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Multiple environmental changes induce interactive effects on bacterial degradation activity in the Arctic Ocean

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Abstract

The Arctic Ocean faces multiple environmental changes induced by climate change on both global and regional scale. In addition to global changes in seawater temperature and pH, Arctic waters receive organic matter enrichment due to increasing pelagic primary production, enhanced sea ice melting and increasing terrestrial carbon loads. We experimentally tested individual and combined effects of warming, acidification and organic matter amendment on growth, biomass production and extracellular enzyme activities of bacterioplankton in Fram Strait during early summer. Results reveal pH optima of 6.7–7.6 for extracellular leucine-aminopeptidase and below pH 6.0 for beta-glucosidase in the West Spitsbergen Current. These optima well below the current seawater pH imply increasing hydrolytic activity with ongoing ocean acidification. However, the new synthesis of extracellular enzymes during 4-d incubations obscured the biochemical pH effects. Elevated temperature and carbohydrate supply had strongly interactive effects on bacterial biomass production in both Atlantic Water of the West Spitsbergen Current and Polar Water of the East Greenland Current. Activation energies ranged from 45 kJ mol^{-1} to 52 kJ mol^{-1} at in situ substrate concentration, while substantially higher values of 122–174 kJ mol^{-1} could be estimated from incubations with carbohydrate addition. The net loss of total amino acids in carbohydrate-amended incubations was significantly reduced at elevated temperature in all experiments, suggesting enhanced de novo synthesis. Our findings show that the complexity of combined effects must be considered to better assess the potential of climate change to alter biogenic carbon and energy fluxes in marine systems.

The Arctic Ocean is experiencing severe consequences of climate change that become most obvious in the strongly declining summer sea ice extent coincident with intense loss of multi-year ice (Polyakov et al. 2010; Wassmann 2011; Stroeve et al. 2012). The rate of warming in the Arctic exceeds three times the global average and may result in a 6°C temperature increase in the 21st century (ACIA 2004). Numerous studies on rate-temperature relationships revealed remarkable heterogeneity of thermal sensitivity among species and metabolic processes, thereby demonstrating the complexity of community responses to upcoming temperature changes in marine environments (e.g., Pomeroy and Deibel 1986; Rose and Caron 2007; Hall et al. 2008). A threshold of 5°C was experimentally identified, beyond which metabolism in Arctic plankton communities shifts from net autotrophic to net heterotrophic (Holding et al. 2013).

Concomitantly with warming, the Arctic Ocean is expected to be among the marine ecosystems most susceptible to ocean acidification. Model simulations project that the absorption of anthropogenic CO_2 will drop seawater pH in the Arctic Ocean by 0.45 units within the 21st century (Steinacher et al. 2009). Freshening by increased ice melting and river runoff as well as the enhanced CO_2 uptake by larger ice-free areas will further reduce the low buffering capacity of the Arctic seawater, which results from naturally low carbonate ion concentrations. Recently, results of a mesocosm manipulation study demonstrated effects of rising seawater CO_2 on community structure and performance of Arctic bacterioplankton (Piontek et al. 2013; Sperling et al. 2013). In the same experiment, elevated CO_2 stimulated primary production (Engel et al. 2013) and the growth of autotrophic picoplankton, thereby suggesting impacts on higher trophic levels in the pelagic food web (Brussaard et al. 2013). Tipping the balance of autotrophic and heterotrophic microbial metabolism has a high potential to change biogenic carbon fluxes in the ocean. CO_2 fixation by phytoplankton photosynthesis in sunlit surface waters drives the biological flux of atmospheric CO_2 into the ocean. However, the

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transfer of organic carbon to depth and the subsequent long-term removal of CO_2 from the atmosphere are strongly attenuated by heterotrophic recycling processes that regenerate inorganic nutrients and CO_2 (Volk and Hoffert 1985; Buesseler et al. 2007; Honjo et al. 2008).

In the Arctic Ocean, warming and acidification are accompanied by ecosystem-specific secondary effects that may alter organic matter resources in seawater. In particular, the input of organic matter by enhanced phytoplankton production during the extended ice-free season will likely increase the availability of reactive organic compounds (Arrigo et al. 2008). Most recently, it has also been shown that dissolved organic matter released from melting sea ice can stimulate bacterial activity when added to surface waters (Niemi et al. 2014). Studies investigating whether elevated loads of terrestrial carbon discharged by rivers contribute additional bioavailable organic matter to the Arctic Ocean do not show consistent results (Meon and Amon 2004; Holmes et al. 2008). Recent studies revealed a multifactorial control of bacterioplankton activity in the Arctic Ocean and the adjacent Greenland Sea that includes, besides low temperature, also the availability of labile dissolved organic matter as a major constraint (Kirchman et al. 2009; Sala et al. 2010; Ortega-Retuerta et al. 2014; Piontek et al. 2014). Hence, the relaxation of bacterial substrate limitation combined with elevated seawater temperature and lowered pH suggests multiple and potentially interactive effects of global change on bacterial activity in the Arctic Ocean.

In Fram Strait, the main gateway between the North Atlantic and the central Arctic Ocean, net heterotrophic communities have been frequently observed during summer, indicating that a further increase in heterotrophic activity induced by ongoing environmental changes can significantly weaken the biological capacity of the Arctic Ocean to act as a CO_2 sink (Arrigo et al. 2010; Regaudie-de-Gioux and Duarte 2010; MacGilchrist et al. 2014). Time-series observations in Fram Strait reveal decreasing numbers of diatoms in sedimented organic matter, concurrent with decreasing amounts of biogenic silicate and less fecal pellets during a warm anomaly from late 2004 to 2008 (Bauerfeind et al. 2009; Lalande et al. 2012). These changes likely reflect deteriorated feeding conditions for copepods due to reduced diatom abundances. In the same time period, increasing abundances of picophytoplankton and bacterioplankton indicate enhanced organic matter recycling in the microbial loop in the Canada Basin (Li et al. 2009).

This study experimentally tested individual and combined effects of warming, acidification and the enrichment of labile carbon on the activity of natural bacterioplankton in Fram Strait. Natural microbial plankton communities were collected at 78.8°N–79.7°N during early summer, 2011. The experiments aim to complement results of field surveys and time series studies with an improved mechanistic understanding of environmental constraints on heterotrophic bacterial metabolism in the West Spitsbergen Current and the East Greenland Current that are potentially altered by global change. More specifically, the following hypotheses were tested:

i. Changes in seawater temperature and pH, as projected for the next decades, will not act as stressor on Arctic bacterioplankton but enhance heterotrophic metabolic activity.
ii. Due to the multifactorial environmental control of bacterial activity, simultaneous changes in temperature, pH and labile organic carbon will have interactive effects on bacterial activity.
iii. Environmental changes that enhance bacterial activity induce effects on the concentration and composition of labile and semi-labile organic matter in seawater.

Methods

Site description

Experimental studies were accomplished during the RV *Polarstern* expedition ARK 26/1+2 with surface seawater samples collected in Fram Strait at 78.8°N–79.4°N between 25 June 2011 and 23 July 2011 (Fig. 1). In the eastern Fram Strait, Atlantic Water of the northward-heading West Spitsbergen Current is located, characterized by salinities of 34.1 to 35.2 and temperatures between 3.7°C and 6.7°C. In the western section of Fram Strait, Polar Water of lower temperature (−1.5°C to −1.0°C) and salinity (30.9-31.4) is exported to the Atlantic Ocean on the eastern Greenland shelf (Schlichtholz and Houssais 2002).

Experimental designs

Two types of on-board manipulation experiments, acidification assays and temperature-pH incubation studies, were conducted to investigate the response of Arctic bacterioplankton to changes in temperature, pH and the availability of organic carbon. Samples for acidification assays were collected in Atlantic Water at stations KH (79°3′N, 7°0′E), N4 (79°43′N, 4°27′E) and HG9 (79°8′N, 2°45′E). Temperature-pH incubation studies were conducted with two samples of Atlantic Water (AW-1: 78°50′N, 6°00′E; 25 June 2011; AW-2: 78°45′N, 6°00′E; 09 July 2011) and one sample of Polar Water (PW: 78°45′N, 6°00′E; 03 July 2011) (Fig. 1).

Acidification assays

Three acidification assays carried out within 24 h after sampling tested the immediate impact of changing seawater pH on the reaction velocity of natural extracellular enzyme assemblages. For this purpose, seawater collected at stations KH, N4 and HG9 in 3-5 m depth was manipulated in samples of 200 mL by the addition of 0.1-6.0 mL of 0.1 M hydrochloric acid and 0.1-1 mL 0.1 M sodium hydroxide. After equilibration for 1 h, pH was measured at in situ temperature with a combined pH-temperature electrode (WTW, Sentix 41; calibrated with WTW standard DIN/NBS buffers

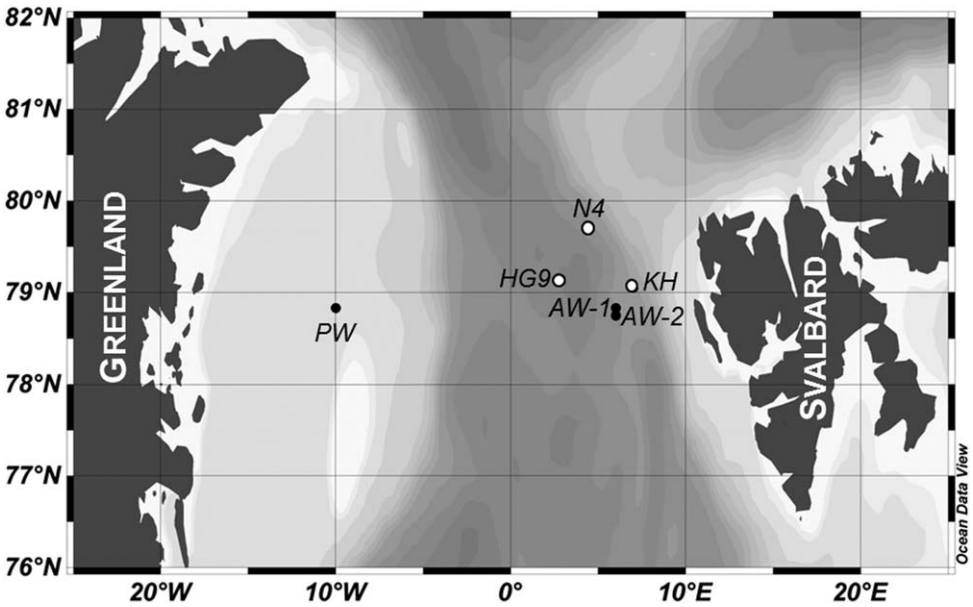

Fig. 1. Sampling stations in Fram Strait. *Closed symbols* show locations chosen for T-pH incubation studies, *open symbols* represent sites of acidification assays.

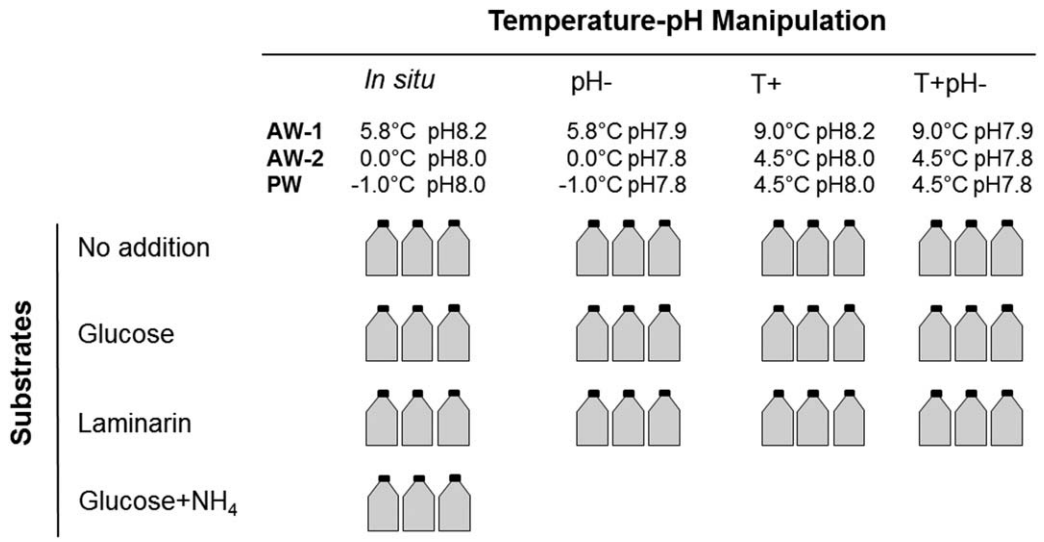

Fig. 2. Setup of the temperature-pH incubation studies AW-1, AW-2 and PW. Samples were incubated at in situ conditions, at lowered seawater pH (pH-), at elevated temperature (T+), and at elevated temperature in combination with lowered pH (T+pH-). Besides incubations without organic matter addition, a series of 12 bottles was supplied with glucose and laminarin, respectively. At in situ T-pH conditions three additional bottles were supplied with glucose and ammonium (NH_4).

PL 4, PL 7 and PL 9). Resulting pH gradients encompassed 10 levels between pH 5.1 and 8.8. Activities of extracellular leucine-aminopeptidase and beta-glucosidase were determined at each pH level.

Temperature-pH (T-pH) incubation studies

Three T-pH incubation studies with a duration of 4 d each, referred to as AW-1, AW-2 and PW, explored the response of bacterioplankton growth and degradation activity to rising temperature and lowered pH. Manipulation of temperature and seawater pH in these studies simulated changes projected for the Arctic Ocean in the 21st century (Fig. 2) (ACIA 2004; Trenberth et al. 2007; Steinacher et al. 2009). The experimental design tested effects of temperature and pH settings at natural substrate availability and in combination with the amendment of glucose and laminarin, two

Table 1. Values of physicochemical parameters, organic matter concentrations and bacterial activity in temperature-pH incubation studies. In PW no initial extracellular enzyme activities could be detected. In AW-1 and AW-2 beta-glucosidase and cellobiase activity were below the detection limit (*nd*: not detectable).

	AW-1	AW-2	PW
	78°50′N, 6°00′E	78°45′N, 6°00′E	78°50′N, 10°00′W
Salinity	35.0	33.5	30.7
Temperature (°C)	5.8	0.0	−1.0
pH	8.2	8.0	8.0
Total alkalinity (μmol kg^{-1})	2321	2166	2230
Total amino acids (μmol monomers L^{-1})	0.78 (±0.05)	0.58 (±0.03)	0.37 (±0.04)
Total combined carbohydrates (μmol monomers L^{-1})	1.86 (±0.91)	0.91 (±0.08)	1.07 (±0.10)
Bacterial abundance (cells mL^{-1})	9.9E+05 (±2.4E+04)	1.3E+05 (±6.9E+03)	5.3E+04 (±4.4E+03)
Bacterial production (μg C L^{-1} h^{-1})	0.03 (±0.001)	0.04 (±0.005)	0.01 (±0.0001)
Leu-aminopeptidase (V_{max}, nmol L^{-1} h^{-1})	13.72 (±2.50)	11.13 (±0.37)	*nd*
Beta-glucosidase (V_{max}, nmol L^{-1} h^{-1})	0.76 (±0.24)	0.35 (±0.05)	*nd*
Cellobiase (V_{max}, nmol L^{-1} h^{-1})	*nd*	*nd*	*nd*

carbohydrates that represent utilizable carbon sources for bacterioplankton.

The experimental setup is depicted in Fig. 2. For each T-pH incubation study a batch of 10 L seawater collected at 3-5 m depth was pre-screened with a 100 μm mesh to exclude larger zooplankton. The 10 L-batch was divided into 39 subsamples of 250 mL each that were transferred into acid-cleaned autoclaved glass bottles. Eighteen samples were acidified by the addition of 0.1 M hydrochloric acid, thereby lowering seawater pH by 0.2-0.3 units (Table 1; Fig. 2). Seawater pH remained constant or decreased slightly by < 0.1 units during 4 d of incubation in the dark. For each experiment two temperature treatments were chosen. Twelve samples with in situ pH and lowered pH, respectively, were kept at in situ temperature, while the other bottles were incubated at a temperature elevated by 3.2-4.5°C (Fig. 2). The different temperature-pH settings are referred to as in situ (in situ T and pH), T+ (elevated temperature at in situ pH), pH- (lowered pH at in situ temperature) and T+pH- (elevated temperature in combination with lowered pH). At each T-pH setting, six samples were amended with carbohydrates, while the remaining samples were incubated without carbohydrate addition. Amended incubations received 5.3 μmol L^{-1} D-(+)-glucose (Sigma, ACS reagent grade, $\geq$99% purity) and 5.3 μmol (glucose equivalents) L^{-1} laminarin, respectively. Laminarin extracted from *Laminaria digitata* (Sigma) has a mean degree of polymerization of 25 glycosyl residues (Read et al. 1996). The molecule is primarily linear with poly(β-Glc-[1→3]) linkages and some β-(1→6) interstrand linkages and branch points.

To test the effect of inorganic nitrogen on bacterial production an additional series of three samples was amended with glucose plus ammonium chloride to a final concentration of 7.5 μmol L^{-1} and incubated at in situ T-pH conditions.

Initial samples for bacterial cell numbers, bacterial production, extracellular enzyme activity and concentrations of amino acids and carbohydrates were taken. Sampling for all parameters was repeated after 4 d. Samples for bacterial cell numbers were taken daily.

Bacterial cell numbers

Bacterial abundance was determined by flow cytometry (FACSCalibur, Becton Dickinson) after staining with the DNA-binding dye SybrGreen I (Invitrogen). Samples were fixed on board with glutardialdehyde at 2% final concentration and stored at −20°C until analysis within 4 months. Bacterial cell numbers were estimated after visual inspection and manual gating of the bacterial population in the cytogram of side scatter vs. green fluorescence. Fluorescent latex beads (Polyscience, Becton Dickinson) were used to normalize the counted events to volume (Gasol and del Giorgio 2000).

Bacterial production and growth rates

Bacterial production was estimated from the incorporation of radioactively labeled leucine at saturating final concentration of 20 nmol L^{-1}. Three replicate samples were incubated for 2-4 h in the dark at in situ temperature and the incubation temperature chosen for the high-temperature treatments, respectively. Incubations were stopped by the addition of trichloroacetic acid (TCA) at a final concentration of 5%. After that, samples were processed by the centrifugation method according to Smith and Azam (1992). Briefly, samples were centrifuged at 14,000 × *g* to gain cell pellets that were washed twice with 5% TCA. Incorporation

into the TCA-insoluble fraction was measured by liquid scintillation counting after resuspension of the cell pellets in scintillation cocktail (Ultima Gold AB, Perkin Elmer). Leucine incorporation was converted into bacterial carbon production applying a factor of 1.5 kg C mol leucine^{-1}, assuming no intracellular isotope dilution (Simon and Azam 1989).

The apparent growth rate (μ_a, d^{-1}) was estimated from initial and final cell numbers (c_{t0}, c_{t4}) after 4 d of incubation by

$$\mu_a = (\ln c_{t4} - \ln c_{t0})/4 \quad (1)$$

Extracellular enzyme activities

Activity of extracellular leucine (leu)-aminopeptidase, β-glucosidase and cellobiase was assessed from the hydrolysis of L-leucyl-4-methylcoumarinylamid-hydrochlorid, 4-methylumbelliferyl-β-glucopyranoside and 4-methylumbelliferyl-β-D-cellobioside (Sigma), respectively, according to Hoppe (1983). The substrate analogues were added to whole seawater samples at final concentrations of 1 μmol L^{-1}, 5 μmol L^{-1}, 10 μmol L^{-1}, 20 μmol L^{-1}, 50 μmol L^{-1}, 80 μmol L^{-1}, 100 μmol L^{-1}, and 200 μmol L^{-1} to achieve concentration 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}. Enzymatic rates were calculated from the increase in MUF and AMC concentration 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 in situ temperature and the incubation temperature chosen for the high-temperature treatments, respectively (Table 1). The range of pH manipulation in our experiments did not affect the fluorescence of AMC. The fluorescence intensity of MUF decreased with decreasing seawater pH. Therefore, rates of beta-glucosidase and cellobiase were calculated with calibration factors determined at the specific pH values of acidification assays and T-pH incubation studies. Rates measured at the eight different substrate concentrations were fitted to a kinetic model using the Michaelis–Menten equation to determine the maximum velocity of the enzymatic reactions (V_{max}). V_{max} does not equal enzymatic rates at substrate concentrations in seawater. Instead, it reflects the maximum rate that can be achieved by the natural enzyme assemblage at saturating substrate concentration.

Amino acids

Total amino acids (TAA) were analyzed by HPLC according to Lindroth and Mopper (1979). Samples of unfiltered seawater were stored in precombusted glass vials at −20°C. Prior to analysis, 1 mL of 30% hydrochloric acid was added to 1 mL sample for hydrolysis at 100°C for 20 h. Analysis of samples was carried out after neutralization and derivatisation with *o*-phthaldialdehyde and mercaptoethanol. The HPLC system (Agilent 1260) was equipped with a C18 column (Phenomenex Kinetex, 150 × 4.6 mm).

Carbohydrates

Total combined carbohydrates of > 1 kDa molecular weight (TCHO) were determined by ion chromatography on a Dionex ICS 3000 system using high performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) (Engel and Händel 2011). Samples of 20 mL unfiltered seawater were stored frozen at −20°C in precombusted glass vials. Prior to analysis desalination was conducted by the use of dialysis membranes with a molecular weight cut-off of 1 kDa (Spectra/Por®). Desalinated samples were hydrolyzed for 20 h at 100°C using hydrochloric acid at a final concentration of 0.8 N. After neutralization through acid evaporation the chromatographic separation of the monomeric carbohydrates was carried out with a CarboPac PA10 analytical column (2 × 250 mm) coupled with a Dionex CarboPac PA10 guard column (2 × 50 mm). The detection limit for this method was 10 nmol monomer L^{-1} with a standard deviation between replicate analysis of < 2%.

Calculations and statistical analysis

Potential growth limitation by resource availability

Growth limitations by utilizable organic carbon were tested by means of *t*-test. Bacterial production determined at in situ T-pH conditions in non-amended incubations were compared with rates in incubations amended with glucose and laminarin. Likewise, potential colimitation by nitrogen was tested by comparing rates in glucose-supplied incubations with those in incubations amended with glucose plus ammonium chloride. Tests were carried out separately for experiments AW-1, AW-2 and PW. Differences were accepted as significant for $p \leq 0.05$.

Effects of temperature and pH on bacterial activity

Two-way analysis of variance (ANOVA) was applied to examine differences of growth rates, bacterial production and leucine-aminopeptidase activity in T-pH incubation studies. Significance was accepted for $p \leq 0.05$. Two-way ANOVA was separately performed for AW-1, AW-2 and PW. Data for extracellular beta-glucosidase and cellobiase activity could not be analyzed by this approach because rates in most incubations were below the detection limit.

Effect size

The ln-transformed response ratio (RR) was calculated according to Hedges et al. (1999) to analyze the effect size of temperature and pH changes and potentially combined effects with carbon supply on bacterial production and leu-

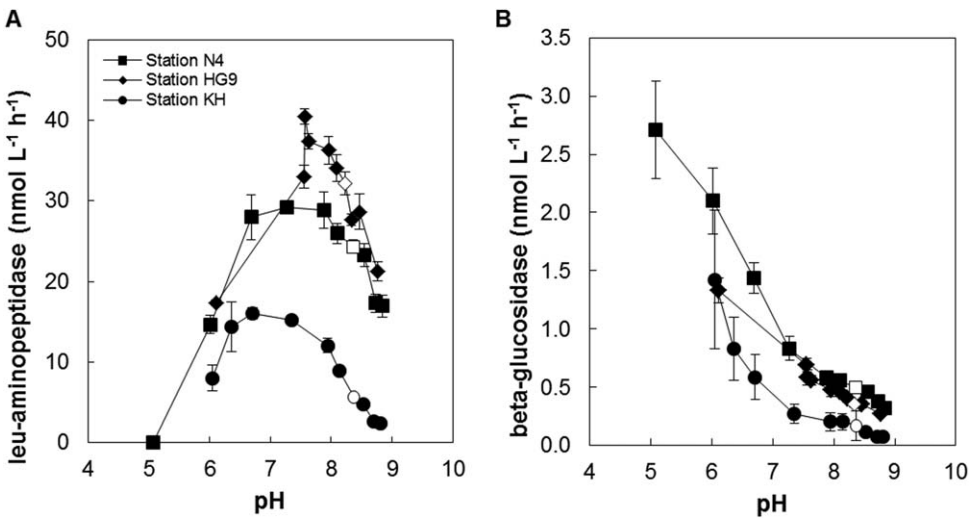

Fig. 3. Response of (A) leucine-aminopeptidase and (B) beta-glucosidase activity to changing pH at stations KH, N4 and HG9. *Open symbols* show rates at non-manipulated seawater pH.

aminopeptidase activity. RR was calculated from rates determined after 4 d of incubation by

$$\ln RR = \ln X_E - \ln X_C \quad (2)$$

where X_E and X_C are the rates in experimental and control treatment, respectively. A positive lnRR indicates a positive effect on the response variable and a negative value indicates suppression under manipulated T-pH conditions.

Activation energy

Activation energy (E_a) for bacterial biomass production was derived from the Arrhenius equation by

$$\ln\ (k_2/k_1)\ = E_a/R \times\ (1/T_1 - 1/T_2) \quad (3)$$

where k_1 and k_2 are rates at in situ and elevated temperature, respectively, R is the gas constant and T_1 and T_2 are in situ temperature and elevated experimental temperature, respectively, converted to Kelvin.

Results

Acidification assays

The response of natural enzyme assemblages to changing seawater pH was tested by short-term acidification assays. Rate measurements were completed within 24 h to minimize effects of bacterial growth and potential metabolic adjustments in response to in vitro changes in organic substrates and nutrients. Although secondary effects cannot be entirely excluded in natural communities, it can be assumed that the short-term response of enzymatic reaction velocities was primarily induced by biochemical effects of manipulated seawater pH. Assays were conducted with surface seawater collected at three different stations in the Atlantic sector of Fram Strait that showed a narrow in situ pH range of 8.2-8.4. Highest maximum velocities (V_{max}) of leu-aminopeptidase and beta-glucosidase were determined at pH values well below in situ conditions (Fig. 3). Leucine-aminopeptidase activity showed optimum curves in all assays. The pH optimum was reached at pH 6.7-7.6. Leucine-aminopeptidase activity at stations N4 and KH shows broad pH optima, suggesting minor changes of rates between pH 6.7 and 7.8. In contrast, a narrow pH optimum at 6.7 was determined for leucine-aminopeptidase at station HG9. Further acidification as well as basification reduced V_{max} of leucine-aminopeptidase. Beta-glucosidase showed highest V_{max} at pH values even lower than the optima for leucine-aminopeptidase. Highest V_{max} of beta-glucosidase were determined at pH 6.1 at stations KH and HG9 and at pH 5.0 at station N4. The optimum pH of beta-glucosidase activity could not be determined because V_{max} did not culminate at lowest pH values tested (Fig. 3). According to the pH-enzyme activity profiles, the pH decrease by 0.45 units expected throughout this century would increase enzymatic rates on average by 6-19%. An exceptionally high pH sensitivity was determined for leu-aminopeptidase activity at the shelf station KH, where the enzymatic rate increased by 56% when seawater pH was lowered from in situ pH of 8.34-8.14.

T-pH incubation studies

Experiments with incubation times of 4 d were conducted to investigate the response of bacterial growth, biomass production and resulting changes in enzymatic activity to manipulation of temperature and pH. Furthermore, effects of organic carbon and inorganic nitrogen supply were tested (Fig. 2). Environmental parameters at sampling sites of AW-1, AW-2 and PW revealed substantial differences as did parameters of bacterial activity in initial samples (Table 1). Highest initial bacterial abundance and extracellular enzyme

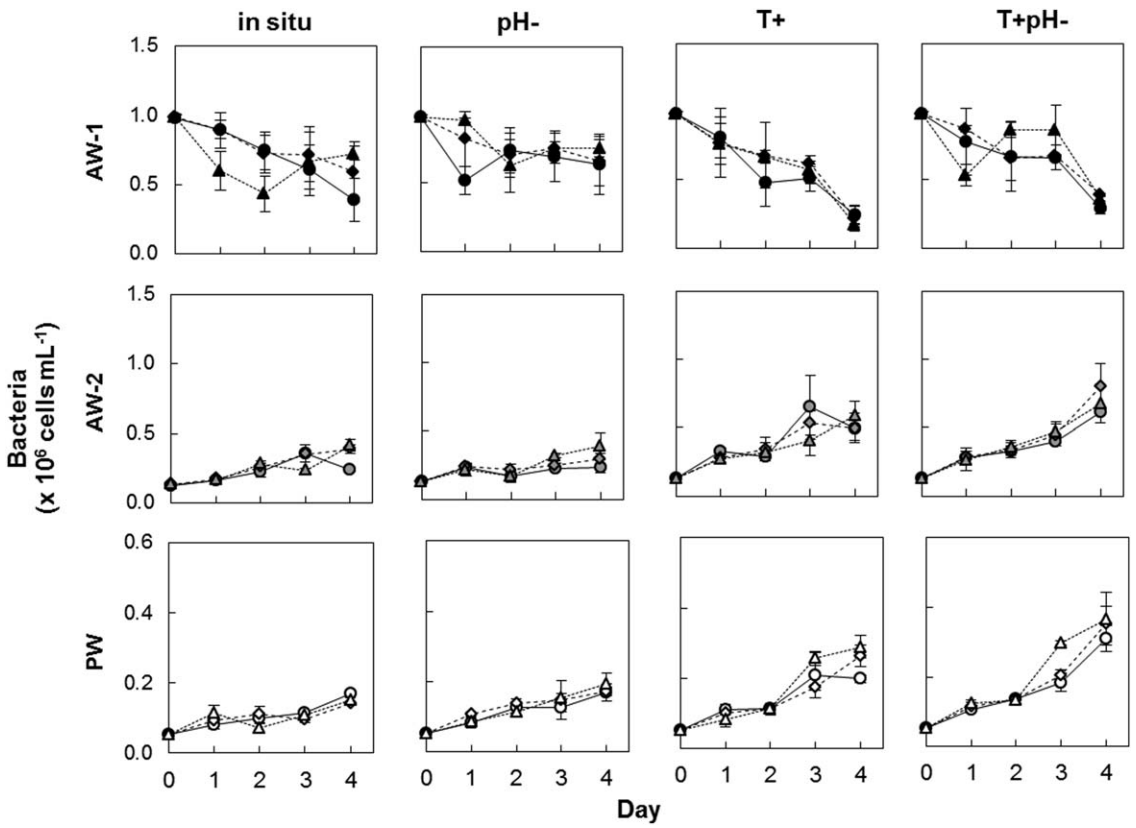

Fig. 4. Bacterial abundances in T-pH manipulation studies AW-1, AW-2 and PW (*circles*: no amendment, *diamonds*: glucose addition, *triangles*: laminarin addition).

activity were determined in Atlantic Water at station AW-1, coinciding with highest temperature, pH and organic matter concentrations. Polar Water collected for experiment PW showed lowest initial bacterial abundance and activity at lowest temperature and amino acid concentrations (Table 1). Bacterial growth and degradation activity in Fram Strait during early summer 2011 were related to compositional differences of organic matter. Bacterial production and leucine-aminopeptidase in Atlantic and Polar Water were significantly correlated with concentrations of amino acids. Q_{10} factors of bacterial production and rates of extracellular enzymes in Atlantic and Polar Water showed high variability (Piontek et al. 2014).

Effects of temperature and pH on bacterial growth and activity

T-pH incubation studies showed different bacterial growth characteristics. Bacterial cell numbers decreased in AW-1 during 4 d of incubation, although bacterial production increased (Figs. 4, 5; Table 1). Hence, high mortality likely induced by grazing and/or viral lysis controlled the standing stock of bacteria in this experiment and, therefore, largely the metabolic activity on the community level. Net growth of bacteria was observed in experiments AW-2 and PW. Depending on T-pH settings, average apparent growth rates of 0.30 d^{-1} and 0.39 d^{-1} were reached in AW-2 and PW, respectively (Fig. 4; Table 2). Elevated temperature increased apparent growth rates in AW-2 and PW, while it decreased apparent growth rates at high mortality in AW-1 (Tables 2, 3). Bacterial biomass production increased in response to elevated temperature throughout all experiments (Fig. 5). Lowered seawater pH significantly increased apparent growth rates and bacterial production in experiment AW-1, while no significant effect could be determined in AW-2 and PW (Tables 2, 3; Fig. 5).

Elevated temperature enhanced the activity of leu-aminopeptidase in experiments AW-1 and AW-2 that were conducted with samples of Atlantic origin. Enhanced rates at elevated temperature integrated effects on both the enzymatic reaction velocity and the new production of extracellular enzymes during 4 d of incubation. The strongest increase in leu-aminopeptidase activity was determined at elevated temperature combined with supply of laminarin in AW-2. In contrast to AW-1 and AW-2, no significant temperature effect on leu-aminopeptidase activity could be determined in experiment PW (Fig. 6). Incubation studies did not consistently show effects of lowered seawater pH on the activity of hydrolytic extracellular enzymes. Only in

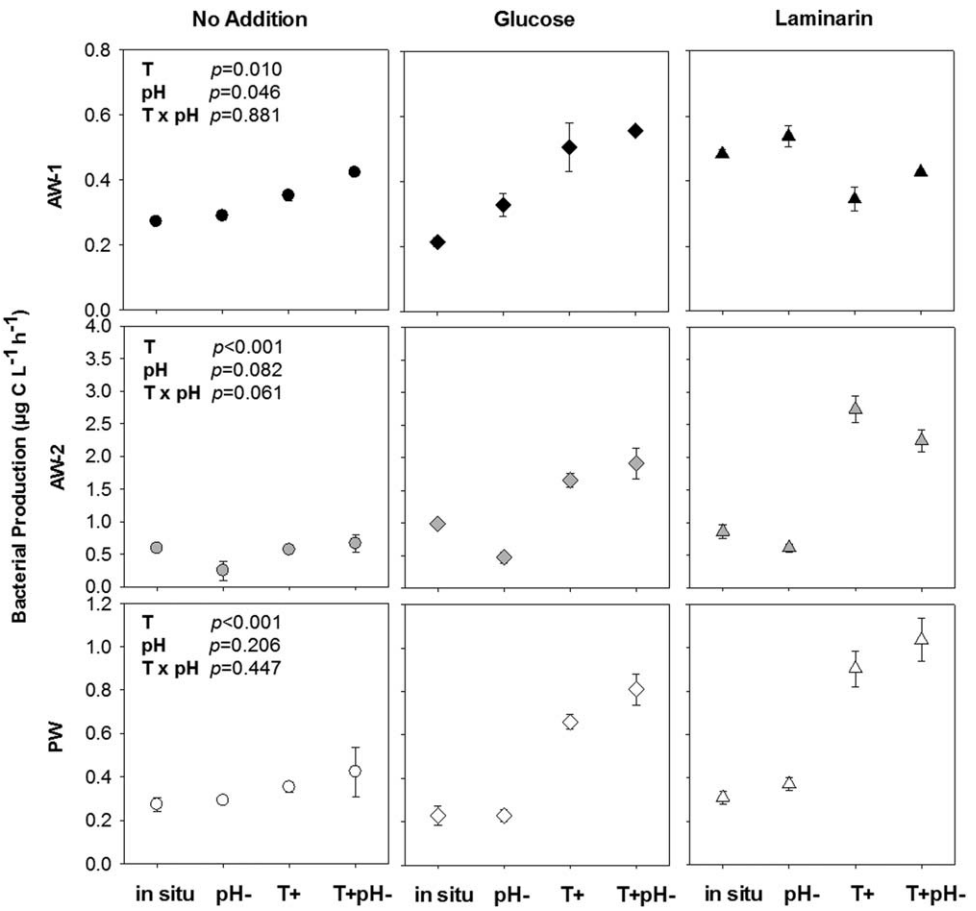

Fig. 5. Bacterial production (µg C L^{-1} h^{-1}) in temperature-pH manipulation studies after 4 d of incubation. Mean values of three replicate incubations (± SD) and results of two-way ANOVA (T × pH, $n = 36$) are shown.

Table 2. Apparent bacterial growth rates (Eq. 1) in T-pH incubation studies.

		Apparent growth rate (d^{-1})		
		AW-1	AW-2	PW
No addition	In situ	−0.24 (±0.09)	0.17 (±0.02)	0.33 (±0.02)
	pH-	−0.12 (±0.10)	0.14 (±0.04)	0.32 (±0.04)
	T+	−0.35 (±0.08)	0.33 (±0.05)	0.37 (±0.01)
	T+pH-	−0.30 (±0.03)	0.39 (±0.002)	0.49 (±0.04)
Glucose	In situ	−0.14 (±0.10)	0.28 (±0.02)	0.33 (±0.02)
	pH-	−0.10 (±0.07)	0.21 (±0.03)	0.32 (±0.04)
	T+	−0.38 (±0.10)	0.33 (±0.05)	0.37 (±0.01)
	T+pH-	−0.22 (±0.01)	0.45 (±0.51)	0.49 (±0.04)
Laminarin	In situ	−0.07 (±0.02)	0.29 (±0.03)	0.29 (±0.01)
	pH-	−0.06 (±0.02)	0.27 (±0.07)	0.35 (±0.05)
	T+	−0.43 (±0.02)	0.38 (±0.04)	0.47 (±0.03)
	T+pH-	−0.24 (±0.01)	0.41 (±0.05)	0.53 (±0.06)

Table 3. Effects of T-pH manipulation on apparent bacterial growth rates (two-way ANOVA T × pH).

	AW-1	AW-2	PW
Factor			
T	<0.001	<0.002	<0.001
pH	0.011	0.955	<0.001
T × pH	0.93	0.024	0.221

experiment AW-1, lowered pH resulted in a significant increase of leucine-aminopeptidase activity. Compiling measurements of all experiments and all incubations, a significant linear relationship of bacterial biomass production and leu-aminopeptidase activity could be determined (Fig. 7). The linear regression indicates an increase in enzyme synthesis proportional to the increase in bacterial biomass production.

Rates of extracellular beta-glucosidase and cellobiase, two carbohydrate-degrading enzymes, were below the detection limit in many samples (Tables 1, 4). Therefore, a statistical evaluation of potential temperature and pH effects similar to the analysis of bacterial growth rates, bacterial production and leucine-aminopeptidase activity was not possible. Elevated temperature roughly doubled rates in non-amended

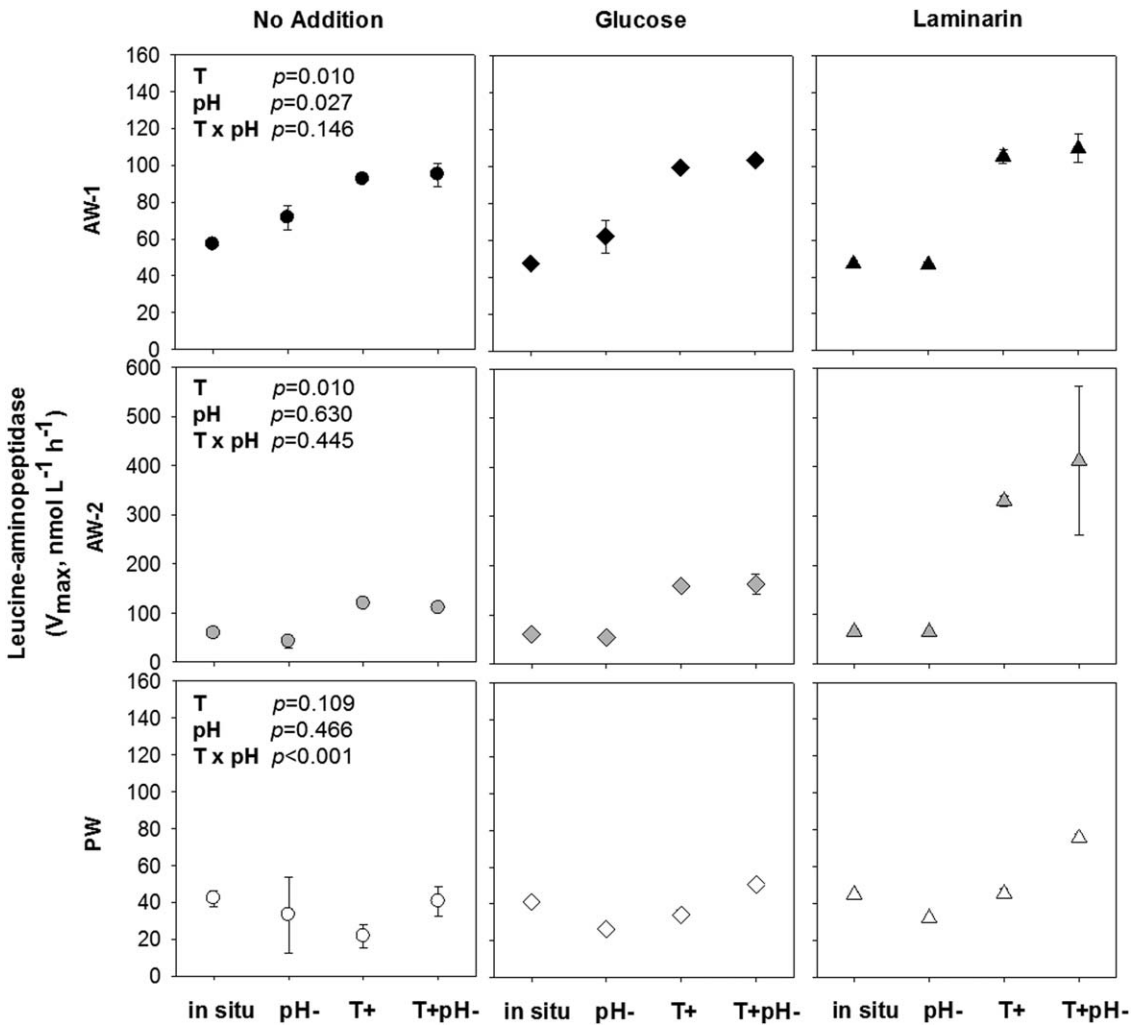

Fig. 6. Maximum velocity (V_{max}) of leucine-aminopeptidase (nmol L^{-1} h^{-1}) in temperature-pH manipulation studies after 4 d of incubation. Mean values of three replicate incubations (± SD) and results of two-way ANOVA (T × pH, $n = 36$) are shown.

incubations of AW-1, while rates at elevated temperature in laminarin-amended incubations were about 30 times higher. It is noteworthy that in all experiments highest beta-glucosidase rates were determined in laminarin-amended incubations at elevated temperature (Table 4).

Response of bacterial activity to carbohydrate supply

To identify growth limiting resources, bacterial production in non-amended samples was compared with rates in incubations amended with glucose, laminarin and ammonium (Table 5). Bacteria could take advantage of added laminarin at in situ T-pH conditions in all experiments, suggesting growth limitation by insufficient utilizable organic carbon resources. Interestingly, glucose that is considered as more labile than laminarin due to its lower molecular weight enhanced bacterial production only in experiment AW-2. In combination with ammonium, glucose had also a beneficial effect on bacterial production in experiment AW-1, pointing to a nitrogen co-limitation of its utilization in this experiment (Table 5). Our results indicate a stimulating effect of laminarin addition on the expression of carbohydrate-cleaving enzymes. Nine of the twelve laminarin-amended treatments showed beta-glucosidase and/or cellobiase activity after 4 d of incubation time, while beta-glucosidase and/or cellobiase activity was detectable only in two out of 12 glucose-amended incubations (Table 4). Hence, the synthesis of new enzymes was likely induced in the presence of a polymeric substrate.

The supply of carbohydrates strongly modulated the size of temperature effects. The addition of glucose and laminarin at least doubled temperature effects on bacterial production in most T-pH incubation studies (Fig. 8), revealing a higher temperature sensitivity of bacterial growth at high substrate availability. Mean effect sizes determined for bacterial production correspond to changes in activation energy from 45-52 kJ mol^{-1} at in situ substrate concentrations to

122-174 kJ mol^{-1} in the presence of high carbohydrate concentrations (Eq. 3). In experiments AW-1 and AW-2, the mean effect size of elevated temperature on leu-aminopeptidase activity was similar to that on bacterial production. In contrast, glucose addition did not increase the temperature effect size of leu-aminopeptidase activity in PW and laminarin addition resulted only in a slight amplification of temperature effects (Fig. 8). Effect sizes reflect synergistic effects of temperature and carbohydrate on rates in experiments AW-1 and AW-2 as both bacterial production and leu-aminopeptidase increased beyond the additive outcome of individual effects.

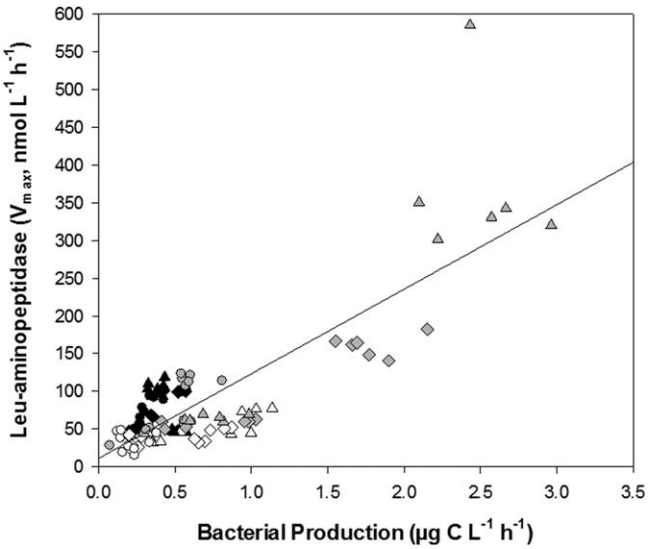

Fig. 7. Leucine-aminopeptidase activity as a function of bacterial production in temperature-pH manipulation studies ($f(x) = 112.2x + 11.0$, $r^2 = 0.67$, $p < 0.0001$) (*black symbols*: AW-1, *grey symbols*: AW-2, *white symbols*: PW, *circles*: incubations without amendment, *diamonds*: incubations with glucose, *triangles*: incubations with laminarin).

Effects of lowered seawater pH on bacterial production and leu-aminopeptidase in experiment AW-1 were strengthened by the addition of glucose and laminarin (Fig. 8). However, the interaction of pH manipulation and carbohydrate addition was not of synergistic nature.

Degradation of proteins and polysaccharides

The composition of TAA in AW-1, AW-2 and PW was dominated by glycine (19.5-30.1 mol%), glutamic acid (14.4-22.0 mol%), aspartic acid (12.6-16.3 mol%), alanine (8.7-11.1 mol%) and serine (6.8-11.1 mol%) while arginine, threonine, tyrosine, valinine, leucine, iso-leucine, penylalanine and GABA contributed less than 8% each. The composition of TCHO was dominated by glucose that contributed 62 mol%, 76 mol%, and 74 mol% to TCHO in AW-1, AW-2, and PW, respectively.

Non-amended incubations showed a net loss of TAA in all experiments. A net loss of TCHO was determined in experiments AW-1 and PW (Fig. 9). Changes in TAA concentration

Table 5. Effect of carbon and nitrogen supply on bacterial production under in situ temperature-pH conditions. Differences between incubations were tested for significance by *t*-tests.

	AW-1	AW-2	PW
Carbon supply Glucose vs. no amendment	$p = 0.10$	$p < 0.01$	$p = 0.08$
Laminarin vs. no amendment	$p < 0.01$	$p = 0.01$	$p < 0.01$
Nitrogen supply Glucose + NH_4 vs. glucose	$p < 0.01$	$p = 0.13$	$p = 0.54$

Table 4. Activity of extracellular beta-glucosidase and cellobiase after 4 d of incubation under different temperature-pH settings (dash: activity below detection limit).

		Beta-glucosidase			Cellobiase		
		V_{max}, nmol L^{-1} h^{-1}			V_{max}, nmol L^{-1} h^{-1}		
		AW-1	AW-2	PW	AW-1	AW-2	PW
No addition	In situ	0.63 (±0.01)	-	-	-	-	-
	pH-	0.74 (±0.07)	-	-	-	-	-
	T+	1.34 (±0.09)	-	-	-	-	-
	T+pH-	1.67 (±0.46)	-	-	-	-	-
Glucose	In situ	-	-	-	-	-	-
	pH-	0.77 (±0.42)	-	-	0.56 (±0.11)	-	-
	T+	-	1.81 (±0.09)	-	-	-	-
	T+pH-	-	-	-	-	-	-
Laminarin	In situ	0.35 (±0.11)	-	1.34 (±0.14)	-	-	-
	pH-	0.70 (±0.16)	-	-	-	-	-
	T+	10.35 (±0.30)	20.57 (±1.23)	3.27 (±0.42)	4.13 (±0.51)	-	2.29 (±0.24)
	T+pH-	19.33 (±1.20)	17.83 (±1.72)	4.73 (±0.30)	10.83 (±1.17)	-	2.73 (±0.10)

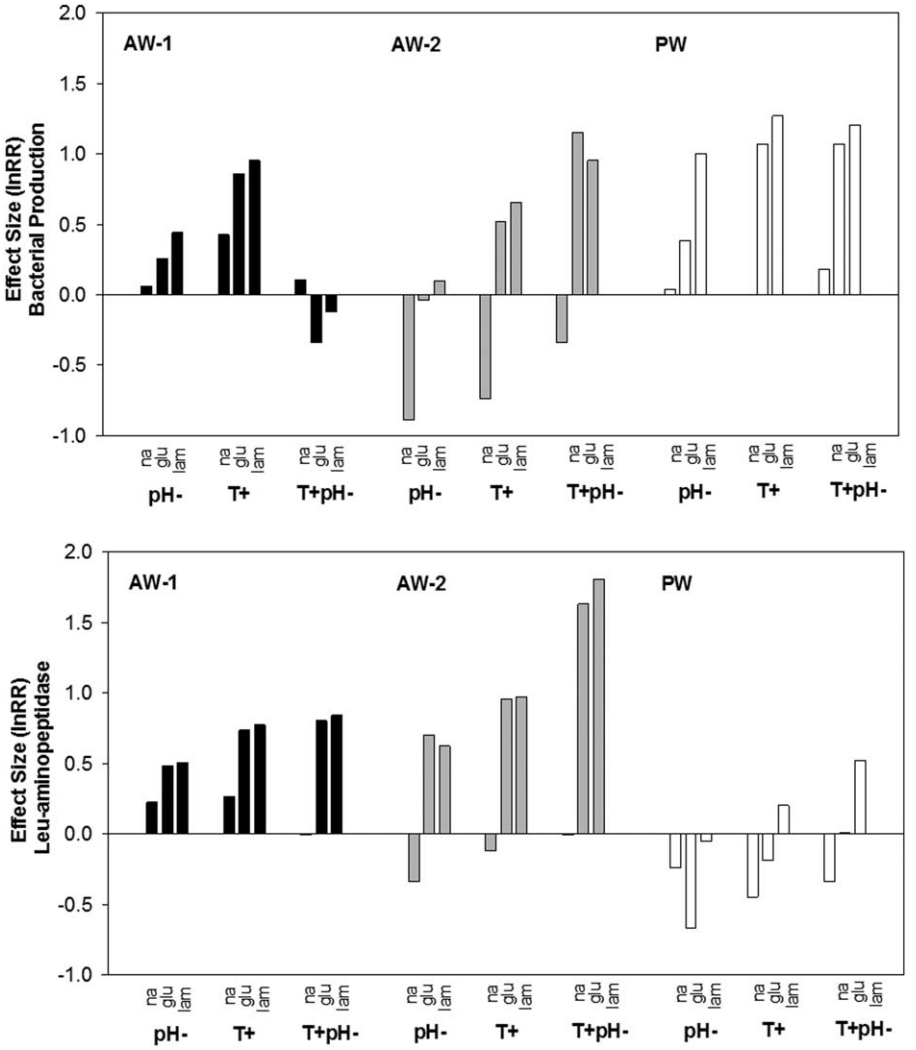

Fig. 8. Effects of temperature and pH changes on bacterial production and leucine (leu)-aminopeptidase activity. Bars represent the mean effect size (lnRR, Eq. 2) (*na*: no amendment, *glu*: glucose addition, *lam*: laminarin addition).

were the net result of remineralization, transformations and the new synthesis of amino acids. Due to methodological constraints changes in TCHO concentrations resulted from remineralization and transformations plus the release of sugars < 1 kDa from polymers by extracellular enzymatic hydrolysis. Temperature had significant effects on the degradation of organic matter in non-amended samples of AW-2, the experiment with highest bacterial activity. Here, elevated temperature enhanced the loss of TAA, while it reduced the loss of TCHO (two-way ANOVA T × pH; $p_{\text{AW-2,TAA}} = 0.031$, $p_{\text{AW-2,TCHO}} = 0.026$) (Fig. 9). The addition of glucose led to temperature effects on amino acid concentrations in all experiments. At elevated temperature the loss of amino acids was significantly reduced in the presence of free glucose that is considered as labile carbon source for bacterial growth (two-way ANOVA T × pH; $p_{\text{AW-1}} = 0.024$, $p_{\text{AW-2}} = 0.008$, $p_{\text{PW}} = 0.021$). In experiment AW-2, glucose addition even led to a net increase in TAA (Fig. 9). Concomitantly with the net increase in TAA, glucose-amended incubations of AW-2 showed 5-6% higher shares of glutamic acid than the initial field sample and the non-amended incubations, strongly suggesting the new synthesis of amino acids from glucose-derived carbon.

Discussion

Manipulation studies are a suitable experimental approach to investigate impacts of environmental changes on biological processes. Conclusions drawn from these studies cannot be directly projected to ecosystem scale but they can provide mechanistic explanations for changes in biological processes under climate change. In particular,

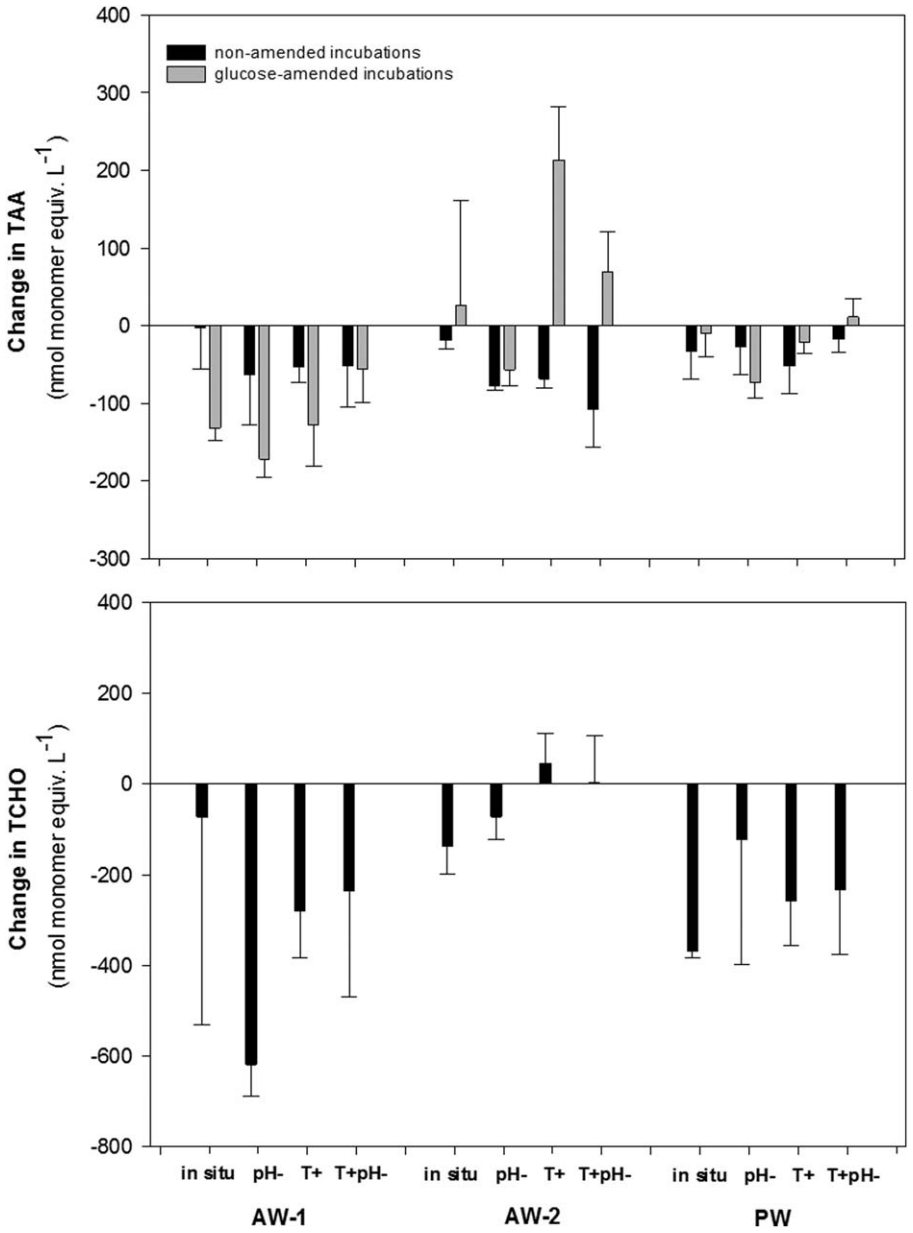

Fig. 9. Concentrations of total amino acids (TAA, upper panel) and total combined carbohydrates (TCHO, lower panel) in temperature-pH manipulation studies. Changes in concentrations after 4 d of incubation are shown.

community-level experimentation has the potential to unravel subtle effects on mixed microbial plankton populations in marine ecosystems that might propagate to impacts on biogeochemical processes and higher trophic levels in food webs.

Effects of pH on extracellular enzymes

A recent review summarized consequences of seawater acidification as projected for the next 100 yr on the morphology and physiology of single marine plankton species as well as on the biological and biogeochemical performance of plankton communities (e.g., Liu et al. 2010 and citations within). We tested the response of hydrolytic extracellular enzymes to seawater acidification. Hydrolytic extracellular enzymes are almost exclusively produced by bacteria and accomplish the initial step in organic matter degradation in seawater. The response of enzymatic activities to gradually changing seawater pH revealed maximum activities of beta-glucosidase and leucine-aminopeptidase well below present-day seawater pH, thereby confirming previous studies showing enhanced enzymatic activities under future-ocean pH conditions (Grossart et al. 2006; Tanaka et al. 2008; Piontek et al. 2010, 2013; Maas et al. 2013; Endres et al. 2014). For the first time, we report full pH-enzyme activity profiles for

Arctic bacterioplankton. Our results reveal pH optima of 6.7-7.6 for leucine-aminopeptidase and below pH 6.0 for beta-glucosidase. In general, optimum curves suggest the abrupt inversion of response processes once a critical tipping point is reached. However, pH simulations for the Arctic Ocean by Steinacher et al. (2009) suggest that optimum pH conditions for the activity of tested enzymatic reactions are not likely to be reached by the year 2100. Hence, near-future changes in ocean pH have the potential to progressively increase the hydrolytic turnover of peptides and polysaccharides in seawater. We determined the maximum velocities of enzymatic reactions. Since V_{max} reflects the potential rate at saturating substrate concentration, changes in V_{max} at changing seawater pH are likely induced by pH effects on the catalytic step of the enzymatic reactions and not by effects on the enzyme's affinity for its substrate. So far, reasons for the evolution of marine extracellular enzymes with acidic pH optima are unknown. One hypothesis could be that acidic pH optima are an adaptation to pH conditions in beneficial microenvironments like the interstitial of particle aggregates. Aggregates are nutrient-rich hotspots of bacterial growth in the ocean (Simon et al. 2002). Due to intense respiratory activity aggregates show steep gradients in oxygen and pH from their periphery to enclosed inner parts (Ploug et al. 1997, 1999). An alternative hypothesis considers the stability and the resulting lifetime of enzymes. The lifetime of extracellular enzymes after release must be long enough to balance the costs for production. From this perspective, a protein configuration ensuring a long lifetime might be of higher value for the microbial producer even though this configuration does not provide optimum reaction velocity at ambient seawater pH.

The biochemical pH effect on hydrolytic extracellular enzymes identified by acidification assays was apparent in only one out of three T-pH manipulation studies. Leu-aminopeptidase activity concomitant with bacterial biomass production increased at lowered seawater pH in experiment AW-1, while no significant pH effect was determined in experiments AW-2 and PW. The reason for this inconsistency is likely related to the variability in enzyme production between replicate incubations during 4 d. Measurements of leu-aminopeptidase activity in replicate incubations after 4 d showed average standard deviations of 4.6%, 10.4%, and 13.0% in AW-1, AW-2, and PW, respectively. Assuming that these standard deviations approximate the variability in new enzyme production, variability in experiments AW-2 and PW would exceed the size of pH effects as inferred from acidification assays. Enzymatic reaction velocities depend on both physicochemical parameters like seawater temperature and pH and on enzyme concentration. Across all T-pH manipulation studies, bacterial production was directly related to V_{max} of leu-aminopeptidase after 4 d of incubation, suggesting that the release of extracellular enzymes was proportional to the production of intracellular proteins (Fig. 7). Therefore, it can be assumed that leu-aminopeptidase activity in the T-pH manipulation studies was largely determined by the rate of new enzyme synthesis. Hence, the effect of changing seawater pH on extracellular enzyme activity was apparent when the existing pool of enzymes in seawater was investigated by short-term acidification assays, but variability in new enzyme production among replicates likely masked biochemical pH effects apparent in T-pH manipulation studies.

Temperature effects on bacterial biomass production and leucine-aminopeptidase activity

A moderate temperature increase by 3.2-5.5°C significantly enhanced bacterial biomass production throughout all experiments as well as apparent growth rates in AW-2 and PW. The addition of glucose and laminarin amplified temperature effects and led to synergistic combined effects. Incubations were amended with glucose and laminarin to simulate increasing availability of carbohydrate-rich reactive organic matter as it will likely originate from increased pelagic primary production at receding sea ice. Carbohydrates are the primary product of photosynthesis and comprise the largest share of chemically identified organic matter in the surface ocean (Aluwihare et al. 1997; Borch and Kirchman 1997; Skoog and Benner 1997). In particular, extracellular release during phytoplankton blooms is a major source of carbohydrates in marine dissolved organic matter (Myklestad 1977, 1995; Ittekkot et al. 1981). Glucose is the most abundant free sugar in seawater and represents usually the largest fraction in combined carbohydrates of marine origin (Skoog and Benner 1997; Kaiser and Benner 2009). Free glucose can be directly taken up and metabolized by bacterial cells, while the phytoplankton storage glucan laminarin, a soluble β-1,3-D-glucose polymer of 20-30 units, is too large for direct uptake and requires extracellular cleavage by hydrolytic enzymes (Keith and Arnosti 2001; Alderkamp et al. 2007). Both monomeric sugars and polysaccharides are major utilizable substrates for Arctic bacterioplankton, although patterns of substrate utilization vary with season (Sala et al. 2008; Fernández-Gómez et al. 2014). In samples collected offshore from Alaska, and in the Beaufort and Chukchi Seas the abundances of major bacterial groups matched their contribution to total glucose uptake (Nikrad et al. 2012). The temperature response of bacterial production and growth in glucose-amended samples predominantly integrates effects on two processes: substrate diffusion to the cell surface and substrate uptake by membrane transporters. The use of laminarin is additionally influenced by temperature effects on the extracellular enzymatic hydrolysis driven by extracellular glucanase and glucosidase. Synergistic combined effects of temperature and carbohydrate amendment in our experiments cannot be explained with kinetic models based on Arrhenius-type reactions. According to these

models, the degradation of structurally complex organic compounds is more temperature sensitive than the use of reactive compounds with a simple molecule structure since complex structures require higher activation energy for enzymatic reworking. In soil science, studies on the remineralization of litter and soil organic matter are in line with the Arrhenius concept (Bosatta and Ågren 1999; Fierer et al. 2005; Wetterstedt et al. 2010; Erhagen et al. 2013), implying that heterotrophic microbial activity is mainly limited by biochemical restrictions of enzymes involved in the decomposition of complex structures. Assuming that simple carbohydrate compounds like glucose and laminarin should represent a more reactive carbon source than the complex mixture of natural organic matter, enzyme kinetic models would predict higher temperature sensitivity of rates in non-amended controls than in carbohydrate-amended incubations. Our experiments, however, show that the availability of reactive substrates strengthens effects of elevated temperature on bacterial productivity in both Atlantic Water of the West Spitsbergen Current and Polar Water in the East Greenland Current. Therefore, it can be suggested that also in summer, when fresh organic matter has already been produced by phytoplankton blooms, the dilute concentration of labile substrate is a stronger constraint on bacterial growth and activity than substrate quality that is co-determined by molecule structures. Experimental studies have recently shown that temperature sensitivity of bacterial metabolism in marine systems can be linked to resource availability (Kritzberg et al. 2010; Degerman et al. 2013). The relevance of substrate control for heterotrophic bacterial metabolism in the Arctic Ocean is still under debate. There is growing evidence from field studies that extremely low seawater temperature is not the predominant environmental constraint on bacterial growth and activity. Instead, substrate limitation is likely of equal importance for growth of Arctic bacterioplankton (Kirchman et al. 2005, 2009; Sala et al. 2010; Piontek et al. 2014). Our experimental results suggest that this multifactorial environmental control of bacterial activity has a high potential to sustain combined effects of rising temperature and increasing substrate availability that exceed the sum of its parts. In response to insufficient substrate supply, down-regulation of the cellular metabolism and its restriction to basal processes is frequently observed in marine bacterial populations during periods of non-growth and dormancy (Kjelleberg et al. 1987). Increasing temperature, however, results in a net increase of energy demand for basal metabolic processes (Clarke 2006). Our results suggest that these higher energetic costs required a high share of energy generated from natural organic matter in our non-amended incubations, while higher demands for basal metabolism at elevated temperature could be easily overcome in incubations supplied with carbohydrates. Consequently, high shares of energy derived from carbohydrate utilization at elevated temperature remained to fuel protein production.

Since our study does not include the analysis of the bacterial community structure and composition, differences between communities in experiments AW-1, AW-2, and PW cannot be resolved. Differences in intrinsic metabolic capacities, however, can have a strong influence on the temperature sensitivity of bacterial metabolism on the community level (Yager and Deming et al. 1999; Piontek et al. 2013). In our experiments, the bacterioplankton community in experiment PW likely included psychrophilic strains. Reactions catalyzed by enzymes of psychrophilic bacteria typically show a lower activation energy, which results from a decrease in activation enthalpy and renders the enzymatic reactions less dependent on variations in temperature (e.g., Feller and Gerday 2003). It can be assumed that the low activation energy of cold-adapted enzymes impeded a temperature effect on leu-aminopeptidase in experiment PW. Furthermore, changes in community composition in response to confinement and experimental substrate enrichment have been frequently observed (Eilers et al. 2000; Øvreås et al. 2003). Shifts in community composition during 4 d of incubation may have contributed to interactive effects of temperature and organic matter enrichment in our experiments. A mesocosm study conducted with Fram Strait bacterioplankton did not reveal consistent changes in assemblage diversity after glucose addition and pCO_2. Nevertheless, *Flavobacteria* seemed to positively respond to acidification when glucose was added at or in excess the Redfield ratio, while *Gammaproteobacteria* responded negatively (Ray et al. 2012). Further studies need to test whether community shifts in response to organic matter enrichment can be linked to systematic changes in community function.

Treatment effects on the loss of organic matter

Less than 20% of dissolved organic matter (DOM) in the ocean consists of characterizable biochemicals (Benner 2002; Kaiser and Benner 2009). Carbohydrates and amino acids are the dominant compounds identified in marine DOM. Free sugars and amino acids are recycled on time scales of hours to days, while reactive polymers that contribute the by far larger percentage to DOM are remineralized within days to months (Hansell 2013). Hence, the limited incubation time of 4 d allowed for the turnover of the labile compounds and the most reactive fraction of the semi-labile pool. Temperature effects on bacterial activity did not consistently translate into significant effects on concentrations of carbohydrates and amino acids. Only in AW-2, the experiment with highest leucine-aminopeptidase activity and bacterial biomass production, elevated temperature significantly affected the turnover of carbohydrates and amino acids in non-amended incubations. Here, elevated temperature increased the loss of amino acids but reduced the loss of carbohydrates. The addition of glucose, however, changed temperature effects on amino acid turnover. In all three experiments, elevated temperature reduced the loss of amino

acids or even led to a net production if incubations received glucose amendment. Hence, temperature effects on the bacterial turnover of amino acids seem to be strongly dependent on the composition of organic matter and the resulting availability of substrates for growth.

Combined effects of seawater pH, temperature and substrate availability on heterotrophic bacterial activity and implications for the future Arctic Ocean

Our experimental results show that changes in seawater temperature and pH as projected for the next 100 yr have the potential to significantly enhance growth and degradation activity of Arctic marine bacterioplankton.

Hydrolytic extracellular enzymes produced by bacterioplankton in the West Spitsbergen Current showed pH optima below the present-day seawater pH. The accelerated enzymatic hydrolysis at lowered pH likely facilitates the utilization of polymeric organic matter and potentially enhances bacterial organic matter turnover at proceeding ocean acidification. We investigated the response of natural enzyme assemblages to changing seawater pH. These assemblages included an unknown number of isoenzymes that catalyze the same reaction but can differ with respect to other kinetic and biochemical characteristics (Ferenci 1996; Wick et al. 2001). Potential shifts in the composition of enzyme assemblages as a consequence of changes in community composition or in response to environmental factors like substrate concentrations may create spatial and temporal differences in pH optima for reaction velocities.

It is noteworthy, that the turnover of proteins was enhanced in our incubations when bacterial growth was stimulated by the addition of carbohydrates. Leucine-aminopeptidase increased proportionally with growth, confirming the strongly constitutive nature of its bacterial production (Christian and Karl 1998). This is in contrast to glucosidase activity in Fram Strait that is largely substrate-induced (Piontek et al. 2014). Hence, our findings suggest that the spectrum of extracellular enzyme activity in Fram Strait will not be determined by organic matter composition alone but also by constitutive enzyme expression accompanying enhanced bacterial growth.

Interactive effects of temperature and carbohydrate supply in our experiments strongly support the hypothesis that the availability of labile organic matter acts beside temperature as the major environmental constraint on bacterial carbon turnover. In line with earlier studies, activation energies estimated for bacterial growth and production from our experiments suggest that rising temperature alone will not have a disproportionately stimulating effect on bacterial activity in the Arctic Ocean relative to temperate marine systems (Kirchman et al. 2005, 2009; Kritzberg et al. 2010; Piontek et al. 2014). Instead, the bioavailability of labile organic matter will modulate the effect size of elevated temperature. Thus, the concentrations of freshly produced organic matter will likely co-determine the metabolic balance in a warming Arctic Ocean. Recent studies have shown that the decreasing minimum ice extent in summer and a longer growing season enhance primary production by marine phytoplankton (Arrigo et al. 2008; Leu et al. 2011) and, thereby, likely increase the input of labile organic matter to the Arctic Ocean. Furthermore, a mesocosm study conducted in Kongsfjorden (Svalbard Archipelago) suggests that Arctic phytoplankton communities will increase primary production also in response to elevated seawater pCO_2 (Engel et al. 2013). It has been demonstrated that increased substrate availability for bacteria reduces diatom growth by stimulated competition for mineral nutrients between bacterioplankton and phytoplankton of Kongsfjorden (Thingstad et al. 2008). Our experimental results reveal that higher availability of labile compounds amplifies beneficial temperature effects on heterotrophic bacterial metabolism that can further strengthen the heterotrophic component of microbial communities in the future Arctic Ocean. The synergistic effect of elevated temperature and substrate availability implies seasonal differences in temperature sensitivity of bacterial activity. Temperature effects should be most pronounced at high primary production during summer, while synergistic effects are likely of minor importance during the unproductive winter season.

Time series observations in Fram Strait revealed that warming and freshening by ice melt favor small-sized phytoplankton over diatoms, resulting in deteriorated feeding conditions for copepods (Bauerfeind et al. 2009; Lalande et al. 2012). Photosynthetic key species in a changing Arctic Ocean are *Phaeocystis* and the picoeukaryote *Micromonas* (Tremblay et al. 2009; Balzano et al. 2012; Kilias et al. 2013). Phytoplankton communities dominated by small-sized species usually cooccur with retentive pelagic food webs that recycle carbon and nutrients at high rates (Ryther 1969; Azam et al. 1983). It can be suggested that enhanced bacterial productivity and increasing primary production will increase the demand for inorganic nutrients in the future Arctic Ocean, thereby promoting the competition of autotrophic and heterotrophic microbes. It seems likely that small-sized autotrophic and heterotrophic plankton can benefit from large cellular surface-area-to-volume ratio at low nutrient concentrations and further replace diatom-dominated communities associated with classical grazing food chains.

Warming and sea ice melting causes an atlantification of the Atlantic sector of the Arctic Ocean (Wassmann et al. 2006). Sea-ice associated species are replaced by species of the North Atlantic (Wassmann et al. 2006; Kraft et al. 2013). It is unclear whether atlantification will also lead to changes in microbial communities that, in turn, might be linked to changes in community metabolic capabilities. A comparison of the T-pH incubation studies shows a similar temperature sensitivity of biomass production in all experiments but a clearly higher temperature sensitivity of extracellular leu-

aminopeptidase in Atlantic Water of the West Spitsbergen Current than in Polar Water of the East Greenland Current. Hence, the persistence of Atlantic communities in the future Arctic Ocean might be accompanied by stronger temperature effects on the hydrolytic turnover of organic matter.

Overall, the results of our experiments strongly suggest that an improved understanding of individual and combined effects induced by warming, acidification and organic matter enrichment is crucial to better project the regional impact of climate change on Polar and Atlantic Water in Fram Strait. Biochemical effects of seawater pH and synergistic effects of temperature and reactive organic matter reveal high complexity of multiple environmental changes on marine bacterial metabolism. Our study suggests that further community-level experimentation investigating combined environmental effects is required to improve model projections for consequences of global change in the ocean.

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