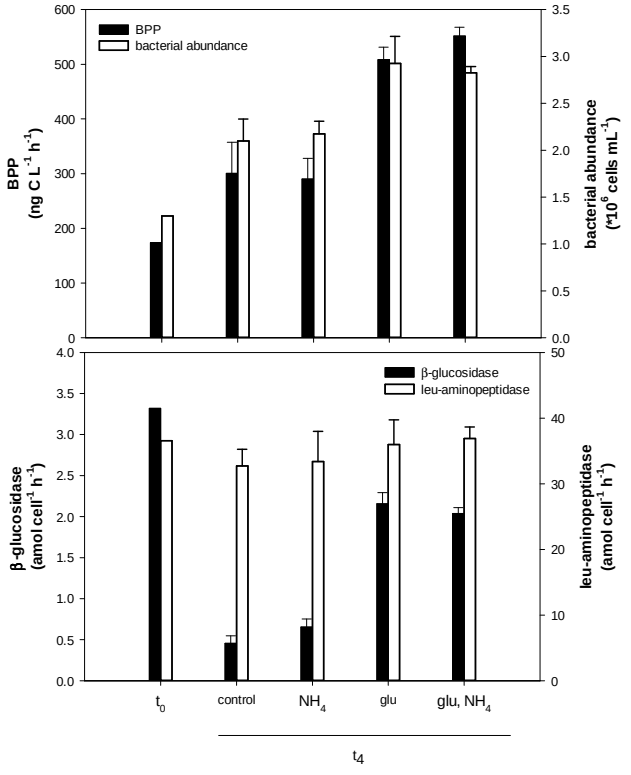

Fig. 1. Bacterial growth characteristics (upper panel; BPP: bacterial protein production) and extracellular enzyme activities (lower panel) in samples of the Kongsfjorden with and without amendments of ammonium (NH_4) and glucose (glu) (t0: initial sampling, t4: sampling after 4 days of incubation).

V_{max} of 3.3 amol cell^{-1} h^{-1} decreased during incubation in all treatments. Cell-specific V_{max} of β-glucosidase in glucose-amended incubations were reduced by approximately 37 % to 2.0–2.2 amol cell^{-1} h^{-1}, while rates in controls and ammonium-amended samples decreased strongly by approximately 83 % to 0.5–0.7 amol cell^{-1} h^{-1} (attomol [amol] = 10^{-18} mol) (Fig. 1).

3.2 Bacterial activity in Kongsfjorden during early summer

In mid-June, during days 3–12 of the mesocosm study, a chlorophyll *a* maximum of 2.3 µg L^{-1} developed in the Kongsfjorden (Schulz et al., 2012). Concomitant with maximum chlorophyll *a* concentration highest $BPP_{2\,^\circ C}$ of 421 ng C L^{-1} h^{-1} was determined in the fjord on day 10 of the experiment, when the rate was almost twice the maximum mesocosm value (Figs. 2–3). Elevated rates of β-glucosidase and leu-aminopeptidase were measured during and shortly after the chlorophyll *a* maximum. After day 16 rates of both enzymes did not show substantial variation in the fjord despite continuously increasing bacterial cell numbers (Fig. 2; Brussaard et al., 2012). Phosphatase activity

Table 1. Statistical analysis of the enrichment assays. Significance levels of ANOVA (p_{ANOVA}) and post-hoc comparisons versus the control ($p_{post\text{-}hoc\ test}$) are given. One-way ANOVA with post-hoc comparison by the Holm-Sidak method was carried out for bacterial protein production (BPP), bacterial abundance and leu-aminopeptidase. Data for β-glucosidase activity were not normally distributed and, therefore, ANOVA on ranks combined with post-hoc comparison by Dunnett's Method was applied.

	BPP	Bacterial abundance	β-glucosidase	leu-aminopeptidase
p_{ANOVA}	< 0.001	0.02	0.02	0.413
$p_{post\text{-}hoc\ test}$				
glu	< 0.001	0.003	< 0.05	–
NH_4	0.783	0.655	> 0.05	–
glu, NH_4	< 0.001	0.004	< 0.05	–

in the fjord increased from the beginning of the experiment until day 10. After a strong decrease of rates during days 12–14, phosphatase activity in Kongsfjorden samples was below the detection limit from day 16 until the end of the experiment (Fig. 2). Fjord and mesocosms showed similar K_m values for leu-aminopeptidase activity with mean values of 54 and 50 µmol L^{-1}, respectively. In contrast, the K_m values of β-glucosidase were significantly lower in the fjord (average 52 µmol L^{-1}) (Mann-Whitney rank sum test, $p < 0.05$), revealing a higher affinity to substrates than in the mesocosms (average 52 µmol L^{-1}) (Table 2). Extracellular phosphatase activity in the fjord was significantly related to chlorophyll *a* concentrations over the whole duration of the experiment (linear regression, $r^2 = 0.55$, $p < 0.001$, $n = 17$; not shown).

3.3 Bacterial protein production and specific growth rates

The synthesis of cellular proteins by heterotrophic bacterioplankton showed a similar temporal development in all mesocosms (Fig. 3). Lowest $BPP_{2\,^\circ C}$ of 19–56 ng C L^{-1} h^{-1} was determined shortly after CO_2 manipulation (days 2–7). Thereafter, $BPP_{2\,^\circ C}$ did not show a continuous increase, but elevated values during days 12–16 and 22–29 after the first and the second maximum of chlorophyll *a* concentrations, respectively. Maximum $BPP_{2\,^\circ C}$ of 168–268 ng C L^{-1} h^{-1} was attained either on day 22 or day 28, representing on average an increase by a factor of 6.4 over 18–22 days of the experiment (Fig. 3). Specific growth rates of bacteria showed an opposite temporal development. Maximum specific growth rates of 0.24–0.37 d^{-1} were determined during the initial days of the experiment. Specific growth rates declined strongly after day 8 so that highest $BPP_{2\,^\circ C}$ at the end of the experiment coincided with lowest specific growth rates of 0.02–0.09 d^{-1} (Fig. 3).

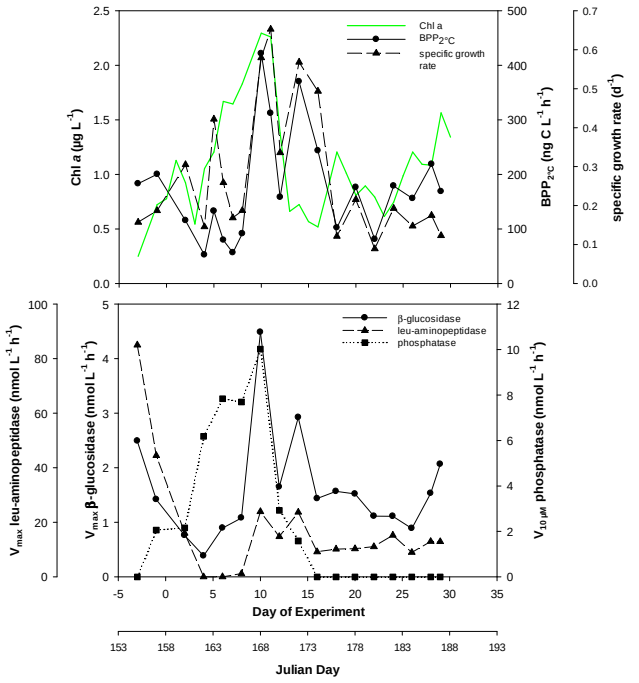

Fig. 2. Temporal development of bacterial production and growth ($BPP_{2\,^{\circ}C}$, specific growth rate; upper panel) and extracellular enzyme activities (leu-aminopeptidase, β-glucosidase, phosphatase; lower panel) in the Kongsfjorden.

3.4 Activity of hydrolytic extracellular enzymes

Rates of extracellular β-glucosidase, leu-aminopeptidase and phosphatase were measured in mesocosm and fjord samples to assess the hydrolytic activity of the three major types of extracellular enzymes that drive the turnover of organic polymers in seawater. V_{max} of β-glucosidase and leu-aminopeptidase started to increase in all mesocosms during days 6–8, shortly after CO_2 manipulation and the development of a first chlorophyll *a* maximum (Figs. 4–5). Rates of β-glucosidase increased further until the end of the experiment (Fig. 4). Also leu-aminopeptidase activity increased rapidly, but declined 2–4 days before the end of the experiment (Fig. 5). Maximum V_{max} in the mesocosms ranged from 3.1–4.8 nmol L^{-1} h^{-1} and 62.1–86.7 nmol L^{-1} h^{-1} for β-glucosidase and leu-aminopeptidase, respectively, and were determined during the last days of the experiment.

The temporal development of cell-specific rates was different from that of bulk rates. Cell-specific V_{max} in the mesocosms increased from days 4 to 12 up to 0.9–1.4 amol $cell^{-1}$ h^{-1} and 12.2–20.0 amol $cell^{-1}$ h^{-1} for β-glucosidase and leu-aminopeptidase, respectively, but showed only minor variation afterwards (Figs. 4–5). Hence, an increasing amount of bacterial enzymes released by a growing bacterioplankton community and not enhanced cellular metabolic activity increased the hydrolytic potential of the bacterioplankton community after day 12.

Table 2. Half-saturation constant K_m for β-glucosidase and leucine-aminopeptidase activity in mesocosms and Kongsfjorden samples.

	β-glucosidase		leu-aminopeptidase	
	mesocosms	fjord	mesocosms	fjord
mean K_m (µmol L^{-1})	94	52	54	50
median K_m (µmol L^{-1})	50	34	51	40
range (µmol L^{-1})	3–696	3–252	2–188	19–126

The temporal development of Δ *hydrolysis potential* reveals an elevated enzymatic potential for polysaccharide and protein degradation in the two mesocosms of highest pCO_2 during the first 20 days of the study (Fig. 6). After the second chlorophyll *a* maximum on day 21 bacterial abundances in the mesocosms diverged (Brussaard et al., 2012) and the differences between the two high-CO_2 mesocosms and the controls were either reduced or balanced (Fig. 6).

The ratio of leu-aminopeptidase: β-glucosidase activity is a measure for the protein relative to polysaccharide hydrolysis. This activity ratio changed in mesocosms and fjord over time (Fig. 7). The temporal development in mesocosms and Kongsfjorden diverged after day 12 when the ratio increased in the mesocosms, but remained lower in the fjord samples. Hence, the comparison of mesocosms and fjord reveals a higher potential for protein turnover at relatively lower polysaccharide degradation in the mesocosms after day 12. The divergence in the activity ratio of the two enzymes coincided with the divergence of the molar ratios of DON:DOC in mesocosms and fjord (Fig. 7).

Extracellular phosphatase activity did not follow Michaelis-Menten kinetics at several time points. The reasons remain speculative, but potential explanations could be either extracellular phosphatase activity of microzooplankton that may not follow Michaelis-Menten kinetics (Gambin et al., 1999; Hoppe, 2003) or artefacts induced by MUF-phosphate addition in excess of saturation (Sebastian and Niell, 2004). In the following, phosphatase rates determined at 10 µmol L^{-1} substrate concentration ($V_{10\,\mu M}$) are shown that do not represent the reaction velocity at substrate saturation (Fig. 8). Hence, phosphatase rates are not directly comparable with rates of β-glucosidase and leu-aminopeptidase that represent V_{max} (Figs. 4–5). The temporal development of phosphatase activity shows a clearly different pattern than β-glucosidase and leu-aminopeptidase. Rates show three activity maxima in all mesocosms during the experiment (Fig. 8). The first maximum in phosphatase activity during days 4–8 coincided with the first maximum in chlorophyll *a* concentrations. The second phosphatase maximum occurred at chlorophyll *a* minimum concentrations between the first and the second chlorophyll *a* peak during days 12–18. A third maximum in phosphatase activity developed simultaneously with the third

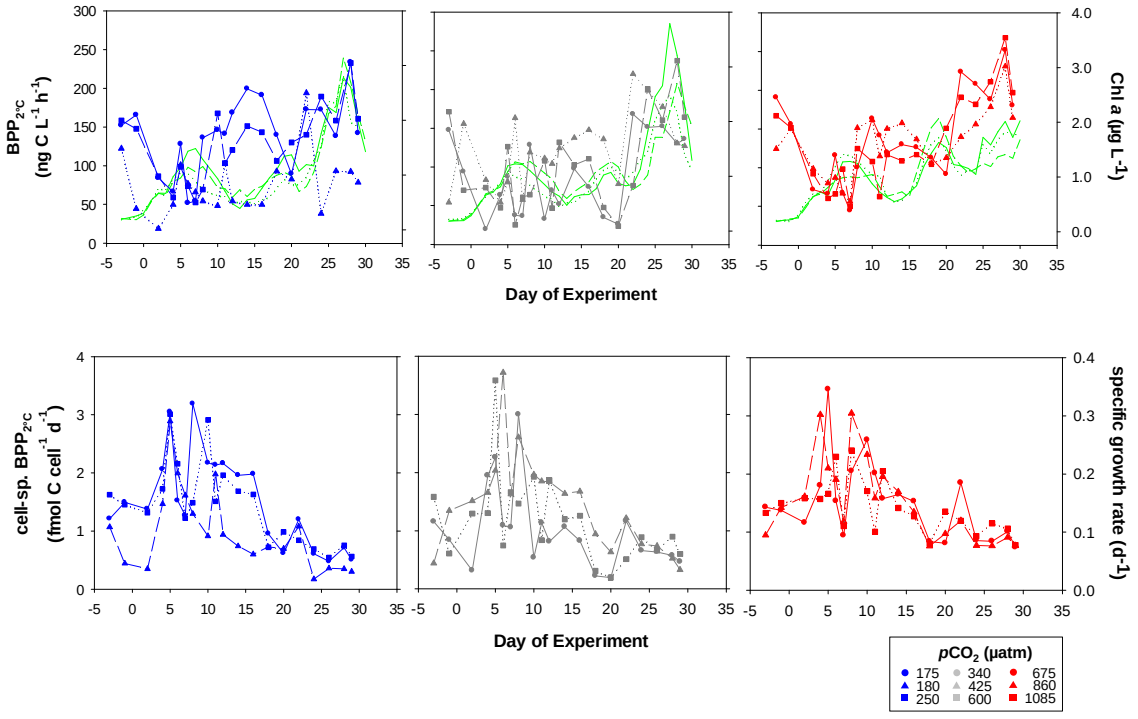

Fig. 3. Temporal development of bacterial protein production ($BPP_{2\,^\circ C}$) and specific growth rates (d^{-1}) at 2 °C in mesocosms of low (175–250 µatm; blue symbols), medium (340–600 µatm; grey symbols) and high pCO_2 (675–1085 µatm; red symbols). Upper panel: $BPP_{2\,^\circ C}$ normalised to volume (ng C L^{-1} h^{-1}), green lines represent chlorophyll *a* concentrations (Chl *a*, µg L^{-1}). Lower panel: $BPP_{2\,^\circ C}$ normalised to bacterial abundance (cell-specific $BPP_{2\,^\circ C}$, fmol C $cell^{-1}$ d^{-1}) and corresponding specific growth rate (d^{-1}).

chlorophyll *a* maximum between days 22 and 28. However, this third phosphatase maximum had lower rates than the two previous maxima in most mesocosms (Fig. 8).

3.5 Temperature dependence of bacterial protein production and extracellular enzymes

The seawater temperature increased during the mesocosm study from 2.0 °C at the beginning of June to 5.2 °C one month later (Schulz et al., 2012). There were no temperature differences between the nine mesocosms (ANOVA on ranks, $p > 0.05$) and also Q_{10} and Q^*_{10} values determined for extracellular enzyme activity (Eq. 2) and BPP (Eq. 3), respectively, did not show significant variation between the mesocosms on the individual sampling days (β-glucosidase: ANOVA, $p > 0.05$; leu-aminopeptidase and BPP: ANOVA on ranks, $p > 0.05$). However, Q_{10} and Q^*_{10} varied significantly over the duration of the study, revealing that the temperature sensitivity of the enzyme assemblages changed within two weeks. Q_{10} values for β-glucosidase and leu-aminopeptidase determined at four time points between day 12 and 24 ranged from 2.0–11.4 and 1.1–4.4 (mean values of all mesocosms), respectively. Activity of β-glucosidase was consistently more temperature sensitive than leu-aminopeptidase activity. Also Q^*_{10} values determined for BPP at nine sampling days showed high temporal variability and ranged from 0.1–11.7 (Fig. 9). The Q_{10} values for both enzymes and Q^*_{10} values for BPP were related to phytoplankton exudation that can be considered as a measure for the input of labile organic matter. The Q_{10} values determined for the two extracellular enzymes at lower exudation levels of 0.9 and 0.7 µmol C L^{-1} d^{-1}, respectively, were significantly higher than those at increased exudation of 1.5 µmol C L^{-1} d^{-1} (Mann-Whitney rank sum test, $p < 0.001$). Also Q^*_{10} values for BPP decreased significantly with increasing exudation (linear regression, $r^2 = 0.51$, $p = 0.03$) (Fig. 9).

Q^*_{10} values for BPP are derived from a comparison of rates determined at 2 °C and at in situ temperature. Since 2 °C represents the initial fjord temperature during mesocosm deployment and CO_2-manipulation, the difference between BPP at the two temperatures gives an estimate for the surplus of bacterial biomass production that can be attributed to seawater warming during the experiment. Summing up this surplus over the experiment, it can be estimated that rising in situ temperature alone increased BPP in the mesocosms by a factor of 1.3–1.7, corresponding to 27–61 µg C L^{-1} in the 26 days between CO_2-manipulation and the end of the experiment.

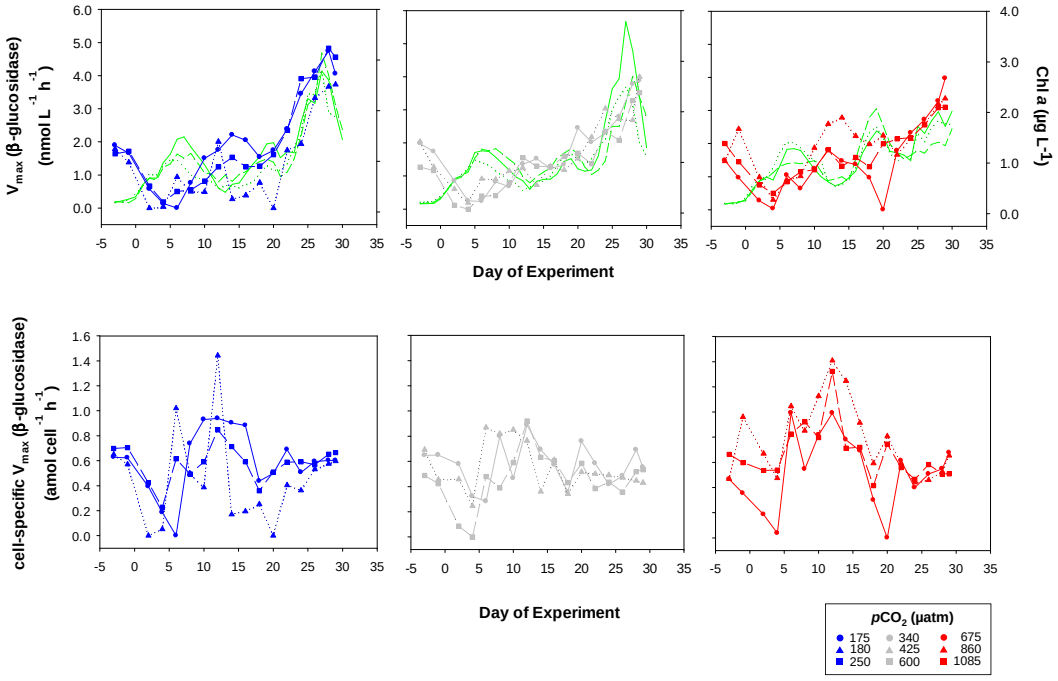

Fig. 4. Temporal development of extracellular β-glucosidase activity in mesocosms of low (175–250 µatm; blue symbols), medium (340–600 µatm; grey symbols), and high pCO_2 (675–1085 µatm; red symbols). Maximum reaction velocities (V_{max}) are normalised to volume (nmol L^{-1} h^{-1}; upper panel) and bacterial abundance (amol $cell^{-1}$ h^{-1}; lower panel). Green lines represent chlorophyll *a* (Chl *a*) concentrations in the mesocosms.

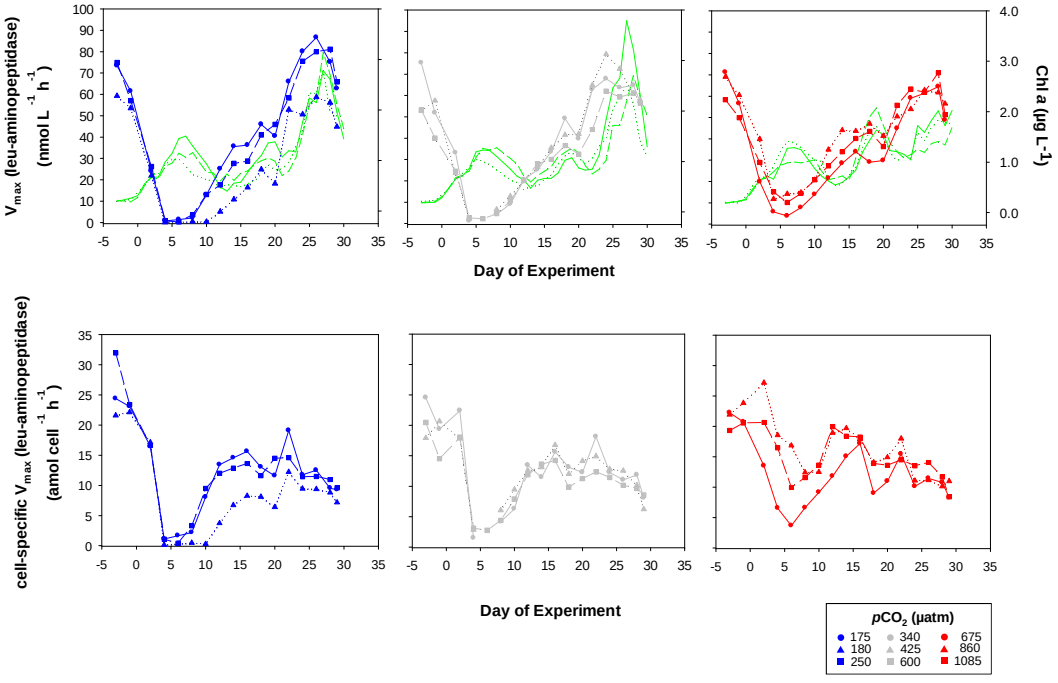

Fig. 5. Temporal development of extracellular leucine (leu)-aminopeptidase activity in mesocosms of low (175–250 µatm; blue symbols), medium (340–600 µatm; grey symbols), and high pCO_2 (675–1085 µatm; red symbols). Maximum reaction velocities (V_{max}) are normalised to volume (nmol L^{-1} h^{-1}; upper panel) and bacterial abundance (amol $cell^{-1}$ h^{-1}; lower panel). Green lines represent chlorophyll *a* (Chl *a*) concentrations in the mesocosms.

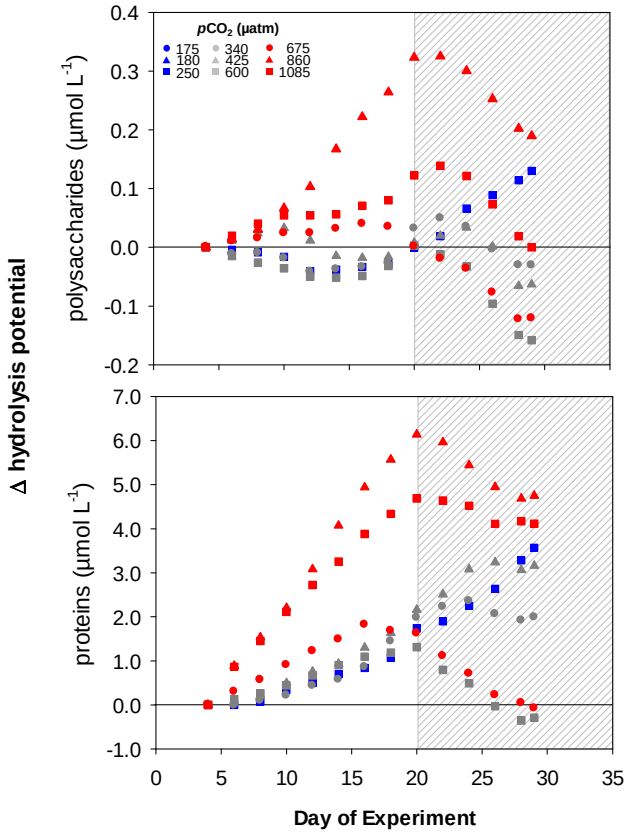

Fig. 6. Temporal development of Δ *hydrolysis potential* for polysaccharide (upper panel) and protein degradation (lower panel) in acidified mesocosms. The shaded area represents the time period after the second chlorophyll *a* maximum when bacterial abundances in the mesocosms diverged (see text for further explanation).

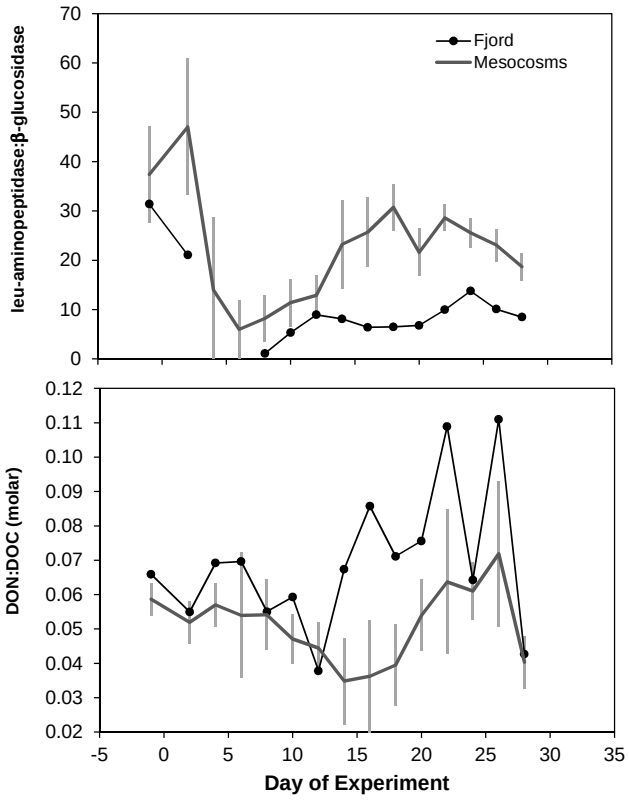

Fig. 7. Activity ratios of leucine (leu)-aminopeptidase: β-glucosidase (upper panel) and molar ratios of DON:DOC (lower panel) in mesocosms (grey line) and Kongsfjorden (black circles). Mean values (±SD) of all mesocosms are shown.

3.6 Testing for effects of different mesocosm pCO_2 levels on bacterial activity

The design of the mesocosm experiment with 8 different pCO_2 levels suggests the use of a regression model to test data for significant relationships between seawater pCO_2 and bacterial activity. It has been shown that running each experimental unit at a different treatment level is an appropriate experimental design for large-scale facilities that would otherwise allow only for a limited number of replicates (Havenhand et al., 2010). Physiological performance and metabolic activity of heterotrophic microbial organisms like bacteria may not be directly affected by changes in seawater pCO_2, but by co-occurring changes in pH. The range of mesocosm pCO_2 from 175 to 1085 µatm corresponded to pH values of 7.63–8.34. Regression analysis of our dataset was carried out with differences in proton concentration that could be calculated between each acidified mesocosm and the non-acidified control mesocosms (Δ $[H]^+$, nmol L^{-1}). The resulting mean for Δ $[H]^+$ over time were correlated with time-integrated rates of cell-specific $BPP_{2\,^\circ C}$, β-glucosidase and leu-aminopeptidase (Eqs. 4 and 5) (Fig. 10). The temporal development of Δ *hydrolysis potential* reveals an elevated enzymatic potential for polysaccharide and protein degradation in the three mesocosms of highest pCO_2 during the first 20 days of the study when similar bacterial abundances were determined in all mesocosms (Fig. 6). Therefore, regression analyses of time-integrated cell-specific β-glucosidase and leu-aminopeptidase rates were accomplished for the whole duration of the experiment (days 4–29) and also separately for the period of similar bacterial cell numbers (days 4–20). Cell-specific leu-aminopeptidase activity was directly related to differences in proton concentration over the whole experiment ($r^2 = 0.57$, $p < 0.05$) (Fig. 10). Hence, the cellular potential for enzymatic protein hydrolysis increased with gradual acidification over the applied pCO_2 range. Correlations of Δ $[H]^+$ with cell-specific enzymatic rates integrated over days 4–20 revealed significant linear relationships for both β-glucosidase ($r^2 = 0.53$, $p < 0.05$) and leu-aminopeptidase activity ($r^2 = 0.79$, $p < 0.05$) (Fig. 10), and confirmed the trend of rising hydrolytic activity mediated by bacterial extracellular enzymes at lowered seawater pH. A significant relationship between Δ $[H]^+$ and cell-specific $BPP_{2\,^\circ C}$ could not be determined.

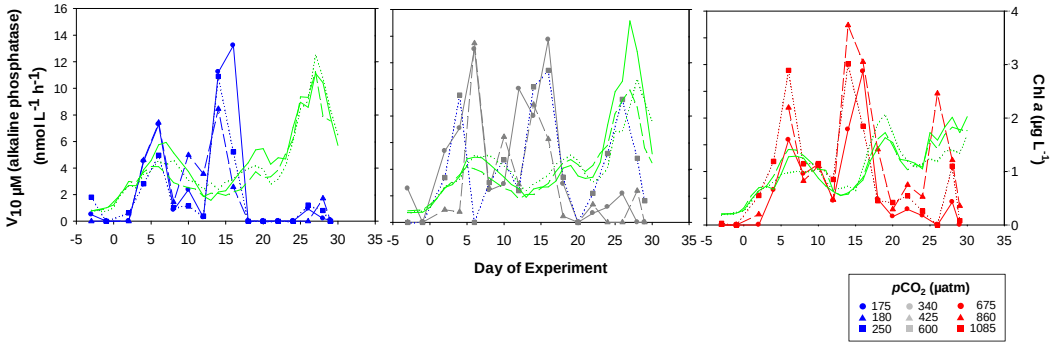

Fig. 8. Temporal development of extracellular phosphatase activity in mesocosms of low (175–250 µatm; blue symbols), medium (340–600 µatm; grey symbols) and high pCO_2 (675–1085 µatm; red symbols). Reaction velocities at 10 µmol L^{-1} substrate concentration ($V_{10\,\mu M}$) are normalised to volume (nmol L^{-1} h^{-1}). Green lines represent chlorophyll *a* (Chl *a*) concentrations in the mesocosms.

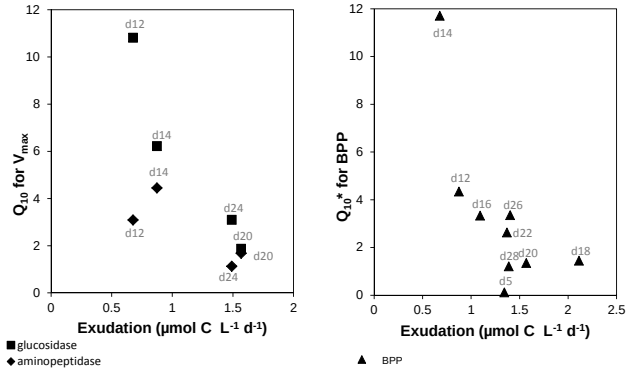

Fig. 9. Q_{10} values for extracellular enzyme activities (β-glucosidase, leu-aminopeptidase) and Q_{10}* values for bacterial protein production (BPP) in relation to phytoplankton exudation at different sampling days (e.g., d14: day 14 of the experiment).

The pH manipulation was the primary source of variation in this experiment but a comparison of bacterial activity in the mesocosms at different pCO_2 should also consider impacts of changing phytoplankton productivity under different pCO_2 that are analysed in detail by Engel et al. (2012). Particulate primary production was significantly higher at elevated pCO_2 after nutrient addition. Dissolved primary production was significantly higher in CO_2-enriched mesocosms before as well as after nutrient addition, suggesting a direct influence of CO_2 on the production of DOC (Engel et al., 2012). Regression analysis revealed direct linear correlations of time-integrated total primary production with cell-specific $BPP_{2\,^\circ C}$ ($r^2 = 0.77$, $p < 0.05$) and cell-specific leu-aminopeptidase activity ($r^2 = 0.57$, $p < 0.05$) (Fig. 11). Hence, bacterioplankton responded with rising biomass production and protein degradation to elevated photosynthetic production of organic substrates. Leu-aminopeptidase activity was significantly related to both seawater pH and primary production.

3.7 Acidification assay

To directly test the influence of seawater pH on β-glucosidase and leu-aminopeptidase activity samples of the Kongsfjorden and one control mesocosm (M3) were acidified with dilute hydrochloric acid on day 25 of the experiment. The pH manipulation was the only source of variation in this experiment, while a comparison of the mesocosms at different pCO_2 includes the divergence of other environmental and biological parameters like phytoplankton community composition (Brussaard et al., 2012), primary production (Engel et al., 2012), net community production (Silyakova et al., 2012) and nutrient utilisation (Schulz et al., 2012). Rates of extracellular β-glucosidase and leu-aminopeptidase in non-manipulated subsamples with in situ pH were compared with subsamples adjusted to three lower pH-levels ranging from 7.79–8.17 (Fig. 12). Both enzymes showed significantly higher rates at lowest pH (Wilcoxon signed rank test, $p = 0.008$), revealing that the in situ pH of the Kongsfjorden and the control mesocosm (M3) did not provide optimum conditions for these enzymatic reactions. Cell-specific V_{max} of β-glucosidase and leu-aminopeptidase in acidified Kongsfjorden samples increased by a factor of 2.1 and 1.8, respectively, when the in situ pH of 8.29 was reduced to 7.79. The effect of acidification was less pronounced in mesocosm samples. Here, cell-specific V_{max} of both enzymes increased by a factor of 1.2 at pH 7.79 (Fig. 12).

4 Discussion

In the present mesocosm study, the microbial community of an open Arctic fjord system was exposed to a pCO_2 gradient ranging from in situ values of 175 µatm to 1085 µatm, according to projections for the future ocean. Hence, changing pCO_2 was the primary manipulation in this experiment. The parameters of the seawater carbonate system must be considered as environmental factors that contribute among

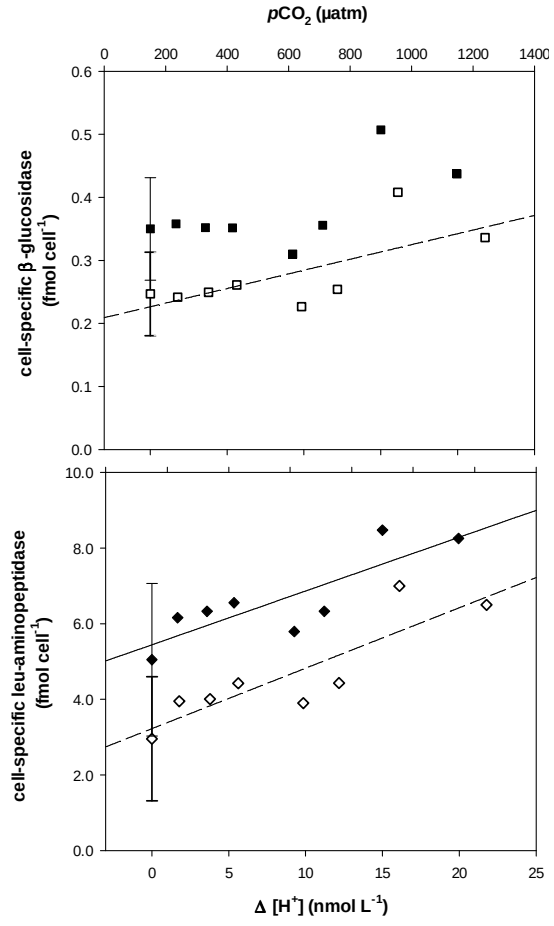

Fig. 10. Relationships between seawater pCO_2/delta proton concentration (Δ [H^+]) and cell-specific extracellular enzyme activities. Separate correlations are shown for rates integrated over days 4–20 (open symbols) and the whole experiment (filled symbols). Lines represent significant ($p < 0.05$) linear regressions (β-glucosidase, d 4–20: $r^2 = 0.53$; leu-aminopeptidase, d 4–20: $r^2 = 0.79$, whole experiment: $r^2 = 0.57$).

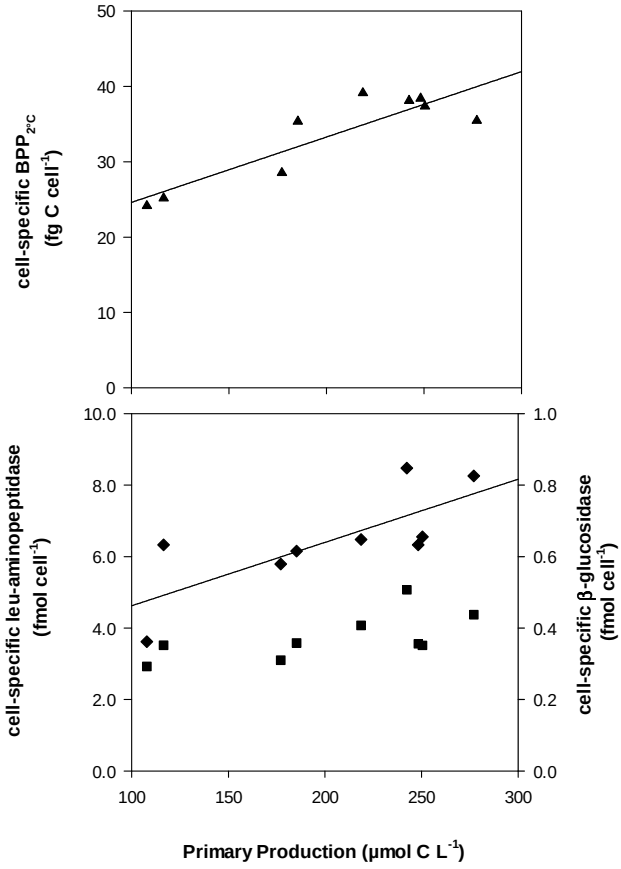

Fig. 11. Relationships between primary production and cell-specific bacterial protein production (BPP) (upper panel) and primary production and cell-specific extracellular enzyme activities (diamonds: leu-aminopeptidase, squares: β-glucosidase) (lower panel). All rates are time-integrated over the whole experiment. Lines represent significant ($p < 0.05$) linear regressions ($BPP_{2\,^\circ C}$: $r^2 = 0.77$; leu-aminopeptidase: $r^2 = 0.57$).

others to the bottom-up control of the microbial community. In the present study, also temperature that increased substantially during the experiment and phytoplankton production that changed over time and in response to different pCO_2 had a high potential to affect growth and metabolic activity of bacterioplankton. In the following the influence of seawater pH, temperature and phytoplankton production on heterotrophic bacterial activity and their role in the bottom-up regulation of bacterioplankton activity will be discussed.

4.1 Phytoplankton-bacterioplankton coupling

The coupling of phytoplankton and bacterioplankton becomes evident from a phasing of biomass and production of the two groups (Ducklow and Yager, 2006; Kirchman et al., 2009b). This co-variation of phytoplankton and bacterioplankton biomass and production over time and space is characterised by a succession of phytoplankton followed by bacterioplankton after a temporal lag of days to weeks. The duration of this lag period in response to increasing phytoplankton biomass and production is decisive for the degradation efficiency and the export of organic matter produced in bloom situations (Kirchman et al., 2009b; Bird and Karl, 1999). The bacterioplankton community in the Kongsfjorden and in the mesocosms responded after a lag of only some days with increasing activities to rising chlorophyll a concentrations, revealing that there was neither a substrate threshold that needed to be exceeded nor any community intrinsic factor impeding a fast bacterial response (Figs. 2–5). In the framework of this mesocosm experiment, ^{13}C added as bicarbonate was used to follow the carbon flow through the microbial food web. The rapid increase of bacterioplankton activity in the mesocosms coincided with increasing shares

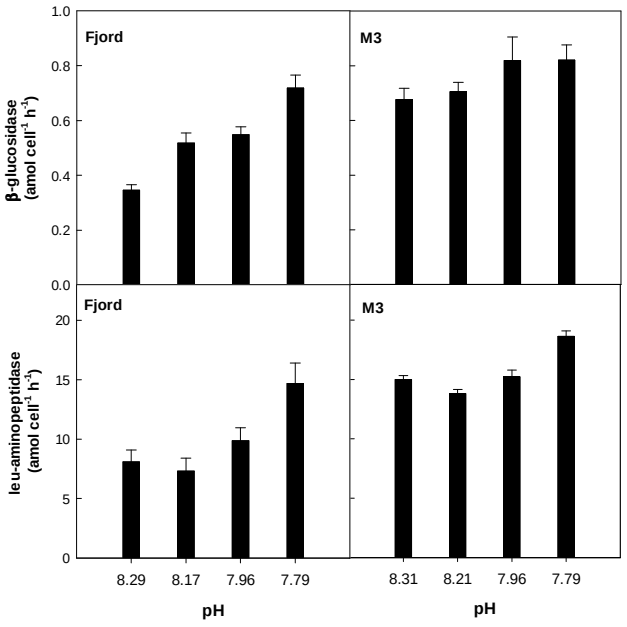

Fig. 12. Activity of extracellular β-glucosidase and leu-aminopeptidase in samples of Kongsfjorden and one control mesocosm (M3) at in situ pH (fjord: 8.29, M3: 8.31) and after acidification with dilute hydrochloric acid (pH 7.79–8.17). Rates at pH 7.79 were significantly higher than at in situ pH (Wilcoxon signed rank test, $p = 0.008$).

of ^{13}C in bacterial biomass, revealing a fast transfer of carbon recently fixed by phytoplankton to the bacterioplankton community (de Kluijver et al., 2012). Previous studies have shown that bacterial communities in polar marine systems can lag behind the development of vernal phytoplankton blooms by weeks (Billen and Becquevort, 1991; Bird and Karl, 1999). Our study, however, was conducted in early summer after the main *Phaeocystis* spring bloom in the Kongsfjorden. Therefore, it seems likely that bacterial cells did not return from a starvation induced dormancy that allows maximum conservation of carbon and energy during winter. A study accomplished in the Chuckchi Sea during summer has shown that the fraction of actively respiring bacterial cells and the cells' substrate affinity increased at elevated chlorophyll levels within some days, suggesting a substantial physiological and metabolic flexibility of bacterioplankton communities at this time of the year (Yager et al., 2001). Hence, the physiological state of the bacterial cells and the community composition after the vernal bloom might facilitate a faster response to subsequent bloom events during summer and, thus, promote a faster onset of organic matter recycling in cold marine systems.

In addition to the lag phase, also the magnitude of bacterial activity achieved in response to elevated phytoplankton production determines the rate at which freshly produced organic matter is recycled. Absolute values for growth rates and extracellular enzyme activities are a measure to assess the overall magnitude of bacterial activity, although information is limited due to broad ranges observed in marine environments and overlapping limits for cold and temperate marine ecosystems. The specific growth rates of bacteria in polar marine systems are not necessarily lower than in oligotrophic temperate regions (Rivkin et al., 1996; Rich et al., 1997; Ducklow, 1999). Specific growth rates of 0.02–0.65 d^{-1} determined in Kongsfjorden and the mesocosms are in the range of values published for both the Arctic Ocean (Kirchman et al., 2009b) and temperate marine ecosystems (Ducklow et al., 1993; Ducklow, 1999) (Fig. 3). Rates derived from the uptake of radio-labelled leucine in our study are similar to bacterial growth rates of 0.13–0.68 calculated from the incorporation of ^{13}C (de Kluijver et al., 2012). This accordance emphasises the importance of freshly produced organic matter for bacterial growth in the mesocosms. High variability was also found for cellular β-glucosidase and leu-aminopeptidase rates in this study. Maximum and mean cell-specific rates of β-glucosidase and leu-aminopepitdase in the mesocosms and in Kongsfjorden are in good accordance with values determined off the coast of Spitsbergen during summer (Sala et al., 2010) (Figs. 2, 4–5). The activities are at the lower limit of ranges published for temperate marine systems like the Central Atlantic (Baltar et al., 2009) and the Baltic Sea (Nausch et al., 1998). Overall, the magnitude of bacterial activity in mesocosms and Kongsfjorden represents the overlap of higher levels in cold marine environments and lower levels in the temperate ocean.

4.2 Microbial acquisition of organic matter by the use of extracellular enzymes

Organic substrates for bacterial utilisation are mainly derived from complex high-molecular-weight compounds that are enzymatically hydrolysed outside the microbial cells into molecules sufficiently small for uptake. In the marine environment, heterotrophic bacteria must be considered as the main producers of β-glucosidase and leu-aminopeptidase, the most important polysaccharide- and protein-degrading extracellular enzymes, respectively. Culture studies have shown that some species of marine phagotrophic protozoa, mainly flagellates, produce and release glucosidases and proteases, but to the best of our knowledge there is no evidence to date that non-bacterial producers are able to significantly influence the bulk rate of these two enzymes in natural marine communities (Karner et al., 1994). The temporal development of cell-specific β-glucosidase and leu-aminopeptidase activity reveals information on the modulation of enzyme activity in the mesocosms. Cell-specific enzymatic rates increased during the first chlorophyll peak, demonstrating a response of bacterial activity to changing environmental conditions on the cellular level in this first phase of the experiment. Afterwards increasing bulk activity was driven by the growing community and thereby increasing numbers of bacterial enzyme producers, but not by the

adjustments of cellular rates. Accordingly, the bulk activity of extracellular enzymes was highest towards the end of the mesocosm experiment when highest bacterial cell numbers were determined, revealing that the enzymatic turnover of organic matter was strongly affected by growth characteristics of the bacterioplankton community in this study.

A variety of heterotrophic and autotrophic microbial organisms contributes substantially to the enzymatic hydrolysis of organic phosphorus compounds. In addition to bacteria, also phytoplankton species and protozoa can be strong producers of extracellular phosphatase in natural marine communities (Hoppe, 2003). In the present study, two maxima of phosphatase activity coincided with chlorophyll *a* maxima, while only the phosphatase peak during days 12–18 developed at low chlorophyll *a* concentrations (Fig. 8). Therefore, it seems likely that in particular before nutrient addition on day 12 autotrophic organisms recycled a significant share of organic phosphorus. As a consequence, high chlorophyll *a* concentrations could be achieved without the availability of inorganic phosphorus (Schulz et al., 2012).

4.3 Growth limitation of bacterioplankton by organic matter resources

The availability of labile and semilabile carbon is considered to be a major factor regulating bacterial activity in the marine pelagic environment, including subpolar and polar oceans (Kirchman et al., 2005, 2009a; Granéli et al., 2004; Cuevas et al., 2011). Results of the enrichment assay conducted with Kongsfjorden samples at the beginning of the mesocosm study are in accordance with this conclusion, since the amendment of labile carbon stimulated bacterial biomass production and growth (Fig. 1, Table 1). A recent study revealed high spatial variability of the limiting resource for bacterial growth at coastal and fjord stations located in the west of Spitsbergen. Enhanced carbon consumption was evident in incubations of coastal samples, while mineral nutrient deficiency limited bacterioplankton production in fjord samples of high phytoplankton biomass (Vadstein, 2011). Our incubations were accomplished during the initial days of the mesocosm experiment when low levels of phytoplankton biomass and primary production prevailed (Engel et al., 2012; Schulz et al., 2012). Therefore, it can be assumed that regeneration of nutrients was high and prevented mineral nutrient limitation, while the input of fresh and labile organic compounds was low and, thus, limiting at that time. High levels of leu-aminopeptidase activity in glucose-amended Kongsfjorden samples show that also the demand for amino acids released from protein hydrolysis was high. Hence, the limitation of bacterial production and growth by the availability of labile carbon was evident, but also a deficiency of labile organic nitrogen compounds must be assumed. The enclosure of the bacterioplankton community into the mesocosms likely intensified the shortage in organic nitrogen for bacterial consumption as implied by higher activity ratios of leu-aminopeptidase: β-glucosidase in the mesocosms than in the fjord (Fig. 7). Higher activity ratios in the mesocosms coincided with a lower share of dissolved and particulate organic nitrogen (Fig. 7; Schulz et al., 2012), suggesting that the high organic nitrogen demand of the strongly growing bacterioplankton populations in the mesocosms enhanced the need for enzymatic protein hydrolysis.

Overall, the enrichment assay and the activity ratios of leu-aminopeptidase: β-glucosidase in mesocosms and fjord demonstrate that organic matter resources had a high potential to affect bacterial growth characteristics in this study. Furthermore, results reveal an efficient adjustment of hydrolytic extracellular enzyme production to changes in substrate availability and to potentially changing requirements.

4.4 Effects of temperature on bacterial activity

Temperature is an extensively investigated constraint on bacterial activity in polar marine systems that has a high potential to change metabolic rates (e.g., Rivkin et al., 1996; Pomeroy and Wiebe, 2001; Kirchman et al., 2009a). In the present study, in situ temperature increased over time and enhanced the daily production of bacterial biomass in the mesocosms substantially. The daily values for Q_{10}^{*} varied significantly from values ≤ 1 up to 12 over the duration of the experiment (Fig. 9). Values ≤ 2 are characteristic for psychrophilic bacteria in polar waters that show low-temperature optima for metabolic processes, while higher values were observed for psychrotolerant bacterial strains that can cope with low seawater temperatures, but show temperature optima around 20 °C (Morita, 1975). Hence, Q_{10}^{*} values determined for BPP in the mesocosms suggest that the bacterial community metabolism was mainly driven by the activity of psychrotolerant instead of phsychrophilic bacterial strains. Q_{10} values for β-glucosidase, leu-aminopeptidase and Q_{10}^{*} values for BPP were related to rates of phytoplankton exudation, strongly suggesting interactions of bacterial temperature sensitivity and substrate availability. Low Q_{10} and Q_{10}^{*} values for bacterial activity at elevated rates of phytoplankton exudation reveal lower temperature sensitivity at high substrate availability. A similar relationship between ambient substrate concentration and temperature sensitivity of bacterial permeases was determined in an Arctic polynya. Here, Q_{10} values up to 11 were determined for bacterial amino acid uptake at amino acid concentrations $\leq 50\,\mathrm{nmol\,L^{-1}}$, while Q_{10} decreased at higher amino acid concentrations (Yager and Deming, 1999). The differences in temperature sensitivity determined in our study cannot be induced by direct substrate – enzyme interactions, since the fluorescent and radioactive markers for enzyme and BPP measurements, respectively, were added at saturating concentrations. It is likely that the variability in Q_{10} and Q_{10}^{*} is related to metabolic adaptations of the bacterioplankton community to different levels of substrate input by phytoplankton production. These adaptations to different substrate concentrations can be achieved

by the use of different isoenzymes. Isoenzymes catalyse the same reaction, but can differ with respect to other kinetic and biochemical characteristics (Ferenci, 1996; Wick et al., 2001). The co-existence of isoenzymes in natural marine assemblages was shown by biphasic and multiphasic kinetics of extracellular enzyme activity and bacterial substrate uptake and by the isolation of diverse isoenzymes for extracellular polysaccharide hydrolysis during a bloom situation (Unanue et al., 1999; Tholosan et al., 1999; Arrieta and Herndl, 2002). Furthermore, laboratory studies revealed that extracellular isoenzymes produced by polar marine bacteria can show different optimum temperatures for their catalytic reaction (Nichols et al., 1999). Even isoenzymes produced by a single bacterial strain can differ with regard to their temperature characteristics (Maki et al., 2006). Hence, a modulation of the community metabolism by isoenzymes that are produced in response to different levels of substrate availability and show different temperature sensitivities could explain short-term variability in Q_{10} values for bacterial activity in natural communities.

4.5 Bacterial activity under elevated pCO_2

The general dependence of enzymatic reactions on pH is well-known for more than one hundred years and was confirmed for extracellular enzymes of aquatic habitats about 20 yr ago (Arrhenius, 1889; Münster, 1991). Also extracellular enzyme activity of cold-adapted marine isolates was shown to respond with characteristic optimum curves to a pH range from 2 to 12 (Groudieva et al., 2004). Recent studies revealed that also moderate decreases in seawater pH as projected for the near future can significantly impact bulk rates of hydrolytic extracellular enzymes produced from natural marine bacterioplankton communities (Grossart et al., 2006; Tanaka et al., 2008; Piontek et al., 2010; Yamada and Suzumura, 2010). Most of these studies show elevated activity of different extracellular enzymes in response to seawater acidification. A meta-analysis of ocean acidification effects on microbially driven biogeochemical processes has shown that effects on extracellular enzyme activity are among the most sensitive ones (Liu et al., 2010). During our mesocosm study, the direct response of the natural enzyme assemblages to lowered seawater pH was tested by an acidification assay with dilute hydrochloric acid. A decrease of 0.5 pH units doubled rates of β-glucosidase and leu-aminopeptidase in the Kongsfjorden samples and strongly suggests direct biochemical effects of lowered seawater pH on the enzymatic reaction velocities (Fig. 12). A comparison with the mean Q_{10} values for β-glucosidase and leu-aminopeptidase activity in the Kongsfjorden reveals that the rate increase after reduction of seawater pH by 0.3–0.5 units equals the rate increase at a temperature elevated by 3 °C. Therefore, our results indicate that future changes in seawater temperature and pH have a similar potential to increase the hydrolytic activity of extracellular enzymes in this fjord system,

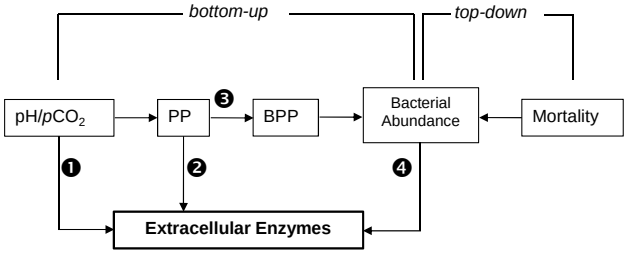

Fig. 13. Pathways of direct pH effects and indirect pCO_2 effects on extracellular enzyme activity in the mesocosm plankton community. (1) Biochemical pH effect on enzymatic reaction velocity, (2) effect of primary production (PP) on bacterial enzyme production, (3) effect of PP on bacterial protein production (BPP), (4) effect of bacterial abundance on bulk enzymatic activity. See text for further explanation.

suggesting a high potential for combined temperature and pH effects. Also extracellular enzyme activities in the high-CO_2 mesocosms were enhanced and directly related to the rising hydrogen ion concentrations over the applied pCO_2 gradient (Fig. 10). Hence, results of both acidification assay and mesocosm study confirm an acceleration of organic matter-degrading extracellular enzyme activity at seawater pH projected for the near future. Enhanced extracellular enzyme activity in the mesocosms, however, cannot be exclusively attributed to direct biochemical pH effects. Instead, it must be assumed that bacterial activity in the mesocosms was additionally stimulated by increased primary production under elevated pCO_2. Both particulate and dissolved primary production supply heterotrophic bacterial communities with organic substrates. Phytoplankton exudation contributed about 20 % and 15 % to total primary production in the mesocosms before and after nutrient addition, respectively, and directly provided dissolved substrates that are easily accessible for bacterial utilisation (Engel et al., 2012). However, also processes like sloppy feeding by zooplankton (Möller, 2005) and viral lysis of phytoplankton cells (Fuhrman, 1999) provide labile substrates by the release of dissolved compounds. A positive linear correlation of primary production with rates of leu-aminopeptidase strongly suggests that an increased supply of organic matter to bacterioplankton under elevated pCO_2 enhanced the cellular production of protein-degrading extracellular enzymes. Furthermore, a direct relationship between primary production and BPP shows that enhanced primary production under elevated pCO_2 stimulated not only the hydrolysis of organic matter, but also its assimilation into bacterial biomass. Thus, our results reveal that the bacterioplankton community of the Kongsfjorden had the potential to efficiently degrade surplus organic substrates derived from increased autotrophic carbon fixation under elevated pCO_2. This is supported by carbon fluxes in the pelagic food web derived from the incorporation of ^{13}C. Carbon fixed by phytoplankton was efficiently transferred to bacterioplankton.

Bacterial production accounted for 34 % of phytoplankton production, while a low share of only 6 % of phytoplankton carbon was grazed by mesozooplankton (de Kluijver et al., 2012). However, neither bacterial respiration nor community respiration showed significant differences between the mesocosms (Motegi et al., 2012; Tanaka et al., 2012). Hence, it can be suggested that on the time scale of this experiment enhanced bacterial degradation activity under high CO_2 induced a more pronounced effect on the re-assimilation and transfer of organic matter in the microbial loop than on carbon remineralisation.

In the present mesocosm study lower bacterial abundances developed in the high-CO_2 mesocosms after day 20 due to higher viral lysis rates (Brussaard et al., 2012). This top-down regulation of bacterial cell numbers and, thus, enzyme producers strongly affected the bulk hydrolytic potential of β-glucosidase and leu-aminopeptidase in the mesocosms (Fig. 6). In accordance with the bottom-up regulation by seawater pH and primary production, high-CO_2 mesocosms showed a higher hydrolytic potential until day 20. This pattern changed and the trend of enhanced bulk hydrolysis under high pCO_2 was attenuated or even inverted when bacterial abundances diverged during the last phase of the experiment and top-down regulation led to lower bacterial cell numbers at high pCO_2 (Fig. 6; Brussaard et al., 2012). Also the community composition of bacterioplankton changed after day 20, and showed a higher diversity of particle-attached bacteria in high-CO_2 mesocosms (Sperling et al., 2012). Increased competitive relationships at high diversity can evoke a negative effect on the activity of communities when competitively strong species or groups do not contribute a large part to specific community functions (Jiang et al., 2008). Hence, also changes in the bacterioplankton community structure during the last ten days of the experiment may have reduced community performance with regard to extracellular enzyme activities (Sperling et al., 2012). A complex multifaceted regulation of extracellular enzyme activity within natural plankton communities can partly explain why bulk enzymatic rates were affected to a varying degree under high pCO_2 in recent perturbation studies (Liu et al., 2010; Grossart et al., 2006; Tanaka et al., 2008; Yamada and Suzumura, 2010).

5 Concluding remarks

The present study investigates how elevated seawater pCO_2 can impact the metabolic activity of a natural bacterioplankton community in an Arctic fjord system. A short lag phase of extracellular enzyme activities and BPP after increasing phytoplankton production revealed high flexibility and pulse-responsiveness of two bacterial processes that substantially contribute to the heterotrophic carbon recycling and nutrient remineralisation. Changes in seawater pCO_2 induced beneficial effects on bacterial activity by two principal mechanisms (Fig. 13). Firstly, acidification increased rates of extracellular β-glucosidase and leu-aminopeptidase, strongly suggesting direct pH effects to enhance extracellular hydrolytic activity under elevated pCO_2. Secondly, BPP and leu-aminopeptidase activity were directly related to primary production at changing pCO_2. This suggests that elevated phytoplankton production under high CO_2, as observed in the present mesocosm experiment (Engel et al., 2012) and in previous CO_2-perturbation studies (e.g., Egge et al., 2009), is efficiently channelled into the microbial food web in natural plankton communities. Therefore, enhanced heterotrophic carbon utilisation may counterbalance increased autotrophic carbon fixation under high CO_2 and prevent enhanced carbon export. However, the top-down control of bacterial abundances led to higher cell losses at high CO_2 in the present mesocosm study and partly counteracted effects of lowered pH and enhanced primary production on the bulk hydrolysis of polysaccharides and proteins (Figs. 6 and 13). Our results strongly suggest further investigation of acidification effects on both the biochemical sensitivity of bacterial isoenzymes and the regulation of bacterial activity in natural communities to better evaluate impacts of climate change on heterotrophic microorganisms and their feedback potential to ocean acidification.

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