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Effect of CO$_2$ enrichment on bacterial metabolism in an Arctic fjord

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Abstract. The anthropogenic increase of carbon dioxide (CO$_2$) alters the seawater carbonate chemistry, with a decline of pH and an increase in the partial pressure of CO$_2$ (pCO$_2$). Although bacteria play a major role in carbon cycling, little is known about the impact of rising pCO$_2$ on bacterial carbon metabolism, especially for natural bacterial communities. In this study, we investigated the effect of rising pCO$_2$ on bacterial production (BP), bacterial respiration (BR) and bacterial carbon metabolism during a mesocosm experiment performed in Kongsfjorden (Svalbard) in 2010. Nine mesocosms with pCO$_2$ levels ranging from ca. 180 to 1400 µatm were deployed in the fjord and monitored for 30 days. Generally BP gradually decreased in all mesocosms in an initial phase, showed a large (3.6-fold average) but temporary increase on day 10, and increased slightly after inorganic nutrient addition. Over the wide range of pCO$_2$ investigated, the patterns in BP and growth rate of bulk and free-living communities were generally similar over time. However, BP of the bulk community significantly decreased with increasing pCO$_2$ after nutrient addition (day 14). In addition, increasing pCO$_2$ enhanced the leucine to thymidine (Leu : TdR) ratio at the end of experiment, suggesting that pCO$_2$ may alter the growth balance of bacteria. Stepwise multiple regression analysis suggests that multiple factors, including pCO$_2$, explained the changes of BP, growth rate and Leu : TdR ratio at the end of the experiment. In contrast to BP, no clear trend and effect of changes of pCO$_2$ was observed for BR, bacterial carbon demand and bacterial growth efficiency. Overall, the results suggest that changes in pCO$_2$ potentially influence bacterial production, growth rate and growth balance rather than the conversion of dissolved organic matter into CO$_2$.

1 Introduction

Bacteria are the main organisms that incorporate and mineralise dissolved organic carbon in the ocean, recycling about 50 % of daily primary production. Since bacteria take up carbon into anabolic and catabolic processes, measuring both bacterial production (BP) and respiration (BR) is crucial to estimating carbon metabolism (e.g., bacterial carbon demand (BCD) and bacterial growth efficiency (BGE)) as well as to improving our understanding of the impact of bacteria on global marine carbon flux (del Giorgio and Cole, 2000; Robinson, 2008). Compared to extensive BP measurements, far less is known about the control of BR and carbon metabolism due to methodological difficulties (e.g., long-time incubation, requires filtration to separate bacteria from the rest of the plankton community). Previous studies have

suggested that over a broad range of marine environments, BGE tends to increase with decreasing temperature (Rivkin and Legendre, 2001) or with an increasing level of organic resources (Lopez-Urrutia and Morán, 2007). However, the effect of environmental change, which presumably influences global marine carbon flux, on bacterial carbon metabolism is still largely unknown.

Recent studies have reported that the world ocean is absorbing about 25 % of the atmospheric partial pressure of CO$_2$ (pCO$_2$) and that pCO$_2$ will increase from 280 to nearly 384 µatm over the next 250 yr (IPCC, 2007). The increase in pCO$_2$ reduces ocean pH (ocean acidification), which may affect calcifying organisms (e.g., Riebesell et al., 2000) and primary production (reviewed by Liu et al., 2010; Weinbauer et al., 2011). However, few studies have focused on pCO$_2$ effects on bacterial carbon metabolism. Previous studies have examined the effect of pCO$_2$ on microbial communities and found that pCO$_2$ potentially alters BP (Coffin et al., 2004; Grossart et al., 2006; Yamada et al., 2010), growth rate (Grossart et al., 2006), enzymatic activity (Grossart et al., 2006; Piontek et al., 2010; Yamada and Suzumura, 2010) and community structure (Allgaier et al., 2008; Yamada et al., 2010); whereas little or no effect of pCO$_2$ was found for BP (Allgaier et al., 2008; Arnosti et al., 2011) and abundance (Rochelle-Newall et al., 2004; Grossart et al., 2006; Allgaier et al., 2008; Arnosti et al., 2011) or chromophoric dissolved organic matter (Rochelle-Newall et al., 2004). It is noteworthy that changes in phytoplankton growth or community composition, or a modification of the quality and quantity of dissolved organic matter production by changing pCO$_2$ levels could indirectly influence bacterial parameters (Grossart et al., 2006; Engel et al., 2004). A recent review paper suggests that unlike calcifying organisms, the effect of pCO$_2$ on biogeochemical processes driven by microbes or microbial function in the oceans might be minor (Joint et al., 2011). However, this has been challenged by Liu et al. (2010). For example, there is evidence that some functions such as nitrogen fixation (Wannicke et al., 2012), nitrification and bacterial activity are influenced by changing pCO$_2$ levels, which would influence biogeochemical processes (summarised in Liu et al., 2010 and in Weinbauer et al., 2011). Also, there is no study on the effect of pCO$_2$ on specific carbon metabolism components such as BR, BGE and BCD of natural bacterial communities in aquatic environments.

Arctic Ocean surface waters are specifically affected by aragonite undersaturation due to relatively high solubility of CO$_2$ in cold water (Steinacher et al. 2009). High-latitude seas are, thus, a bellwether for prospective impacts of ocean acidification on marine organisms (Fabry et al. 2009). In the present study, we determined the potential impact of changes in pCO$_2$ on BP, BR and bacterial carbon metabolism in an Arctic fjord. We hypothesised that changing pCO$_2$ levels would not influence bacterial carbon metabolism of natural communities in mesocosm experiments. Particularly, we focused on how changes in pCO$_2$ may influence (1) BP,

(2) BR, (3) the amount of new bacterial biomass produced per unit of organic C substrate assimilated (i.e., BGE), (4) the amount of organic C assimilated by bacteria (i.e., BCD), and (5) the ratio of biomass produced to substrate assimilated (i.e., Leucine (Leu) : Thymidine (TdR) ratio) in the Arctic fjord. The present study is part of the joint mesocosm experiment of the European Project on Ocean Acidification (EPOCA) conducted in Kongsfjorden (Svalbard) in 2010.

2 Materials and methods

2.1 Experimental set-up and sample collection

The mesocosm experiment was conducted over a period of 30 days, between June 7 (day t0) and July 7 (day t30) 2010, at Kongsfjord, Svalbard (78°56.2' N, 11°53.6' E). Details of the mesocosm set-up are described by Riebesell et al. (2013). Briefly, nine Kiel off-shore mesocosms (KOSMOS) were deployed at $t - 10$, and seven days after closing of the mesocosms, a stepwise addition of CO$_2$ saturated water was applied between t1 and t4 to obtain 8 different CO$_2$ levels: 185 µatm (M3 and M7, two controls with no CO$_2$ addition), 270 µatm (M2), 375 µatm (M4), 480 µatm (M8), 685 µatm (M1), 820 µatm (M6), 1050 µatm (M5) and 1420 µatm (M9). No further CO$_2$ manipulation was done after initially targeted pCO$_2$ levels were reached (for details see Riebesell et al., 2013). Due to gas exchange and photoautotrophic uptake, pCO$_2$ levels declined in the mesocosms during the experiment, and final CO$_2$ levels ranged from 160 to 855 µatm. At day 13 of the experiment (t13), inorganic nutrients (nitrate, silicate and phosphate: 5, 2.5, and 0.31 µmol L^{-1}, respectively) were added. Based on pCO$_2$ manipulations and the temporal development of chlorophyll a (Chl a), four distinct phases were defined: Phase 0 (after closing of the mesocosms until end of CO$_2$ manipulation, $t - 4$ to t3), Phase 1 (from the end of CO$_2$ manipulation until nutrient addition, t4 to t12), Phase 2 (after nutrient addition until 2nd chlorophyll minimum, t13 to t21), and Phase 3 (from the 2nd chlorophyll minimum until end of experiment, t22 to t30) (for more details see Schulz et al., 2013; Riebesell et al., 2013). Subsamples for BP and BR were obtained every 2 and 4 days, respectively. Water samples were collected using clean depth integrated sampler (5 L volume) at depths between the surface and 12 m for all mesocosms, transferred to 2 L polycarbonate bottle (Nalgene) and brought back to the laboratory. Containers and plastic wares used for the sampling were rinsed before use with 1.2 N HCl followed by vigorous rinsing with Milli-Q water. During sample collection and handling, gloves were worn, and care was taken to minimise contamination.

2.2 Bacterial production (BP)

Bacterial production rates of unfiltered (BP$_{Total}$) and filtered (0.8 µm, Nuclepore, Millipore) water (BP$_{Free}$) were determined from the incorporation rate of ^{3}H-thymidine

(Kirchman, 2001) using a centrifugation method. Triplicate subsamples and one trichloroacetic acid (TCA)-killed control (1.5 mL) were spiked with [methyl-^{3}H] TdR (1.77 TBq mmol^{-1}, PerkinElmer, NET027W, final conc. 10 nmol L^{-1}) and incubated for 1 h at 2 °C in the dark. The reaction was stopped by the addition of 1 mL of ice cold TCA (5 % final concentration). Prior to the mesocosm experiments, the optimum incubation time of TdR incorporation in this environment was determined (Fig. S1).

Macromolecules were precipitated with 5 % cold TCA, followed by cold ethanol rinsing using a temperature controlled desktop centrifuge (18 000 × g at 4 °C for 10 min for each run; Sigma, 1–15K). The extracts were then completely dried and mixed with scintillation cocktail (1 mL, Ultima Gold, PerkinElmer) for the detection of incorporated label using a Packard Tri-Carb 1600CA liquid scintillation counter with corrections for quenching. The coefficient of variation (CV) of the triplicate measurement ranged from 0 to 28.3 %. The ^{3}H-TdR incorporation rates were converted to cell production by the conversion factor 2×10^{18} cells per mole of TdR (Fuhrman and Azam, 1982). Cell-specific bacterial growth rate of unfiltered (csBP$_{Total}$, d^{-1}) and filtered fractions (csBP$_{Free}$, d^{-1}) were estimated as BP$_{Total}$ or BP$_{Free}$ (cells L^{-1} d^{-1}) divided by bulk bacterial abundance (cells L^{-1}). Bacterial production rates of attached fraction (BP$_{Att}$) were estimated by BP$_{Free}$ subtracting from BP$_{Total}$.

2.3 Bacterial respiration (BR)

BR was determined from the decrease of dissolved oxygen concentration during 48 h incubations of filtered water samples. Sample water was filtered through a 0.8 μm filter (Nucleopore, Millipore) by applying a weak positive pressure (< 67 cm Hg) with an air pressure pump and the filtrate was dispensed into biochemical oxygen demand bottles (BOD; 60 mL capacity). BOD bottles were incubated in the laboratory incubator for 48 h at the mean temperature in the top 12 m of water on the day of sampling (2 to 4 °C). Dissolved oxygen concentration was determined by Winkler titration using an automated titrator with a potentiometric end-point detector (Mettler Toledo, Titrando 888) (Knap et al., 1996). Oxygen consumption was estimated from the difference in oxygen concentration between time zero and the end of the incubation (48 h). O$_2$ consumption rate (μmol O$_2$ L^{-1} d^{-1}) was converted to carbon C respired by assuming that the respiratory quotient = 1 (del Giorgio and Cole, 1998). Cell-specific bacterial respiration (csBR, fg C cell^{-1} d^{-1}) was estimated as BR (fg C L^{-1} d^{-1}) divided by bulk bacterial abundance (cells L^{-1}) at the start of the incubation.

2.4 Bacterial growth efficiency (BGE) and bacterial carbon demand (BCD)

To estimate BGE and BCD, BP and BR of filtered fractions were used. BGE and BCD were estimated with the following equations:

$$BGE = \frac{BP}{BP + BR} \tag{1}$$

$$BCD = BP + BR \tag{2}$$

where BP (TdR incorporation was converted to C flux by assuming a conversion factor of 20 fg C per cell; Cho and Azam, 1990) and BR was estimated as described above.

2.5 Leucine to Thymidine (Leu : TdR) ratio

To estimate the Leu (pmol Leu L^{-1} d^{-1}) and TdR incorporation (pmol TdR L^{-1} d^{-1}) ratio, data on incorporation rates of ^{14}C-leucine were obtained from Piontek et al. (2013). These measurements were carried out on unfiltered water samples. Details of the method are described in Piontek et al. (2013).

2.6 Bacterial abundance

Total bacterial abundance and 2 clusters (i.e., high and low DNA fluorescent) were determined by flow cytometer. Details on the method are described in Brussaard et al. (2013). Briefly, samples for bacterial abundance were fixed for 30 min at 7 °C with glutaraldehyde (25 %, EM-grade) at a final concentration of 0.5 % before snap freezing in liquid nitrogen and storage at −80 °C until analysis. Bacterial abundance was determined by flow cytometer (FACScalibur, Becton Dickinson) after staining of samples with SYBR-Green I (Molecular Probes, Invitrogen Inc.).

2.7 Statistical analysis

A stepwise multiple regression analysis was conducted to examine the relationship between bacterial variables (i.e., BP$_{Total}$, csBP$_{Total}$, BP$_{Free}$, csBP$_{Free}$, BR, csBR, BGE, BCD or Leu : TdR ratio) and other environmental parameters (i.e., temperature (Temp; Schulz et al., 2013), salinity (Sal; Schulz et al., 2013), pCO$_2$ (Bellerby et al., 2012), nitrate (NO$_3$, Schulz et al., 2013), phosphate (PO$_4$, Schulz et al., 2013), silicate (Si, Schulz et al., 2013), ammonium (NH$_4$, Schulz et al., 2013), Chl a (Schulz et al., 2013) and viral abundance (VA; Brussaard et al, 2013)) using the software package R (R Development Core Team, 2012). BP$_{Total}$, BP$_{Free}$, BR and VA were log-transformed (i.e., log-BP$_{Total}$, log-BP$_{Free}$, log-BR and log-VA).

Linear regression analysis was conducted to examine the relationship between pCO$_2$ or bacterial carbon metabolism using Sigma Plot 12.0. Relationships were considered significant at a probability level (p) of < 0.05.

3 Results

3.1 Temporal variations of BP and BR

The range of BP_{Total}, BP_{Free} and BR in the mesocosms before adding CO_2 saturated water ($t-1$) were 3.4–4.4×10^8 cells L^{-1} d^{-1}, 2.8–3.7×10^8 cells L^{-1} d^{-1}, and 10.9–51.4 µg C L^{-1} d^{-1}, respectively (Fig. 1a, b and c). Generally, BP_{Total} decreased until $t7$, after which it showed a 2.4 to 5.5-fold increase (Fig. 1a; Phase 1). Although there was no pronounced enhancement by nutrient addition, BP_{Total} gradually increased towards the end of the experiment (Fig. 1a; Phase 2 and 3). BP_{Free} decreased between $t0$ and $t7$ and gradually increased towards the end of the experiment (Fig. 1b). BP_{Total} and BP_{Free} varied from 0.7 to 5.6, and 0.5 to 4.6×10^8 cells L^{-1} d^{-1}, respectively, during the experiment (Fig. 1a and b; $t1$–$t28$). When BP_{Free} were subtracted from BP_{Total}, 12 out of 62 measurements showed negative value; however, these 12 measurements were not significantly different (t test: $p > 0.05$). BR varied between 0.5 and 51 µg C L^{-1} d^{-1}, without a clear pattern with time or pCO_2 (Fig. 1c).

The $csBP_{Total}$, $csBP_{Free}$ and csBR at $t-1$ ranged between 0.16 and 0.22 d^{-1}, 0.13 and 0.17 d^{-1}, and 5.4 and 23.6 fg C cell^{-1} d^{-1}, respectively (Fig. 2a, b and c). $csBP_{Total}$ gradually increased from $t0$ until $t10$, after which it gradually declined to 0.04–0.09 d^{-1} on $t28$ (Fig. 2a). $csBP_{Free}$ slightly decreased at phase 0, then increased after closing the mesocosms (Phase 1). Consistent with $csBP_{Total}$, $csBP_{Free}$ gradually decreased towards the end of the experiment after nutrient addition (Fig. 2b). In contrast to BP, csBR did not show any clear pattern during the experiment (Fig. 2c).

Averaged BP_{Free} % of BP_{Total} at phases 0, 1, 2 and 3 was 70 ± 15, 69 ± 11, 70 ± 19 and 95 ± 9 % ($\pm$SD), whereby phase 3 exhibited significantly higher values than phases 0 and 1 (Fig. 3; t test, both $p < 0.001$). BP_{Att} dominated, especially at $t3$ (the end of pCO_2 manipulation) and $t20$ (the second Chl a peak, Schulz et al., 2013), and ranged from 24 to 56 and 23 to 56 %, respectively.

3.2 Temporal variations of BGE, BCD and Leu : TdR ratio

Average BGE ranged between 13 and 53 % in phase 0, and varied from 4 to 35, 9 to 44, and 21 to 46 % in phases 1, 2 and 3, respectively (Fig. 4a). Average BCD at the beginning of the experiment ranged between 10.5 and 58.7 µg C L^{-1} d^{-1}, and varied from 3.6 to 47.2, 12.8 to 33.7, 19.6 to 32.3 µg C L^{-1} d^{-1} in phases 1, 2 and 3, respectively (Fig. 4b). Although BCD gradually increased after nutrient addition, no obvious trend was observed by the effect of pCO_2 or time of experiment.

The Leu : TdR ratio averaged 10.4 ± 4.0 (range 3.2 to 14.7) at the beginning of experiment ($t-1$), and increased to 25.8 ± 8.1 (range 15.1 to 35.2) on $t5$ (Fig. 5). During phase 1
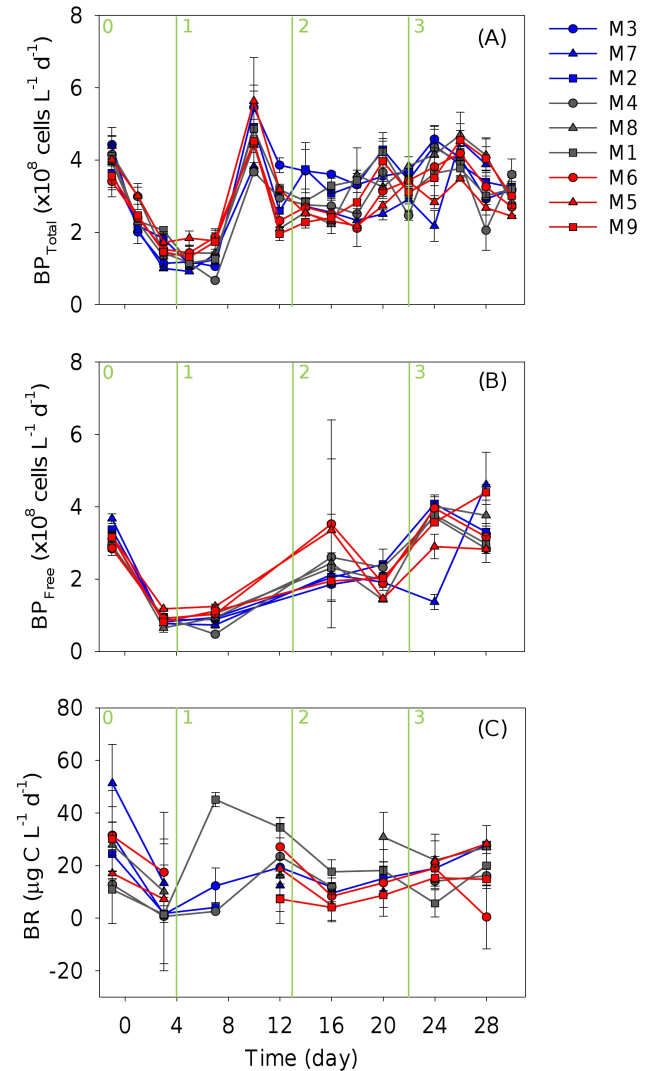

Fig. 1. Temporal variation of bacterial production estimated by TdR incorporation of unfiltered fraction **(A)**, of filtered fraction **(B)** and bacterial respiration **(C)**. Values are means $\pm$ stand deviations for triplicate measurements ($n = 3$). Circles represent mesocosms M3, M4 and M6; triangles M7, M8, M5; and squares M2, M1 and M9, respectively. Low, medium, and high pCO_2 treatments are coloured in blue, grey, and red.

(the time span after the pCO_2 manipulation and just before nutrient addition) the average Leu : TdR ratio in low pCO_2 mesocosms (M3, 7, 2) was 17.8, higher than for the medium (M4, 8, 1) and high pCO_2 mesocosms (M6, 5, 9) with ratios of 14.9 and 13.6, respectively. Contrarily, between the time of nutrient addition and the end of the experiment (phase 2 and 3), a higher average ratio was observed in high pCO_2 mesocosms (16.9; M6, 5, 9) compared to low (12.7; M3, 7, 2) and medium pCO_2 mesocosms (12.5; M4, 8, 1).
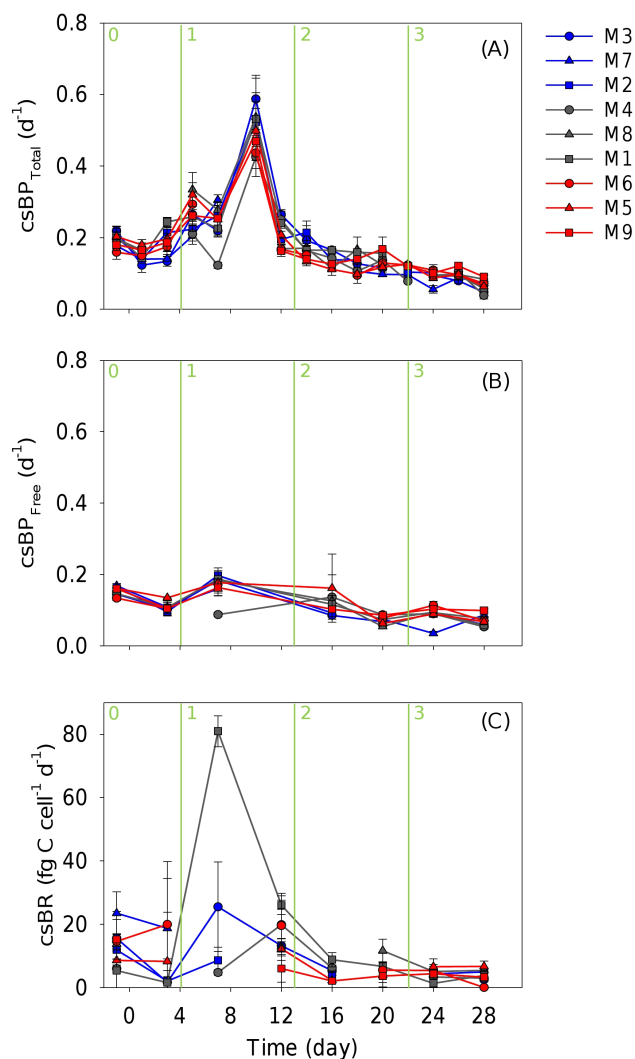
Fig. 2. Temporal variation of bacterial growth rate of the unfiltered fraction **(A)** and bacterial growth rate of filtered fraction **(B)**, and cell specific bacterial respiration **(C)**. Values are means ± standard deviations for triplicate measurements ($n = 3$). For colour and symbol coding, see Fig. 1.

Fig. 3. Percentage of attached (line bars) and free-living bacterial production (solid bars) in phase 0 **(A)**, phase 1 **(B)**, phase 2 **(C)** and phase 3 **(D)**.

3.3 Relationship between pCO$_2$ and bacterial activity in different phases of the experiment

Statistical analysis showed that log-transformed BP$_{Total}$ was negatively correlated with pCO$_2$ concentration after nutrient addition at t14 ($r^2 = 0.51$, $p < 0.05$, $n = 9$; Fig. 6). No significant relationships between pCO$_2$ and csBP$_{Total}$, BP$_{Free}$, csBP$_{Free}$, BR, csBR, BGE or BCD were found otherwise ($p > 0.05$). However, the Leu : TdR ratio was negatively correlated with pCO$_2$ at t5 and t7 (Linear regression, t5; $r^2 = 0.41$, $p < 0.05$, $n = 9$, t7; $r^2 = 0.57$, $p < 0.05$, $n = 9$), but positively correlated at t24 and t26 (Linear regression, t24; $r^2 = 0.51$, $p < 0.05$, $n = 9$, t26; $r^2 = 0.55$, $p < 0.05$, $n = 9$). There was also a positive correlation between aver-
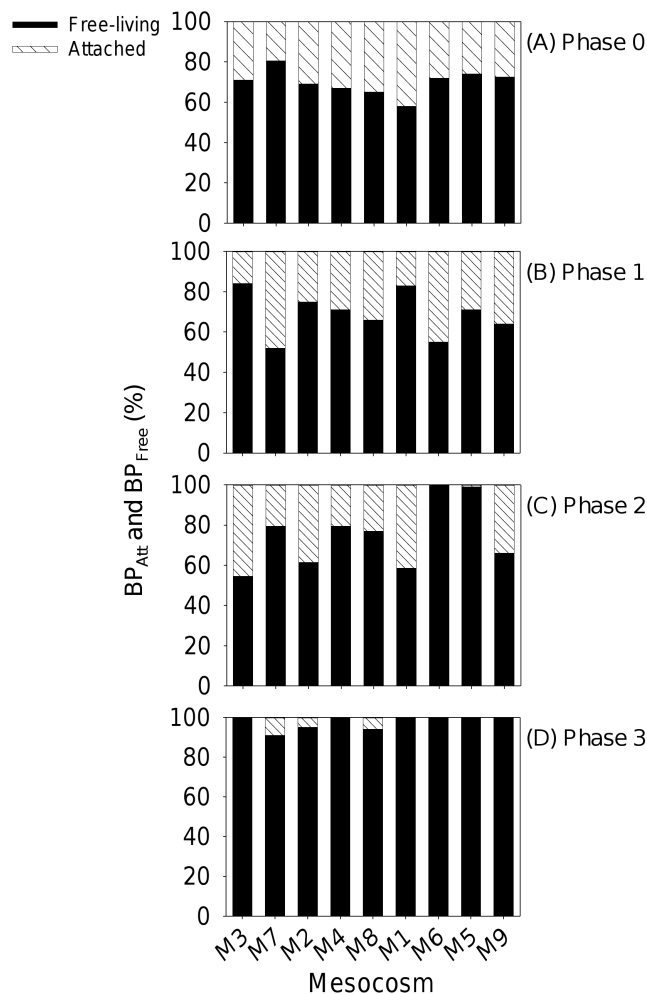
aged pCO$_2$ and averaged Leu : TdR ratio in phase 3 (Linear regression, Phase 3; $r^2 = 0.58$, $p < 0.05$, $n = 9$, Fig. 7).

3.4 Relationship between environmental variables and bacterial activity

We used a stepwise multiple regression analysis to examine the relationship between bacterial parameters and physical and biological environmental parameters in each phase (Tables 1, A1). Fifty-five percent of the variation in log-BP$_{Total}$ was explained by Si, pCO$_2$, NH$_4$, PO$_4$ and Chl a in phase 1, 18 % by pCO$_2$ and log-VA in phase 2, and 80 % by Si, PO$_4$, log-VA, NH$_4$, Temp, pCO$_2$ and Chl a in phase 3. Sixty percent of the variation in csBP$_{Total}$ was explained by NH$_4$, PO$_4$, Chl a, and Temp in phase 1, 24 % by PO$_4$, Chl a and NO$_3$ in phase 2, and 93 % by Si, Temp, pCO$_2$, PO$_4$ NH$_4$, and Sal and log-VA in phase 3. NH$_4$, and Sal explained 37 % of the variation in log-BP$_{Free}$, in phase 2. For csBP$_{Free}$, 63 % of the
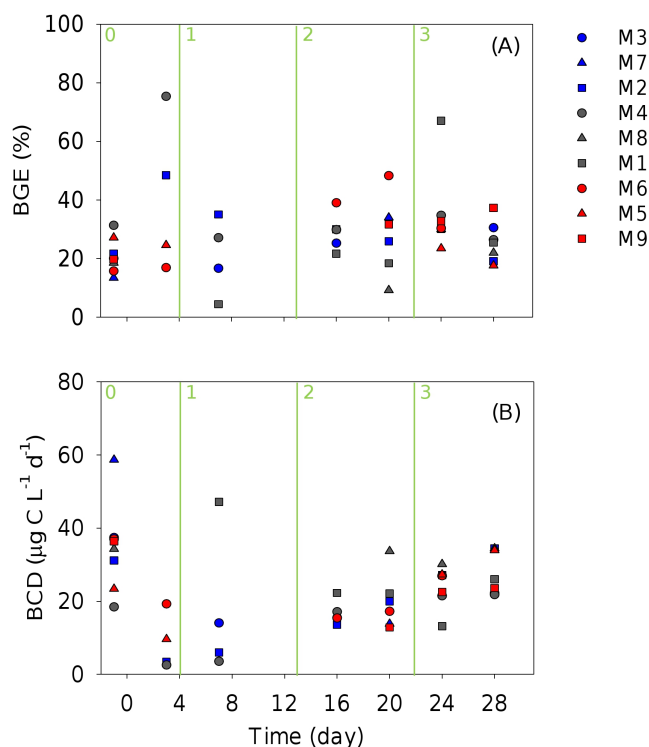
Fig. 4. Temporal variation of bacterial growth efficiency **(A)** and bacterial carbon demand **(B)**. For colour and symbol coding, see Fig. 1.

Fig. 5. Temporal variation of Leu : TdR ratio. For colour and symbol coding, see Fig. 1.

variation was explained by Chl *a* and NH_4 in phase 1 and 73 % by pCO_2, Si, Sal, log-VA and NH_4 in phase 2.

Fifty percent of the variation in log-BR was explained by Temp, PO_4, log-VA and Chl *a* in phase 1. Environmental variables of NH_4, Temp and Sal explained 60 % of the variations in log-BR, 62 % of csBR, 71 % of BCD and 62 % of the Leu : TdR ratio in phase 2. Thirty six percent of the variation in csBR was explained by PO_4 in phase 1 and 82 % by PO_4 and Temp in phase 3. Seventy five percent of the variation in the Leu : TdR ratio was explained by NO_3, NH_4, Si, Temp and log-VA in phase 1, and 81 % by pCO_2, NO_3, log-VA, Chl *a* and Si in phase 3.

No coupling between phytoplankton biomass (i.e., Chl *a*) and bacterial cell production of the free-living fraction (i.e., BP_{Free}) was observed in this study (linear regression, $p > 0.05$).

4 Discussion

4.1 General variation of parameters in the experiment

During the experiment, many physical, chemical and biological parameters were measured and they showed a strong temporal variation (for more details see Bellerby et al., 2012; Brussaard et al., 2013; Schulz et al., 2013). After the tar-
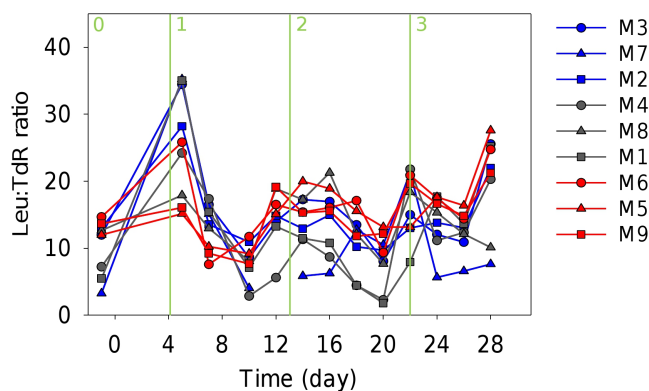
get pCO_2 levels were set at $t4$, pCO_2 declined to a range of ca. 160 to 855 µatm at the end of the experiment (Bellerby et al., 2012). Temperature was 2 °C at the start of the experiment and increased gradually to 5.5 °C at the end of experiment. Before nutrient addition at $t13$, the concentration of NO_3 was close to the detection limit, PO_4 was taken up in the first couple of days and remained low, and Si was low and stable (Schulz et al., 2013). After nutrient addition, NO_3, PO_4 and Si concentrations declined steadily towards the end of the experiment. Concomitant with this decrease, Chl *a* concentrations increased during manipulation and showed 3 peaks (Schulz et al., 2013). A slow and gradual effect of pCO_2 was observed on phytoplankton composition and positive effect on its growth (Brussaard et al., 2013; Schulz et al., 2013). Furthermore, the concentration of NH_4^+ gradually decreased during the experiment (Schulz et al., 2013), whereas the average dissolved organic carbon (DOC) concentration of all mesocosms significantly increased between $t4$ and $t13$ (Engel et al., 2013).

4.2 Temporal variations of bacterial variables

BP_{Total} and BP_{Free} decreased during CO_2 manipulation ($t - 1$ to $t4$), matching the lower abundances of heterotrophic prokaryotes bacteria and lower percentage of bacteria with high DNA fluorescence (Brussaard et al., 2013). The significant increase in the average DOC concentration between $t4$ and $t13$ (Engel et al., 2013) as a consequence of phytoplankton bloom and demise (Brussaard et al., 2013; Schultz et al., 2013) potentially stimulated BP_{Total} and $csBP_{Total}$ at $t10$. Bacterial growth was likely limited by labile carbon and nitrogen in the study site during the days of mesocosm deployment (Piontek et al., 2013). Also, the average Leu : TdR ratio decreased from 25.8 ± 8.1 at $t5$ to 7.8 ± 3.1 at $t10$, possibly as a consequence of labile dissolved organic matter release by phytoplankton (see discussion below). Furthermore, the stepwise multiple regression analysis supports the notion that phytoplankton biomass in combination with

Table 1. Stepwise multiple regression analysis of log-BP_{Total}, $csBP_{Total}$, $csBP_{Free}$, and Leu : TdR in phases 1, 2 and 3. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

	Phase 1				Phase 2				Phase 3			
	F	Adj. r2	n	variables	F	Adj. r2	n	variables	F	Adj. r2	n	variables
log-BP_{Total}	7.6***	0.55	33	Si** pCO2* NH4* PO4 Chl a	4.6*	0.18	33	pCO2* log-VA*	10.1**	0.80	15	Si** PO4* log-VA* NH4 Temp pCO2 Chl a
$csBP_{Total}$	13.1***	0.60	33	NH4*** PO4** Chl a** Temp*	4.3*	0.24	33	PO4* Chl a* NO3	27.3**	0.93	15	Si*** Temp*** PO4** NH4** pCO2 Sal log-VA
$csBP_{Free}$	7.0*	0.63	8	Chl a* NH4	8.7**	0.73	15	pCO2** Si* Sal* log-VA NH4		nd		
Leu : TdR ratio	20.1***	0.75	33	NO3** NH4** Si* Temp log-VA	18.3***	0.62	33	Sal*** NH4** Temp	12.6**	0.81	15	pCO2** NO3** log-VA** Chl a* Si

Please see Table A1 for the result of stepwise multiple regression analysis of log-BP_{Free}, log-BR, csBR, BGE and BCD.

other environmental variables, potentially influenced BP_{Total}, $csBP_{Total}$, $csBP_{Free}$ and BR variability in phase 1. Interestingly, the bacterial community composition determined by T-RFLP showed that species richness and the diversity index increased during pCO_2 manipulation and decreased at $t10$ when we observed the peak of BP_{Total} (Zhang et al., 2012). A previous study showed that active prokaryotic communities can be characterised by low bacterial richness (Winter et al., 2005), suggesting that a few active bacterial groups might have dominated at $t10$ in our study.

After nutrient addition at $t13$, Chl a showed two peaks (Schulz et al., 2013), while BP_{Total} and BP_{Free} increased gradually. This pattern may be due to changes in bacterial community composition after nutrient addition (Roy et al., 2013; Zhang et al., 2012). Furthermore, after nutrient addition, bacterial abundance increased and reached its maximum at the end of the experiment (Brussaard et al., 2013), while the net bacterial growth rate (i.e., $csBP_{Total}$, $csBP_{Free}$) gradually decreased. This pattern was also found for the specific growth rate estimated by Leu incorporation (Piontek et al., 2013). Bacterial abundance is the resultant of gross

bacterial growth and loss factors. After nutrient addition, the virus to prokaryote ratio slightly decreased towards the end of the experiment (Brussaard et al., 2013). This suggests that the encounter rate between bacteria and viruses decreased and, thus, the discrepancy between low growth rate and high abundance might be attributed to lower viral lysis rates. Also, it is possible that grazing losses of bacteria changed during the experiment.

Temporal variations of free-living and attached bacterial production were found in pCO_2 manipulated experiments, i.e., the high percentage of BP_{Free} at the end of experiment. Contrarily, Allgaier et al. (2008) reported similar rates of free-living and attached bacterial production, both of which were tightly coupled to a phytoplankton bloom. Although cell-specific bacterial protein production determined by Leu incorporation was positively correlated with primary production in the present study (Piontek et al., 2013), no coupling between phytoplankton biomass and bacterial cell production of the free-living fraction (i.e., BP_{Free}) was observed in this study. However, there is evidence that mortality due to
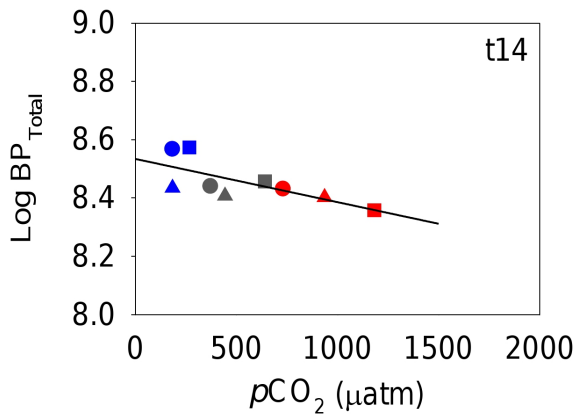
Fig. 6. Relationship between pCO_2 value and log BP_{Total} at $t14$. Liner regression line at $t14$: Log $BP_{Total} = -0.00015 \times pCO_2 + 8.53$ ($r^2 = 0.51$, $p < 0.05$, $n = 9$).

viral lysis rather than mortality due to flagellate grazing decreased at the end of the experiment (unpublished data).

In addition, we used prefiltered water ($< 0.8\,\mu$m pore size and low pressure filtration) to estimate BR and BP_{Free}. Twelve of the 62 measurements of BP_{Free} showed negative values. Higher free-living activity than total activity is potentially due to prefiltration, since it is, for example, known that prefiltration can destroy some protists, thus, resulting in dissolved organic matter production which can be used by bacteria (e.g., Gasol and Morán, 1999). However, these measurements were not significantly different (t test: $p > 0.05$), leading to the assumption that stress due to shear during prefiltration is unlikely at this scale for bacterial cells.

Overall, no single factor could statistically account for the different bacterial parameters throughout the experiment. Rather, multiple regression analysis suggest that bacterial activities were influenced by interactive effects of multiple factors, such as phytoplankton, nutrients, temperature and viruses in our study.

4.3 Effect of pCO_2 on bacterial carbon metabolism

Although a large range of pCO_2 levels was used in the experiment, the general patterns of BP_{Total}, $csBP_{Total}$, BP_{Free} and $csBP_{Free}$ were similar in all mesocosms during the experiment. This indicates that effect of pCO_2 on variability of bacterial production and growth rate of unfiltered and filtered communities was not evident. In accordance with our results, no clear trend with pCO_2 was observed for patterns of bacterial protein production (Piontek et al., 2013), prokaryote and viral abundance (Brussaard et al., 2013) and Chl a (Schulz et al., 2013). However, when regarding a specific time points or phases, the effect of pCO_2 on bacterial variables was found. In our study, BP_{Total} significantly decreased with increasing pCO_2 at $t14$, suggesting that pCO_2 potentially had a negative influence on BP_{Total} under nutrient-rich conditions. This
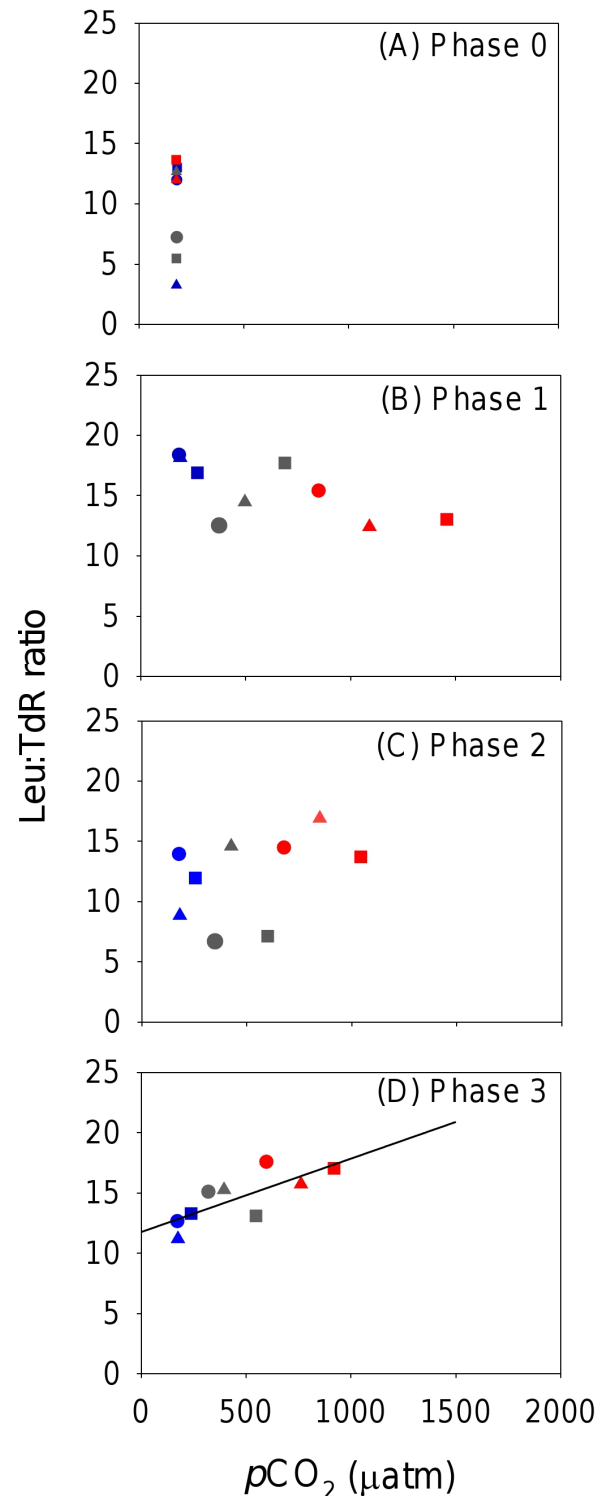

Fig. 7. Relationship between pCO_2 value and the Leu : TdR ratio in phase 0 (**A**), phase 1 (**B**), phase 2 (**C**), and phase 3 (**D**). Liner regression line in phase 3 (**D**): Leu : TdR ratio $= 0.006 \times pCO_2 + 11.7$ ($r^2 = 0.58$, $p < 0.05$, $n = 9$).

relationship disappeared afterward, however, the stepwise multiple regression analysis revealed that log-transformed BP_{Total} and pCO_2 levels were negatively correlated in phases 1 and 2, and positively correlated in phase 3. This suggests that pCO_2, in combination with multiple factors, potentially influenced bulk bacterial production throughout the experiment. This change in the slope direction also suggests that the pCO_2 effects on bacterial production are rather indirect than direct and that they changed during the experiment. Such effects could, for example, be related to the phytoplankton bloom, changing DOM quality and nutrient concentration.

In addition, a significant positive relationship between pCO_2 and the Leu : TdR ratio was found in phase 3. The Leu : TdR ratio is an indicator of the relative importance of protein and nucleic acid synthesis and it may reflect the balance of bacterial growth (Chin-Leo and Kirchman, 1988; Kirchman, 1992; Gasol et al., 1998; Ducklow, 2000; del Giorgio et al., 2011). In our experiment, the initial Leu : TdR ratio was relatively low (i.e., on average 10.4 ± 4.0) compared to the literature (i.e., 16.8, $n = 481$, subarctic Pacific, May–September, 0–80 m; Kirchman, 1992). Previous studies suggested that under favourable environmental conditions (e.g., greater availability of organic matter or higher temperature), bacteria might optimise DNA duplication over protein synthesis to maximise reproduction, resulting in a decline in the Leu : TdR ratio (Shiah and Ducklow, 1997; Gasol et al., 1998). In this regard, the large increase and variability of Leu : TdR ratio at $t5$ suggest that compared to the bacterial growth at $t - 1$, it was less balanced after pCO_2 manipulation; however, this trend changed and bacterial growth became more balanced again afterwards. All major phylogenetic groups of bacteria in aquatic systems assimilate both TdR and Leu and there is no difference in single cell activity for bacterial groups (Cottrell and Kirchman, 2003). Also, no significant difference in microbial community structure between mesocosms was observed after nutrient addition (Roy et al., 2013). Therefore, the variation of the ratio is not likely due to changes of bacterial community composition (Pérez et al., 2010). Throughout the experiment, bacterial growth was more balanced under high than under low pCO_2 levels. This suggests that the significant relationship between pCO_2 and the Leu : TdR ratio was probably due to the large fluctuation of the ratio in the low pCO_2 mesocosms rather than increase in the high pCO_2 mesocosms. Nevertheless, the stepwise multiple regression also revealed that pCO_2 influenced the variability of the Leu : TdR ratio in phase 3, implying that pCO_2 potentially influenced the balance of bacterial growth at the end of the experiment.

The effect of pCO_2 on BR, BGE and BCD of the natural bacterial community was examined for the first time in the present study; however, no clear trend was observed. Teira et al. (2012) examined the effect of CO_2 on two bacterial strains, *Roseobacter* and *Cytophaga*, and demonstrated that respiration of *Cytophaga* was significantly lower and BGE was higher in the elevated CO_2 treatment than control, while *Roseobacter* was not affected. Although an increase in the abundance of the phylum Bacteroidetes, which comprises *Cytophaga*, was observed after nutrient addition (Roy et al., 2013), no effect of pCO_2 on bacterial respiration of the natural community was observed. This could, for example, indicate that other Bacteroidetes genera are influenced differently than *Cytophaga* or that the response of *Cytophaga* is different in the presence of communities. Richardson (2000) suggested that the respiratory system of many bacterial species is highly flexible and that they adapt rapidly to changes within an environment. To support this notion, BGE did not influenced by effect of temperature was observed in the western Arctic Ocean (Kirchman et al., 2009). Hence, our data indicate that bulk bacterial respiration was independent of pCO_2 possibly due acclimation or adaptation to changing environmental conditions in this mesocosm study. Also, it is possible that indirect effects of phytoplankton were small, since the abundance of calcifying phytoplankton, which is mainly influenced by elevated pCO_2 and lower pH levels, was low in the experiment.

5 Summary

The goal of our study was to determine the potential impact of elevated pCO_2 on bacterial production, respiration and carbon metabolism in an Arctic fjord. There was no evidence that elevated pCO_2 levels influenced bacterial carbon metabolism, such as bacterial respiration, BGE and BCD of natural communities in mesocosms experiments. However, in combination with multiple factors, we found evidence that changes in pCO_2 influenced bacterial production, growth rate and growth balance on particular days or in particular phases of the experiment. Overall, our results suggest that changes in pCO_2 potentially influence bacterial production and growth balance rather than the conversion of dissolved organic matter into CO_2.

Appendix A

Table A1. Stepwise multiple regression analysis of log-BP$_{Total}$, csBP$_{Total}$, log-BP$_{Free}$, csBP$_{Free}$, log-BR, csBR, BGE, BCD and Leu : TdR ratio in phase1, 2 and 3. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

	Phase 1				Phase 2				Phase 3			
	F	Adj. r2	n	variables	F	Adj. r2	n	variables	F	Adj. r2	n	variables
log-BP$_{Total}$	7.6***	0.55	33	Si** pCO$_2$* NH$_4$* PO$_4$ Chl a	4.6*	0.18	33	pCO$_2$* log-VA*	10.1**	0.80	15	Si** PO$_4$* log-VA* NH$_4$ Temp pCO$_2$ Chl a
csBP$_{Total}$	13.1***	0.60	33	NH$_4$*** PO$_4$** Chl a** Temp*	4.3*	0.24	33	PO$_4$* Chl a* NO$_3$	27.3**	0.93	15	Si*** Temp*** PO$_4$** NH$_4$** pCO$_2$ Sal log-VA
log-BP$_{Free}$		nd			5.2*	0.37	15	NH$_4$** Sal		nd		
csBP$_{Free}$	7.0*	0.63	8	Chl a* NH$_4$	8.7**	0.73	15	pCO$_2$** Si* Sal* log-VA NH$_4$		nd		
log-BR	4.6*	0.50	12	Temp PO$_4$ log-VA Chl a	6.1*	0.60	11	NH$_4$*** Temp** Sal		nd		
csBR	7.3*	0.36	12	PO$_4$*	6.4*	0.62	11	NH$_4$** Temp* Sal	14.5*	0.82	7	PO$_4$** Temp
BGE		nd				nd				nd		
BCD		nd			7.5*	0.71	9	NH$_4$** Temp* Sal*		nd		
Leu : TdR ratio	20.1***	0.75	33	NO$_3$** NH$_4$** Si* Temp log-VA	18.3***	0.62	33	Sal*** NH$_4$** Temp	12.6**	0.81	15	pCO$_2$** NO$_3$** log-VA** Chl a* Si

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