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► **To cite this version:**

C. Motegi, T. Tanaka, J. Piontek, C. P. D. Brussaard, Jean-Pierre Gattuso, et al.. Effect of CO<sup>2</sup> enrichment on bacterial metabolism in an Arctic fjord. *Biogeosciences*, 2013, 10 (5), pp.3285-3296. 10.5194/bg-10-3285-2013 . hal-01543125

**HAL Id: hal-01543125**

**<https://hal.sorbonne-universite.fr/hal-01543125>**

Submitted on 20 Jun 2017

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## Effect of CO<sub>2</sub> enrichment on bacterial metabolism in an Arctic fjord

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Received: 9 August 2012 – Published in Biogeosciences Discuss.: 31 October 2012

Revised: 30 March 2013 – Accepted: 21 April 2013 – Published: 15 May 2013

**Abstract.** The anthropogenic increase of carbon dioxide (CO<sub>2</sub>) alters the seawater carbonate chemistry, with a decline of pH and an increase in the partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ). Although bacteria play a major role in carbon cycling, little is known about the impact of rising  $p\text{CO}_2$  on bacterial carbon metabolism, especially for natural bacterial communities. In this study, we investigated the effect of rising  $p\text{CO}_2$  on bacterial production (BP), bacterial respiration (BR) and bacterial carbon metabolism during a mesocosm experiment performed in Kongsfjorden (Svalbard) in 2010. Nine mesocosms with  $p\text{CO}_2$  levels ranging from ca. 180 to 1400  $\mu\text{atm}$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  after nutrient addition (day 14). In addition, increasing  $p\text{CO}_2$  enhanced the leucine to thymidine (Leu : TdR) ratio at the end of experiment, suggesting that  $p\text{CO}_2$  may alter the growth balance of bacteria. Stepwise multiple regression analysis suggests that multiple factors, including  $p\text{CO}_2$ , 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  $p\text{CO}_2$  was observed for BR, bacterial carbon demand and bacterial growth efficiency. Overall, the results suggest that changes in  $p\text{CO}_2$  potentially influence bacterial production, growth rate and growth balance rather than the conversion of dissolved organic matter into CO<sub>2</sub>.

### 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<sub>2</sub> ( $p\text{CO}_2$ ) and that  $p\text{CO}_2$  will increase from 280 to nearly 384  $\mu\text{atm}$  over the next 250 yr (IPCC, 2007). The increase in  $p\text{CO}_2$  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  $p\text{CO}_2$  effects on bacterial carbon metabolism. Previous studies have examined the effect of  $p\text{CO}_2$  on microbial communities and found that  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  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<sub>2</sub> 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  $p\text{CO}_2$  on BP, BR and bacterial carbon metabolism in an Arctic fjord. We hypothesised that changing  $p\text{CO}_2$  levels would not influence bacterial carbon metabolism of natural communities in mesocosm experiments. Particularly, we focused on how changes in  $p\text{CO}_2$  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  $t_0$ ) and July 7 (day  $t_{30}$ ) 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<sub>2</sub> saturated water was applied between  $t_1$  and  $t_4$  to obtain 8 different CO<sub>2</sub> levels: 185  $\mu\text{atm}$  (M3 and M7, two controls with no CO<sub>2</sub> addition), 270  $\mu\text{atm}$  (M2), 375  $\mu\text{atm}$  (M4), 480  $\mu\text{atm}$  (M8), 685  $\mu\text{atm}$  (M1), 820  $\mu\text{atm}$  (M6), 1050  $\mu\text{atm}$  (M5) and 1420  $\mu\text{atm}$  (M9). No further CO<sub>2</sub> manipulation was done after initially targeted  $p\text{CO}_2$  levels were reached (for details see Riebesell et al., 2013). Due to gas exchange and photoautotrophic uptake,  $p\text{CO}_2$  levels declined in the mesocosms during the experiment, and final CO<sub>2</sub> levels ranged from 160 to 855  $\mu\text{atm}$ . At day 13 of the experiment ( $t_{13}$ ), inorganic nutrients (nitrate, silicate and phosphate: 5, 2.5, and 0.31  $\mu\text{mol L}^{-1}$ , respectively) were added. Based on  $p\text{CO}_2$  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<sub>2</sub> manipulation,  $t - 4$  to  $t_3$ ), Phase 1 (from the end of CO<sub>2</sub> manipulation until nutrient addition,  $t_4$  to  $t_{12}$ ), Phase 2 (after nutrient addition until 2nd chlorophyll minimum,  $t_{13}$  to  $t_{21}$ ), and Phase 3 (from the 2nd chlorophyll minimum until end of experiment,  $t_{22}$  to  $t_{30}$ ) (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<sub>Total</sub>) and filtered (0.8  $\mu\text{m}$ , Nuclepore, Millipore) water (BP<sub>Free</sub>) were determined from the incorporation rate of <sup>3</sup>H-thymidine

(Kirchman, 2001) using a centrifugation method. Triplicate subsamples and one trichloroacetic acid (TCA)-killed control (1.5 mL) were spiked with [methyl-<sup>3</sup>H] TdR (1.77 TBq mmol<sup>-1</sup>, PerkinElmer, NET027W, final conc. 10 nmol L<sup>-1</sup>) 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 (18000 × *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 <sup>3</sup>H-TdR incorporation rates were converted to cell production by the conversion factor 2 × 10<sup>18</sup> cells per mole of TdR (Fuhrman and Azam, 1982). Cell-specific bacterial growth rate of unfiltered (csBP<sub>Total</sub>, d<sup>-1</sup>) and filtered fractions (csBP<sub>Free</sub>, d<sup>-1</sup>) were estimated as BP<sub>Total</sub> or BP<sub>Free</sub> (cells L<sup>-1</sup> d<sup>-1</sup>) divided by bulk bacterial abundance (cells L<sup>-1</sup>). Bacterial production rates of attached fraction (BP<sub>Att</sub>) were estimated by BP<sub>Free</sub> subtracting from BP<sub>Total</sub>.

### 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<sub>2</sub> consumption rate (μmol O<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>) 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<sup>-1</sup> d<sup>-1</sup>) was estimated as BR (fg C L<sup>-1</sup> d<sup>-1</sup>) divided by bulk bacterial abundance (cells L<sup>-1</sup>) 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:

$$\text{BGE} = \frac{\text{BP}}{\text{BP} + \text{BR}} \quad (1)$$

$$\text{BCD} = \text{BP} + \text{BR} \quad (2)$$

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<sup>-1</sup> d<sup>-1</sup>) and TdR incorporation (pmol TdR L<sup>-1</sup> d<sup>-1</sup>) ratio, data on incorporation rates of <sup>14</sup>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 (FAC-SCalibur, 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<sub>Total</sub>, csBP<sub>Total</sub>, BP<sub>Free</sub>, csBP<sub>Free</sub>, 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), *p*CO<sub>2</sub> (Bellerby et al., 2012), nitrate (NO<sub>3</sub>, Schulz et al., 2013), phosphate (PO<sub>4</sub>, Schulz et al., 2013), silicate (Si, Schulz et al., 2013), ammonium (NH<sub>4</sub>, 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<sub>Total</sub>, BP<sub>Free</sub>, BR and VA were log-transformed (i.e., log-BP<sub>Total</sub>, log-BP<sub>Free</sub>, log-BR and log-VA).

Linear regression analysis was conducted to examine the relationship between *p*CO<sub>2</sub> 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<sub>Total</sub>, BP<sub>Free</sub> and BR in the mesocosms before adding CO<sub>2</sub> saturated water ( $t - 1$ ) were  $3.4\text{--}4.4 \times 10^8 \text{ cells L}^{-1} \text{ d}^{-1}$ ,  $2.8\text{--}3.7 \times 10^8 \text{ cells L}^{-1} \text{ d}^{-1}$ , and  $10.9\text{--}51.4 \mu\text{g C L}^{-1} \text{ d}^{-1}$ , respectively (Fig. 1a, b and c). Generally, BP<sub>Total</sub> decreased until  $t_7$ , 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<sub>Total</sub> gradually increased towards the end of the experiment (Fig. 1a; Phase 2 and 3). BP<sub>Free</sub> decreased between  $t_0$  and  $t_7$  and gradually increased towards the end of the experiment (Fig. 1b). BP<sub>Total</sub> and BP<sub>Free</sub> varied from 0.7 to 5.6, and 0.5 to  $4.6 \times 10^8 \text{ cells L}^{-1} \text{ d}^{-1}$ , respectively, during the experiment (Fig. 1a and b;  $t_1\text{--}t_{28}$ ). When BP<sub>Free</sub> were subtracted from BP<sub>Total</sub>, 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  $51 \mu\text{g C L}^{-1} \text{ d}^{-1}$ , without a clear pattern with time or  $p\text{CO}_2$  (Fig. 1c).

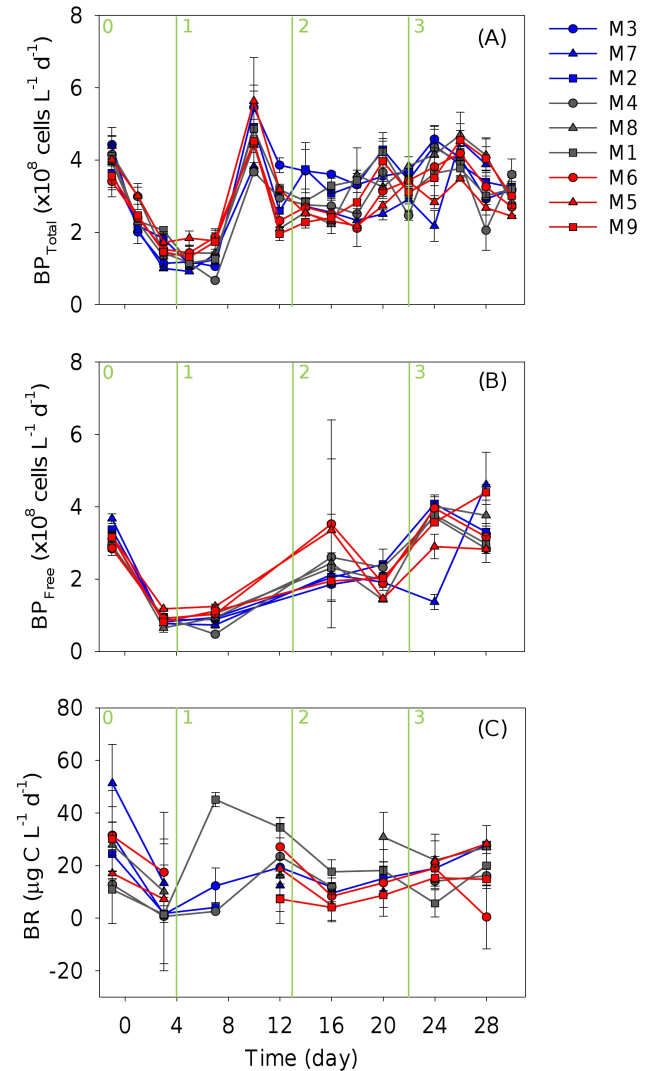
The csBP<sub>Total</sub>, csBP<sub>Free</sub> and csBR at  $t - 1$  ranged between 0.16 and  $0.22 \text{ d}^{-1}$ , 0.13 and  $0.17 \text{ d}^{-1}$ , and 5.4 and  $23.6 \text{ fg C cell}^{-1} \text{ d}^{-1}$ , respectively (Fig. 2a, b and c). csBP<sub>Total</sub> gradually increased from  $t_0$  until  $t_{10}$ , after which it gradually declined to  $0.04\text{--}0.09 \text{ d}^{-1}$  on  $t_{28}$  (Fig. 2a). csBP<sub>Free</sub> slightly decreased at phase 0, then increased after closing the mesocosms (Phase 1). Consistent with csBP<sub>Total</sub>, csBP<sub>Free</sub> 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<sub>Free</sub> % of BP<sub>Total</sub> at phases 0, 1, 2 and 3 was  $70 \pm 15$ ,  $69 \pm 11$ ,  $70 \pm 19$  and  $95 \pm 9 \%$  ( $\pm\text{SD}$ ), whereby phase 3 exhibited significantly higher values than phases 0 and 1 (Fig. 3;  $t$  test, both  $p < 0.001$ ). BP<sub>Att</sub> dominated, especially at  $t_3$  (the end of  $p\text{CO}_2$  manipulation) and  $t_{20}$  (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

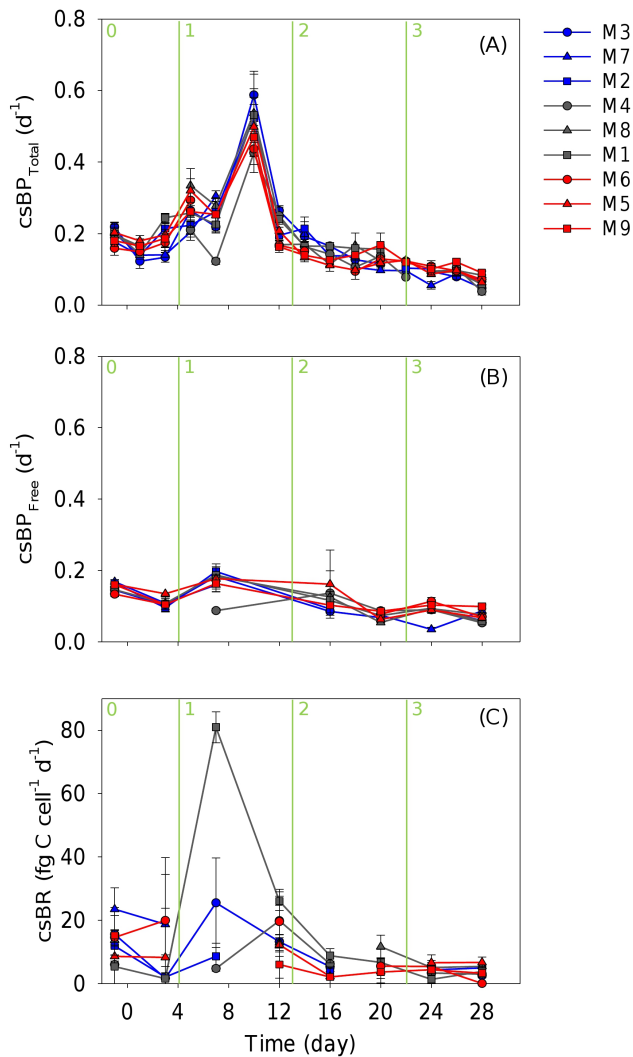
Average BGE ranged between 13 and 53 % in phase 0, and varied from 4 to 35, 9 to 44, and 21 to 46 % in phases 1, 2 and 3, respectively (Fig. 4a). Average BCD at the beginning of the experiment ranged between 10.5 and  $58.7 \mu\text{g C L}^{-1} \text{ d}^{-1}$ , and varied from 3.6 to 47.2, 12.8 to 33.7, 19.6 to  $32.3 \mu\text{g C L}^{-1} \text{ d}^{-1}$  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  $p\text{CO}_2$  or time of experiment.

The Leu : TdR ratio averaged  $10.4 \pm 4.0$  (range 3.2 to 14.7) at the beginning of experiment ( $t - 1$ ), and increased to  $25.8 \pm 8.1$  (range 15.1 to 35.2) on  $t_5$  (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  $p\text{CO}_2$  treatments are coloured in blue, grey, and red.

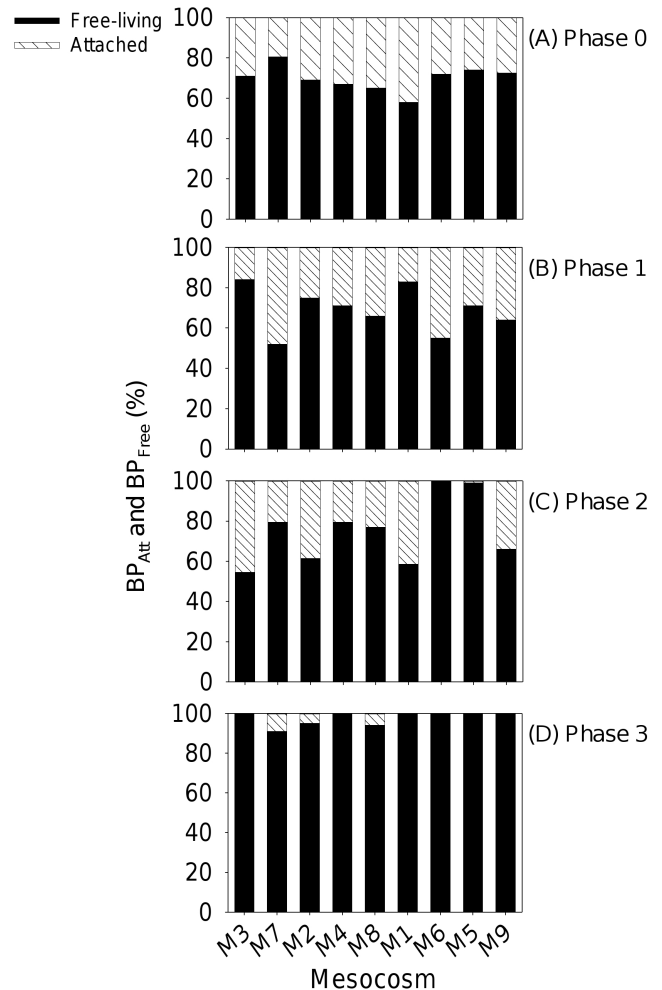
(the time span after the  $p\text{CO}_2$  manipulation and just before nutrient addition) the average Leu : TdR ratio in low  $p\text{CO}_2$  mesocosms (M3, 7, 2) was 17.8, higher than for the medium (M4, 8, 1) and high  $p\text{CO}_2$  mesocosms (M6, 5, 9) with ratios of 14.9 and 13.6, respectively. 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  $p\text{CO}_2$  mesocosms (16.9; M6, 5, 9) compared to low (12.7; M3, 7, 2) and medium  $p\text{CO}_2$  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  $\pm$  stand deviations for triplicate measurements ( $n = 3$ ). For colour and symbol coding, see Fig. 1.

### 3.3 Relationship between $p\text{CO}_2$ and bacterial activity in different phases of the experiment

Statistical analysis showed that log-transformed  $\text{BP}_{\text{Total}}$  was negatively correlated with  $p\text{CO}_2$  concentration after nutrient addition at  $t_{14}$  ( $r^2 = 0.51$ ,  $p < 0.05$ ,  $n = 9$ ; Fig. 6). No significant relationships between  $p\text{CO}_2$  and  $\text{csBP}_{\text{Total}}$ ,  $\text{BP}_{\text{Free}}$ ,  $\text{csBP}_{\text{Free}}$ ,  $\text{BR}$ ,  $\text{csBR}$ ,  $\text{BGE}$  or  $\text{BCD}$  were found otherwise ( $p > 0.05$ ). However, the  $\text{Leu}:\text{TdR}$  ratio was negatively correlated with  $p\text{CO}_2$  at  $t_5$  and  $t_7$  (Linear regression,  $t_5$ ;  $r^2 = 0.41$ ,  $p < 0.05$ ,  $n = 9$ ,  $t_7$ ;  $r^2 = 0.57$ ,  $p < 0.05$ ,  $n = 9$ ), but positively correlated at  $t_{24}$  and  $t_{26}$  (Linear regression,  $t_{24}$ ;  $r^2 = 0.51$ ,  $p < 0.05$ ,  $n = 9$ ,  $t_{26}$ ;  $r^2 = 0.55$ ,  $p < 0.05$ ,  $n = 9$ ). There was also a positive correlation between aver-



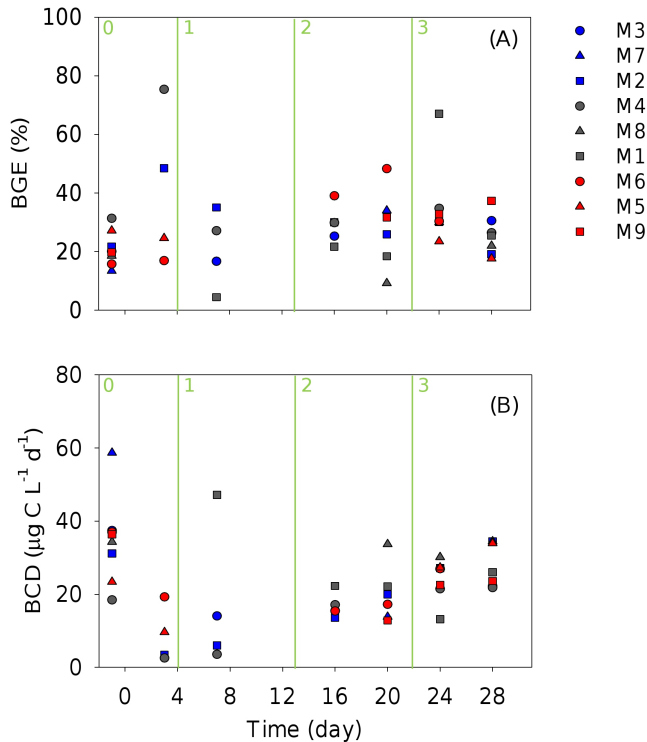
**Fig. 3.** Percentage of attached (line bars) and free-living bacterial production (solid bars) in phase 0 (A), phase 1 (B), phase 2 (C) and phase 3 (D).

aged  $p\text{CO}_2$  and averaged  $\text{Leu}:\text{TdR}$  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  $\log\text{-BP}_{\text{Total}}$  was explained by  $\text{Si}$ ,  $p\text{CO}_2$ ,  $\text{NH}_4$ ,  $\text{PO}_4$  and  $\text{Chl } a$  in phase 1, 18 % by  $p\text{CO}_2$  and  $\log\text{-VA}$  in phase 2, and 80 % by  $\text{Si}$ ,  $\text{PO}_4$ ,  $\log\text{-VA}$ ,  $\text{NH}_4$ ,  $\text{Temp}$ ,  $p\text{CO}_2$  and  $\text{Chl } a$  in phase 3. Sixty percent of the variation in  $\text{csBP}_{\text{Total}}$  was explained by  $\text{NH}_4$ ,  $\text{PO}_4$ ,  $\text{Chl } a$ , and  $\text{Temp}$  in phase 1, 24 % by  $\text{PO}_4$ ,  $\text{Chl } a$  and  $\text{NO}_3$  in phase 2, and 93 % by  $\text{Si}$ ,  $\text{Temp}$ ,  $p\text{CO}_2$ ,  $\text{PO}_4$ ,  $\text{NH}_4$ , and  $\text{Sal}$  and  $\log\text{-VA}$  in phase 3.  $\text{NH}_4$ , and  $\text{Sal}$  explained 37 % of the variation in  $\log\text{-BP}_{\text{Free}}$ , in phase 2. For  $\text{csBP}_{\text{Free}}$ , 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.

variation was explained by Chl *a* and NH<sub>4</sub> in phase 1 and 73 % by *p*CO<sub>2</sub>, Si, Sal, log-VA and NH<sub>4</sub> in phase 2.

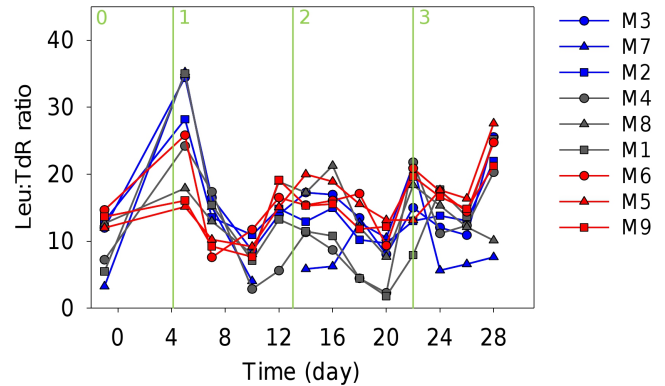
Fifty percent of the variation in log-BR was explained by Temp, PO<sub>4</sub>, log-VA and Chl *a* in phase 1. Environmental variables of NH<sub>4</sub>, Temp and Sal explained 60 % of the variations in log-BR, 62 % of csBR, 71 % of BCD and 62 % of the Leu : TdR ratio in phase 2. Thirty six percent of the variation in csBR was explained by PO<sub>4</sub> in phase 1 and 82 % by PO<sub>4</sub> and Temp in phase 3. Seventy five percent of the variation in the Leu : TdR ratio was explained by NO<sub>3</sub>, NH<sub>4</sub>, Si, Temp and log-VA in phase 1, and 81 % by *p*CO<sub>2</sub>, NO<sub>3</sub>, 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<sub>Free</sub>) 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-



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

get *p*CO<sub>2</sub> levels were set at *t*4, *p*CO<sub>2</sub> 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 *t*13, the concentration of NO<sub>3</sub> was close to the detection limit, PO<sub>4</sub> 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<sub>3</sub>, PO<sub>4</sub> 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 *p*CO<sub>2</sub> was observed on phytoplankton composition and positive effect on its growth (Brussaard et al., 2013; Schulz et al., 2013). Furthermore, the concentration of NH<sub>4</sub><sup>+</sup> gradually decreased during the experiment (Schulz et al., 2013), whereas the average dissolved organic carbon (DOC) concentration of all mesocosms significantly increased between *t*4 and *t*13 (Engel et al., 2013).

### 4.2 Temporal variations of bacterial variables

BP<sub>Total</sub> and BP<sub>Free</sub> decreased during CO<sub>2</sub> manipulation (*t* – 1 to *t*4), 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 *t*4 and *t*13 (Engel et al., 2013) as a consequence of phytoplankton bloom and demise (Brussaard et al., 2013; Schultz et al., 2013) potentially stimulated BP<sub>Total</sub> and csBP<sub>Total</sub> at *t*10. 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  $25.8 \pm 8.1$  at *t*5 to  $7.8 \pm 3.1$  at *t*10, 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<sub>Total</sub>, csBP<sub>Total</sub>, csBP<sub>Free</sub>, and Leu : TdR in phases 1, 2 and 3. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ 

	Phase 1				Phase 2				Phase 3			
	<i>F</i>	Adj. r2	<i>n</i>	variables	<i>F</i>	Adj. r2	<i>n</i>	variables	<i>F</i>	Adj. r2	<i>n</i>	variables
log-BP <sub>Total</sub>	7.6***	0.55	33	Si** <i>p</i> CO <sub>2</sub> * NH <sub>4</sub> * PO <sub>4</sub> Chl <i>a</i>	4.6*	0.18	33	<i>p</i> CO <sub>2</sub> * log-VA*	10.1**	0.80	15	Si** PO <sub>4</sub> * log-VA* NH <sub>4</sub> Temp <i>p</i> CO <sub>2</sub> Chl <i>a</i>
csBP <sub>Total</sub>	13.1***	0.60	33	NH <sub>4</sub> *** PO <sub>4</sub> ** Chl <i>a</i> ** Temp*	4.3*	0.24	33	PO <sub>4</sub> * Chl <i>a</i> * NO <sub>3</sub>	27.3**	0.93	15	Si*** Temp*** PO <sub>4</sub> ** NH <sub>4</sub> ** <i>p</i> CO <sub>2</sub> Sal log-VA
csBP <sub>Free</sub>	7.0*	0.63	8	Chl <i>a</i> * NH <sub>4</sub>	8.7**	0.73	15	<i>p</i> CO <sub>2</sub> ** Si* Sal* log-VA NH <sub>4</sub>	nd			
Leu : TdR ratio	20.1***	0.75	33	NO <sub>3</sub> ** NH <sub>4</sub> ** Si* Temp log-VA	18.3***	0.62	33	Sal*** NH <sub>4</sub> ** Temp	12.6**	0.81	15	<i>p</i> CO <sub>2</sub> ** NO <sub>3</sub> ** log-VA** Chl <i>a</i> * Si

Please see Table A1 for the result of stepwise multiple regression analysis of log-BP<sub>Free</sub>, log-BR, csBR, BGE and BCD.

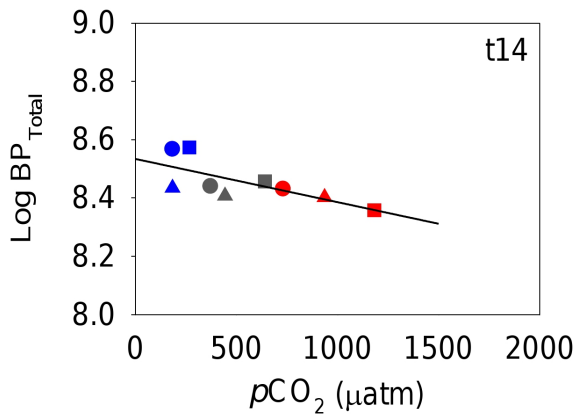
other environmental variables, potentially influenced BP<sub>Total</sub>, csBP<sub>Total</sub>, csBP<sub>Free</sub> and BR variability in phase 1. Interestingly, the bacterial community composition determined by T-RFLP showed that species richness and the diversity index increased during *p*CO<sub>2</sub> manipulation and decreased at *t*10 when we observed the peak of BP<sub>Total</sub> (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 *t*10 in our study.

After nutrient addition at *t*13, Chl *a* showed two peaks (Schulz et al., 2013), while BP<sub>Total</sub> and BP<sub>Free</sub> 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<sub>Total</sub>, csBP<sub>Free</sub>) 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 *p*CO<sub>2</sub> manipulated experiments, i.e., the high percentage of BP<sub>Free</sub> 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<sub>Free</sub>) was observed in this study. However, there is evidence that mortality due to





**Fig. 6.** Relationship between  $p\text{CO}_2$  value and  $\log \text{BP}_{\text{Total}}$  at  $t14$ . Linear regression line at  $t14$ :  $\log \text{BP}_{\text{Total}} = -0.00015 \times p\text{CO}_2 + 8.53$  ( $r^2 = 0.51$ ,  $p < 0.05$ ,  $n = 9$ ).

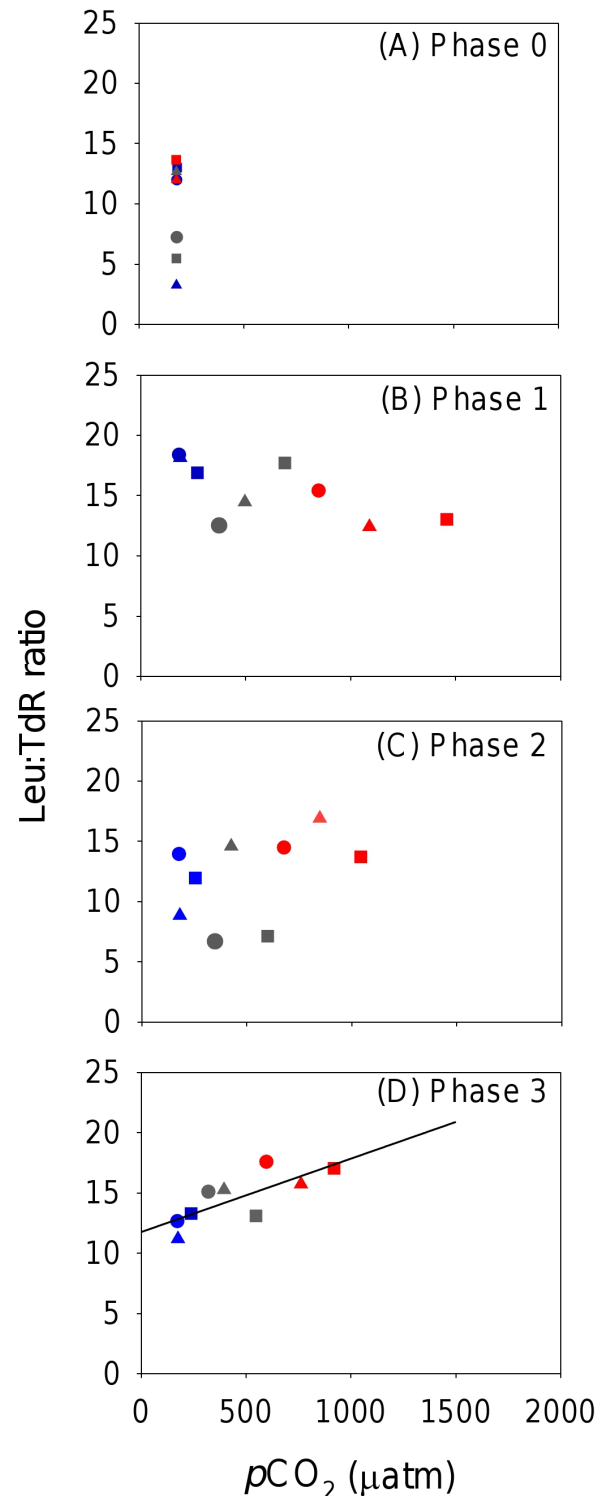
viral lysis rather than mortality due to flagellate grazing decreased at the end of the experiment (unpublished data).

In addition, we used prefiltered water ( $< 0.8 \mu\text{m}$  pore size and low pressure filtration) to estimate BR and  $\text{BP}_{\text{Free}}$ . Twelve of the 62 measurements of  $\text{BP}_{\text{Free}}$  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 $p\text{CO}_2$ on bacterial carbon metabolism

Although a large range of  $p\text{CO}_2$  levels was used in the experiment, the general patterns of  $\text{BP}_{\text{Total}}$ ,  $\text{csBP}_{\text{Total}}$ ,  $\text{BP}_{\text{Free}}$  and  $\text{csBP}_{\text{Free}}$  were similar in all mesocosms during the experiment. This indicates that effect of  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  on bacterial variables was found. In our study,  $\text{BP}_{\text{Total}}$  significantly decreased with increasing  $p\text{CO}_2$  at  $t14$ , suggesting that  $p\text{CO}_2$  potentially had a negative influence on  $\text{BP}_{\text{Total}}$  under nutrient-rich conditions. This



**Fig. 7.** Relationship between  $p\text{CO}_2$  value and the Leu : TdR ratio in phase 0 (A), phase 1 (B), phase 2 (C), and phase 3 (D). Linear regression line in phase 3 (D):  $\text{Leu} : \text{TdR ratio} = 0.006 \times p\text{CO}_2 + 11.7$  ( $r^2 = 0.58$ ,  $p < 0.05$ ,  $n = 9$ ).

relationship disappeared afterward, however, the stepwise multiple regression analysis revealed that log-transformed BP<sub>Total</sub> and  $p\text{CO}_2$  levels were negatively correlated in phases 1 and 2, and positively correlated in phase 3. This suggests that  $p\text{CO}_2$ , in combination with multiple factors, potentially influenced bulk bacterial production throughout the experiment. This change in the slope direction also suggests that the  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $10.4 \pm 4.0$ ) 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  $p\text{CO}_2$  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  $p\text{CO}_2$  levels. This suggests that the significant relationship between  $p\text{CO}_2$  and the Leu:TdR ratio was probably due to the large fluctuation of the ratio in the low  $p\text{CO}_2$  mesocosms rather than increase in the high  $p\text{CO}_2$  mesocosms. Nevertheless, the stepwise multiple regression also revealed that  $p\text{CO}_2$  influenced the variability of the Leu:TdR ratio in phase 3, implying that  $p\text{CO}_2$  potentially influenced the balance of bacterial growth at the end of the experiment.

The effect of  $p\text{CO}_2$  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<sub>2</sub> on two bacterial strains, *Roseobacter* and *Cytophaga*, and demonstrated that respiration of *Cytophaga* was significantly lower and BGE was higher in the elevated CO<sub>2</sub> 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  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  and lower pH levels, was low in the experiment.

## 5 Summary

The goal of our study was to determine the potential impact of elevated  $p\text{CO}_2$  on bacterial production, respiration and carbon metabolism in an Arctic fjord. There was no evidence that elevated  $p\text{CO}_2$  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  $p\text{CO}_2$  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  $p\text{CO}_2$  potentially influence bacterial production and growth balance rather than the conversion of dissolved organic matter into CO<sub>2</sub>.

## Appendix A

**Table A1.** Stepwise multiple regression analysis of log-BP<sub>Total</sub>, csBP<sub>Total</sub>, log-BP<sub>Free</sub>, csBP<sub>Free</sub>, 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			
	<i>F</i>	Adj. r2	<i>n</i>	variables	<i>F</i>	Adj. r2	<i>n</i>	variables	<i>F</i>	Adj. r2	<i>n</i>	variables
log-BP <sub>Total</sub>	7.6***	0.55	33	Si** pCO <sub>2</sub> * NH <sub>4</sub> * PO <sub>4</sub> Chl <i>a</i>	4.6*	0.18	33	pCO <sub>2</sub> * log-VA*	10.1**	0.80	15	Si** PO <sub>4</sub> * log-VA* NH <sub>4</sub> Temp pCO <sub>2</sub> Chl <i>a</i>
csBP <sub>Total</sub>	13.1***	0.60	33	NH <sub>4</sub> *** PO <sub>4</sub> ** Chl <i>a</i> ** Temp*	4.3*	0.24	33	PO <sub>4</sub> * Chl <i>a</i> * NO <sub>3</sub>	27.3**	0.93	15	Si*** Temp*** PO <sub>4</sub> ** NH <sub>4</sub> ** pCO <sub>2</sub> Sal log-VA
log-BP <sub>Free</sub>		nd			5.2*	0.37	15	NH <sub>4</sub> ** Sal		nd		
csBP <sub>Free</sub>	7.0*	0.63	8	Chl <i>a</i> * NH <sub>4</sub>	8.7**	0.73	15	pCO <sub>2</sub> ** Si* Sal* log-VA NH <sub>4</sub>		nd		
log-BR	4.6*	0.50	12	Temp PO <sub>4</sub> log-VA Chl <i>a</i>	6.1*	0.60	11	NH <sub>4</sub> *** Temp** Sal		nd		
csBR	7.3*	0.36	12	PO <sub>4</sub> *	6.4*	0.62	11	NH <sub>4</sub> ** Temp* Sal	14.5*	0.82	7	PO <sub>4</sub> ** Temp
BGE		nd				nd				nd		
BCD		nd			7.5*	0.71	9	NH <sub>4</sub> ** Temp* Sal*		nd		
Leu : TdR ratio	20.1***	0.75	33	NO <sub>3</sub> ** NH <sub>4</sub> ** Si* Temp log-VA	18.3***	0.62	33	Sal*** NH <sub>4</sub> ** Temp	12.6**	0.81	15	pCO <sub>2</sub> ** NO <sub>3</sub> ** log-VA** Chl <i>a</i> * Si

Supplementary material related to this article is available online at: <http://www.biogeosciences.net/10/3285/2013/bg-10-3285-2013-supplement.pdf>.

**Acknowledgements.** This work is a contribution to the “European Project on Ocean Acidification” (EPOCA) which received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 211384. We gratefully acknowledge the logistical support of Greenpeace International for its assistance with the transport of the mesocosm facility from Kiel to Ny-Ålesund and back to Kiel. We also thank the captains and crews of M/V *ESPERANZA* of Greenpeace and R/V *Viking Explorer* of the University Centre in Svalbard (UNIS) for assistance during mesocosm transport and during deployment and recovery in Kongsfjorden. We gratefully acknowledge the KOSMOS team of IFM-GEOMAR for support during fieldwork. We thank the staff of the French-German Arctic Research Base at Ny-Ålesund, in particular Marcus Schumacher, for on-site logistical support. Financial support was also provided by IPEV, The French Polar Institute.

Edited by: U. Riebesell



The publication of this article is financed by CNRS-INSU.

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