Biogeosciences Discuss., 9, 15213–15235, 2012
www.biogeosciences-discuss.net/9/15213/2012/
doi:10.5194/bgd-9-15213-2012

Biogeosciences Discussions

Effect of CO_2 enrichment on bacterial production and respiration and on bacterial carbon metabolism in Arctic waters

C. Motegi[1,2], **T. Tanaka**[1,2], **J. Piontek**[3,4], **C. P. D. Brussaard**[5], **J. P. Gattuso**[1,2], **and M. G. Weinbauer**[1,2]

[1]Université Pierre et Marie Curie-Paris 6, Laboratoire d'Océanographie de Villefranche, 06230, Villefranche-sur-Mer Cedex, France
[2]Laboratoire d'Océanographie de Villefranche, CNRS, UMR7093, 06230, Villefranche-sur-Mer Cedex, France
[3]Helmholtz Centre for Ocean Research Kiel (GEOMAR), Germany
[4]Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany
[5]Royal Netherlands Institute for Sea Research (NIOZ), Dept. of Biological Oceanography, BP 59, 1790 AB Den Burg, The Netherlands

Received: 9 August 2012 – Accepted: 2 September 2012 – Published: 31 October 2012

Correspondence to: C. Motegi (chiaki.motegi@takuvik.ulaval.ca)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The impact of rising carbon dioxide (pCO_2) on bacterial production (BP), bacterial respiration (BR) and bacterial carbon metabolism was investigated during the mesocosm experiment in Kongsfjord (Svalbard) in 2010. The mesocosm experiment lasted 30 days and nine mesocosms with pCO_2 levels ranging from ca. 180 to 1400 µatm were used. Generally, BP gradually decreased in all mesocosms in an initial phase, showed a large (3.6-fold in average) but temporary increase on day 10, and increased slightly afterwards. BP increased with increasing pCO_2 at the beginning of the experiment (day 5). This trend became inversed and BP decreased with increasing pCO_2 on day 14 (after nutrient addition). Interestingly, increasing pCO_2 enhanced the leucine and thymidine ratio at the end of experiment, suggesting that pCO_2 may alter the growth balance of bacteria. 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. Our results suggest that (1) the response to elevated pCO_2 had a strong temporal variation, potentially linked to the nutrient status, and (2) pCO_2 had an influence on biomass accumulation (i.e. BP) rather than on the conversion of dissolved organic matter into CO_2 (i.e. BR).

1 Introduction

Bacteria are the main organisms that incorporate and mineralize 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 estimate carbon metabolism components (e.g. bacterial carbon demand and bacterial growth efficiency) for understanding impact of bacteria on global marine carbon flux (del Giorgio and Cole, 2000; Robinson, 2008). A previous study suggests that bacterial carbon metabolism components are mostly connected and potentially influenced by environmental condition (i.e. temperature, energy

BGD
9, 15213–15235, 2012

limitation) (del Giorgio et al., 2011). However, only a few studies have focused on effect of environmental condition on bacterial carbon metabolism.

Recent studies have reported that the world ocean is absorbing about 25 % of 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 threaten calcifying organisms (e.g. Riebesell et al., 2000) and primary production (reviewed by Liu et al., 2010); however, few studies have focused on pCO_2 effects on bacterial metabolism. Previous studies have examined effect of pCO_2 on microbial communities and found that pCO_2 potentially alters bacterial production (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); other studies have found little or no effect of pCO_2on bacterial production (Grossart et al., 2006; Allgaier et al., 2008; Arnosti et al., 2011), 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). 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, there is also evidence that some functions such as nitrification and bacterial production can be changed, which would influence biogeochemical processes (Liu et al., 2011). However, there is no study on the pCO_2 influence on anabolic and catabolic processes of carbon by bacteria and on bacterial carbon metabolism components.

In the present study, a mesocosm experiment (Svalbard 2010 mesocosm experiment of the European Project on Ocean Acidification (EPOCA) project) was performed that was designed to determine the potential impact of changes in pCO_2 on BP, BR and bacterial carbon metabolism in Kongsfjorden, Svalbard. Particularly, we focused on how changes in pCO_2 may influence (1) bacterial cell production, (2) bacterial respiration, (3) the amount of new bacterial biomass produced per unit of organic C substrate

BGD
9, 15213–15235, 2012

assimilated (i.e. bacterial growth efficiency, BGE), (4) amount of organic C assimilated by bacteria (i.e. bacterial carbon demand, BCD), and (5) the ratio of biomass produced to substrate assimilated (i.e. Leucine : Thymidine ratio) in the Arctic Ocean.

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. (2012). 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 t-1 to t4 to obtain 8 different level of CO_2 : 185 µatm (M3 and M7, 2 controls of no CO_2 saturated water addition mesocosms), 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 reached initial pCO_2 levels (for details see Riebesell et al., 2012). Due to gas exchange and photoautotrophic uptake, pCO_2 levels declined in the mesocosms during the experiment, and final levels of CO_2 were range from 160 to 855 µatm. At day 13 of the experiment (t13), inorganic nutrients (nitrate, silicate and phosphate: 5, 2.5, and 0.32 µmol l^{-1}, respectively) were added. Subsamples for BP and BR were obtained every 2 and 4 days. Water samples were collected using clean depth integrated sampler (12-l volume) at depths between the surface and 12 m for all mesocosms, transferred to 2-l polycarbonate bottle (Nalgen) 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 minimize contamination.

BGD
9, 15213–15235, 2012

2.2 Bacterial production (BP)

Bacterial production rates were determined from the incorporation rate of ^{3}H-thymidine (BP_{TdR}, Kirchman 2001) and ^{14}C-leucine (BP_{Leu}, for a detailed method description see Piontek et al., 2012) using a centrifuge method. Triplicate subsamples (1.5 ml) and 1 trichloroacetic acid (TCA)-killed control were spiked with [methyl-^{3}H] TdR (1.77 TBq mmol^{-1}, PerkinElmer, NET027W, final conc. 10 nM) and incubated for 1 h at 2°C in the dark. Extraction by precipitations with 5 % cold TCA was followed by cold ethanol rinsing using a temperature controlled desktop centrifuge (18 000 × g at 4 °C for 10 min for each run; Sigma, 1–15 K). The extracts were then completely dried and mixed with scintillation cocktail (1 ml, Ultima Gold, PerkinElmer) for the radioassay using a Packard Tri-Carb 1600CA liquid scintillation counter with corrections for quenching. The coefficient of variations (CVs) of the triplicate measurement were 0 to 41 %. 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). Net bacterial growth rate (sBP, d^{-1}) was estimated as BP_{TdR} (cells l^{-1} d^{-1}) divided by bacterial abundance (cells l^{-1}). Bacterial production rates of free-living (BP_{Free}) were determined ^{3}H-TdR incorporation rates of 0.8-µm (Nucleopore, Millipore) filtered water, and of attached fraction (BP_{Att}) were estimated by BP_{Free} subtracted from BP_{TdR}. To estimate the Leucine (Leu; pmol Leu l^{-1} d^{-1}) and TdR incorporation (pmol TdR l^{-1} d^{-1}) ratio (the Leu : TdR ratio), BP_{Leu} data was obtained from Piontek et al. (2012).

2.3 Bacterial respiration (BR)

BR was determined from the decrease of dissolved oxygen concentration during 48-h incubations of water samples (< 0.8 µm pore-size filter). Sample water was filtered through 0.8-µm filter (Nucleopore, Millipore) by applying a weak positive pressure (< 67 cm Hg) with an air pressure pump and dispensed into biochemical oxygen demand bottles (60-ml capacity). Dissolved oxygen concentration was determined by Winkler titration using an automated titrator with a potentiometric end-point detector

BGD
9, 15213–15235, 2012

(Mettler Toledo, Titrando 888) (Knap et al., 1996). Cell-specific bacterial respiration (sBR, fg C cell^{-1} d^{-1}) was estimated as BR (fg C l^{-1} d^{-1}) divided by bacterial abundance (cells l^{-1}) at the start of the incubation.

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

BGE and BCD were estimated with the following equations:

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

$$BCD = BP + BR \quad (2)$$

where BR (O_2 consumption rate was converted to C flux by assuming that the respiratory quotient = 1; del Giorgio and Cole 1998) and BP (TdR incorporation was converted to C flux by assuming a conversion factor of 20 fg C per cell, Cho and Azam, 1990) were estimated as described above.

2.5 Bacterial abundance

Bacterial abundance was determined by flow cytometer. Details are described in Brussaard et al. (2012).

3 Results

3.1 Temporal variations of BP_{TdR} and BR

BP_{TdR} and BR in the mesocosms before adding CO_2 saturated water (t-1) ranged between 3.41 ± 0.42 to $4.42 \pm 0.23 \times 10^8$ cells l^{-1} d^{-1} and 10.9 ± 13.0 to 51.4 ± 14.7 µg C l^{-1} d^{-1}, respectively (Fig. 1a and b). Generally, BP_{TdR} decreased to t7 and showed

BGD
9, 15213–15235, 2012

a substantial increase (2.4 to 5.5-fold) at t10 in all treatments (Fig. 1a). Although there was no pronounced enhancement by nutrient addition, BP_{TdR} gradually increased towards the end of the experiment (Fig. 1a). In contrast to BP_{TdR}, BR did not vary greatly and no clear pattern was observed during the experiment (Fig. 1b).

sBP and sBR at the beginning of experiment ranged between 0.16 ± 0.02 to 0.22 ± 0.01 d^{-1} and 5.43 ± 6.47 to 23.55 ± 6.76 fg C $cell^{-1}$ d^{-1}, respectively (Fig. 2a, b). sBP gradually increased after closing the mesocosms and showed a substantial increase at t10; however, after nutrient addition sBP gradually decreased towards the end of the experiment (Fig. 2a). In contrast to BR, sBR did not show any clear pattern during the experiment (Fig. 2b).

BP was positively correlated to real pCO_2 concentration at t5 (Linear regression, $r^2 = 0.50$, $p < 0.05$, $n = 9$; Fig. 3b) and negatively correlated at t14 ($r^2 = 0.51$, $p < 0.05$, $n = 9$; Fig. 3f). sBP was significantly ($p < 0.05$) related to real pCO_2 concentrations at t-1, t14 and t26.

3.2 Temporal variations of BP_{Free} and BP_{Att}

The percentage of BP_{Free} dominated in all mesocosms except at t3 and t20 (Fig. 4). Averaged % of BP_{Free} at t-1, t7, t16, t24 and t28 was 89 ± 22 % (± SD), while the average % of BP_{Free} at t3 and t20 was 58 ± 11 %. At t3 (the end of pCO_2 manipulation) and t20 (the second Chlorophyll *a* peak, Schulz et al., 2012), percentage of BP_{Att} ranged from 24–56 and 23–56 % (Fig. 4b, e). The percentage of BP_{Att} of CO_2 manipulated mesocosms tended to be lower than control mesocosm at t3. BP_{Free} was significantly influenced by changes in pCO_2 at t24 (Linear regression: $r^2 = 0.67$, $p < 0.05$, $n = 9$).

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

The Leu : TdR ratio averaged 10.4 ± 4.05 (range 3.74 to 14.7) at the beginning of experiment, and it increased to 25.8 ± 8.10 (range 15.1 to 35.2) on t5. However, the ratio decreased to 7.81 ± 3.09 (range 2.85 to 11.7) on t10 and remained constant in

BGD
9, 15213–15235, 2012

the range between 8.3 and 16.8. At the end of the experiment, the ratio increased to 20.5 ± 7.0. In addition, during the period after addition of CO_2 saturated water to just before nutrient addition, the average ratio at low pCO_2 mesocosms (17.8; M3, 7, 2) was high compare to medium (15.0; M4, 8, 1) and high pCO_2 mesocosms (14.2; M6, 5, 9). Contrary, in the period after nutrient addition to the end of the experiment, a high average ratio was observed at high pCO_2 mesocosms (16.9) compared to low (12.7) and medium pCO_2 mesocosms (12.5) (Fig. 5). The Leu : TdR ratio negatively correlated with real pCO_2 concentrations at t5 and t7 (Liner regression, t5; $r^2 = 0.48$, $p < 0.05$, $n = 9$, t7; $r^2 = 0.63$, $p < 0.05$, $n = 9$). Contrary, the ratio positively correlated with real pCO_2 concentrations at t24 and t26 (Liner regression, t24; $r^2 = 0.57$, $p < 0.05$, $n = 9$, t26; $r^2 = 0.61$, $p < 0.05$, $n = 9$).

BGE and BCD were estimated based on TdR and Leu incorporation rate. BGE_{Leu} and BGE_{TdR} at the beginning of experiment ranged between 2 ± 1 to 18 ± 2 % and 13 ± 4 to 31 ± 5 %, respectively, and varied greatly from 3 to 61 % and 4 to 75 %, respectively, during the experiment (Fig. 6). BCD_{Leu} and BCD_{TdR} at the beginning of experiment ranged between 15.0 ± 2.5 to 52.4 ± 14.8 μg C l^{-1} d^{-1} and 18.4 ± 2.8 to 58.7 ± 15 μg C l^{-1} d^{-1}, and varied from 3.5 to 46.6 μg C l^{-1} d^{-1} and 2.6 to 47.2 μg C l^{-1} d^{-1} (Fig. 7). There were significant relations between BGE_{Leu} and BGE_{TdR} and between BCD_{Leu} and BCD_{TdR} during the experiment (Liner regression, BGE; $r^2 = 0.825$, $p < 0.001$, $n = 37$, BCD; $r^2 = 0.983$, $p < 0.001$, $n = 37$), but no obvious trends were observed by the effect of pCO_2 or time of experiment.

4 Discussion

4.1 Temporal variations of BP_{TdR} and BR

BP_{TdR} decreased after closure of the mesocosms but strongly increased at t10 in all mesocosms. Concomitantly with BP_{TdR}, abundance of high nucleic acid bacteria

BGD
9, 15213–15235, 2012

(HDNA) declined, thus, suggesting that this decline was most likely due to viral lysis (Brussaard et al., 2012). Middelboe et al. (1996) demonstrated that viral lysates support growth of non-infected bacteria and this indicates that viral lysates are potentially labile compounds. Our results suggest that decline of BP_{TdR} and bacterial abundance potentially attributed by viruses until t7 and viral lysates might enhance BP_{TdR} at t10. Furthermore, peaks of chlorophyll *a* (Schulz et al., 2012), picophytoplankton II and nanophytoplankton I (Brussaard et al., 2012) were found at t6 to t8 and t4 to t6. In addition to the effect of viral lysates of bacteria, these results suggest that phytoplankton exudation of carbon and viral lysates of nanophytoplankton could have also stimulated BP_{TdR} at t10. In support of this notion, average Leu : TdR ratio decreased from 25.8 ± 8.10 at t5 to 7.81 ± 3.09 at t10 potentially as a consequence of labile dissolved organic matter release by phytoplankton (i.e. balanced growth, see discussion below). Moreover, viral lysis of nanoplankton blooms accounts for a portion of our BCD (e.g. 6–28 %, Brussaard et al., 2012). Interestingly, the bacterial diversity index determined by T-RFLP showed that species richness and diversity index increased during pCO_2 manipulation and decreased at t10 when we observed the peak of BP_{TdR} (Zhang et al., 2012). This suggests that active bacteria might have dominated at t10 of incubation. After nutrient addition at t13, chlorophyll *a* (Schulz et al., 2012) showed 2 peaks, while BP_{TdR} exhibited a gradual increase. However, this pattern was inconsistent and could be due to changes in bacterial community composition after nutrient addition (Zhang et al., 2012). Furthermore, after nutrient addition, bacterial abundance increased and reached its maximum at the end of experiment (Brussaard et al., 2012), while net bacterial growth rate gradually decreased. This discrepancy might be attributed to lower bacterial losses rather than increased gross bacterial growth. Although high viral abundance was observed at end of experiment (Brussaard et al., 2012), viral mediated bacterial mortality declined towards the end of experiment (M. G. Weinbauer, personal communication, 2012), suggesting that bacteria might become resistant to viral infection. The free-living community usually dominated during the experiment. Temporal variations of free-living and attached bacterial production were found in pCO_2 manipulated experiments (i.e.

BGD
9, 15213–15235, 2012

the percentage of BP_{Att} increased and reached a percentage similar to BP_{Free} at t20). 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 BP_{Leu} was positively correlated with primary production in the present study (Piontek et al., 2012), no coupling between phytoplankton and rates of free-living and attached bacterial production was observed. Hence, temporal variation of BP_{Free} and BP_{Att} was potentially uncoupled from phytoplankton, e.g. due to grazing or viral lysis (Brussaard et al., 2012).

Our data on BR are within the range reported for the western Arctic Ocean (Kirchman et al., 2009). In contrast to BP_{TdR}, BR and sBR did not show any trend during the experiment. López-Urrutia and Morán (2007) found that bacterial respiration and production responded similarly to changes in temperature, while an extensive dataset of concurrent measurement of bacterial production and respiration revealed that bacterial respiration is much less variable than production across marine systems (del Giorgio and Cole, 2000). Our results are consistent with the general pattern of a small variability of bacterial respiration.

4.2 Effect of pCO_2 on bacterial carbon metabolism

We focused on day-to-day results rather than dividing the experimental period into 4 phases (other papers, this issue) because averaged results did not show trends for microbial parameters and their relationship to pCO_2 concentrations.

BP_{TdR} was significantly correlated with pCO_2 at t5. This indicates that pCO_2 enhanced BP_{TdR} rapidly after the pCO_2 manipulation, which is consistent with Grossart et al. (2006) that bacterial production was enhanced in the highest pCO_2 mesocosm (700 ppmV). However, although no pronounced enhancement of BP_{TdR} by addition of nutrient was found in the present study, BP_{TdR} decreased with increasing pCO_2 at t14. Suppression of bacterial production by the effect of an increase in pCO_2 or decrease in pH was previously reported in experiments using deep sea water (Coffin et al., 2004; Yamada et al., 2010). Yamada et al. (2010) stimulated acidification through enrichments with high CO_2 air or artificial chemical buffers, and found suppression of

BGD
9, 15213–15235, 2012

prokaryotic activities at lower pH (pH ≤ 7.0). This suppression was more profound under the enrichment treatment with artificial chemical buffers, perhaps because artificial chemical buffers contain organic matter. Consistent with this, our results indicate that the effects of changes in pCO_2 on BP_{TdR} were potentially linked to nutrient status.

In addition to BP_{TdR}, the Leu:TdR ratio was significantly influenced by changes in pCO_2. The Leu : TdR ratio is the 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. 10.3 ± 4.3, average ± SD) compared to the whole water column (0 to 80 m) average from May to September in the subarctic Pacific (i.e. 16.8, $n = 481$; Kirchman, 1992); however, the average ratio in the low (M3, 7, 2), medium (M4, 8, 1) and high (M6, 5, 9) pCO_2 mesocosms were slightly enhanced (i.e. low : 13.1, medium : 12.3, high : 15.1) during the experiment. In particular, the Leu:TdR ratio decreased with increasing pCO_2 concentration at t5 and t7 but this trend changed at end of the experiment. Previous studies have suggested that under favorable environmental conditions (e.g. rich in organic matter or temperature increase), bacteria might optimize DNA duplication over protein synthesis to maximize reproduction (i.e. balanced growth), resulting in a decline in the Leu : TdR ratio. But, under unfavorable environmental conditions (i.e. unbalanced growth), the Leu : TdR ratio would increase because bacteria might concentrate on biomass accumulation rather than reproduction to maximize the chance of survival (Shiah and Ducklow, 1997; Gasol et al., 1998). In this regard, our result imply that after the pCO_2 manipulation, bacterial growth became more unbalanced with increasing pCO_2; however this trend changed and bacterial growth became more balanced towards the end of experiment. Previous mesocosm studies showed that the Leu : TdR ratio was more likely associated with algal bloom rather than pCO_2 (Grossart et al., 2006; Arnosti et al., 2011), and a connection between primary production and BP_{Leu} was found in this experiment (Piontek et al., 2012; Engel et al., 2012); however, we did not observe a significant connection between the Leu : TdR ratio and phytoplankton

BGD
9, 15213–15235, 2012

in our experiment. Changes of pCO_2 can modify the quality and quantity of dissolved organic matter production by phytoplankton (Engel et al., 2004), therefore bacterial activity could be indirectly influenced by changes in pCO_2 (Robinson, 2008). These studies suggest that changes in pCO_2 potentially alter bacterial production and balance of bacterial growth in this study.

The effect of pCO_2 on bacterial respiration, BGE and BCD of bulk community was examined for the first time in the present study; however, no clear trend was observed. Teira et al. (2012) examined effect of CO_2 on 2 bacterial strains, *Roseobacter* and *Cytophaga*, and demonstrated that respiration of *Cytophaga* was significantly lower and BGE was higher in the elevated CO_2 treatment (1000 ppmV) than control (380 ppmV), while *Roseobacter* did not show any trend. Their study showed that different bacterial strains responded differently to CO_2; however, bacterial community structure varied during the experiment (i.e. one month, Zhang et al., 2012), so our measurement of bacterial carbon metabolism of whole community potentially hid the effect of pCO_2 on different strains. Although bacterial respiration does not vary greatly compared to production (del Giorgio and Cole, 2000), previous studies have shown that there is a significant relationship between temperature and bacterial respiration (del Giorgio and Cole, 2000; Robinson, 2008) or BGE (Rivkin and Legendre, 2001). Since ocean acidification is expected to occur concurrently with temperature increase, further studies are required to examine the combined effect of pCO_2 and temperature on bacterial respiration, BCD, BGE and total carbon flux through bacteria.

5 Summary

The goal of our study was to determine the potential impact of changes in pCO_2 on bacterial production, respiration and carbon metabolism in the Arctic water. In the present study, we found changes in pCO_2 influenced bacterial cell production, bacterial production of the free-living community, net growth rate and Leu : TdR ratio during mesocosm experiment. On the contrary, no clear trend of the effect of pCO_2 on bacterial

BGD
9, 15213–15235, 2012

respiration, BGE and BCD was observed. Overall results suggest that although there was temporal variation, changes in pCO_2 potentially influence bacterial production and growth balance rather than the conversion of dissolved organic matter into CO_2.

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.

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

References

Allgaier, M., Riebesell, U., Vogt, M., Thyrhaug, R., and Grossart, H.-P.: Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO_2 levels: a mesocosm study, Biogeosciences, 5, 1007–1022, doi:10.5194/bg-5-1007-2008, 2008.

Arnosti, C., Grossart, H. P., Mühling, M., Joint, I., and Passow, U.: Dynamics of extracellular enzyme activities in seawater under changed atmospheric pCO_2: a mesocosm investigation, Aquat. Microb. Ecol., 64, 285–298, 2011.

BGD
9, 15213–15235, 2012

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M. C. J., Schulz, K., Ludwig, A., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated CO_2 levels, Biogeosciences Discuss., in review, 2012.

Chin-Leo, S. and Kirchman, D. L.: Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine, Appl. Environ. Microbiol., 54, 1934–1939, 1988.

Cho, B. C. and Azam, F.: Biogeochemical significance of bacterial biomass in the ocean's eutphotic zone, Mar. Ecol. Prog. Ser. 63, 253–259, 1990.

Coffin, R. B., Montgomery, M. T., Boyd, T. J., and Masutani, S. M.: Influence of ocean CO_2 sequestration on bacterial production, Energy, 29, 1511–1520, 2004.

del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Ann. Rev. Ecol. Syst., 29, 503–541, 1998.

del Giorgio, P. A. and Cole, J. J.: Bacterial energetics and growth efficiency, in: Microbial ecology of the oceans, edited by: Kirchman, D. L., Wiley, New York, 289–325, 2000.

del Giorgio, P. A., Condon, R., Bouvier, T., Longnecker, K., Bouvier, C., Sherr, E., and Gasol, J. M.: Coherent patterns in bacterial growth, growth efficiency, and leucine metabolism along a northeastern Pacific inshore–offshore transect, Limnol. Oceanogr., 56, 1–16, 2011.

Ducklow, H.: Bacterial production and biomass in the ocean, in: Microbial ecology of the oceans, edited by: Kirchman, D. L., Wiley, New York, 85–120, 2000.

Engel, A., Borchard, C., Piontek, J., Riebesell, U., and Schulz, K.: CO_2 increases ^{14}C-primary production in an arctic plankton community, Biogeosciences Discuss., accepted, 2012.

Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbrüggen, A., and Zondervan, I.: Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO_2 concentrations: a mesocosm experiment, Aquat. Microb. Ecol., 34, 93–104, 2004.

Fuhrman, J. A. and Azam, F.: Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluations and field results, Mar. Biol. 66, 109–120, 1982.

Gasol, J. M., Doval, M. D., Pinhassi, J., Calderon-Paz, J. I., Guixa-Boixareu, N., Vaque, D., and Pedros-Alio, C.: Diel variations in bacterial heterotrophic activity and growth in the northwestern Mediterranean Sea, Mar. Ecol. Prog. Ser., 164, 107–124, 1998.

BGD
9, 15213–15235, 2012

Grossart, H. P., Allgaier, M., Passow, U., and Riebesell, U.: Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton, Limnol. Oceanogr., 51, 1–11, 2006.

IPCC Fourth Assessment Report on Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the IPCC, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor M., and Miller, H. L., Cambridge University Press, Cambridge and New York, 996, 2007.

Joint, I., Doney, S. C., and Karl, D. M.: Will ocean acidification affect marine microbes?, ISME J., 5, 1–7, 2011.

Kirchman, D. L.: Incorporation of thymidine and leucine in the subarctic Pacific: application to estimating bacterial production, Mar. Ecol. Prog. Ser., 82, 301–309, 1992.

Kirchman, D. L.: Measuring of bacterial biomass production and growth rates from leucine incorporation in natural aquatic environment, in: Methods in Microbiology, edited by: Paul, J. H., Academic Press, San Diego, 227–237, 2001.

Kirchman, D. L., Hill, V., Cottrell, M. T., Gradinger, R., Malmstrom, R. R., and Parker, A.: Standing stocks, production, and respiration of phytoplankton and heterotrophic bacteria in the western Arctic Ocean, Deep-Sea. Res. II, 56, 1237–1248, 2009.

Knap, A. H., Michaels, A. E., Close, A., Ducklow, H. W., and Dickson, A. G.: Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements, Unesco, Bergen, 1996.

Liu, J., Weinbauer, M. G., Maier, C., Dai, M., and Gattuso, J. P.: Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning, Aquat. Microb. Ecol., 58, 303–310, 2011.

Lopez-Urrutia, A., and Moran, X. A. G.: Resource limitation of bacterial production distorts the temperature dependence of oceanic carbon cycling, Ecology, 88, 817–822, 2007.

Middelboe, M., Jorgensen, N. O. G., and Kroer, N.: Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton, Appl. Environ. Microbiol., 62, 1991–1997, 1996.

Piontek, J., Lunau, M., Händel, N., Borchard, C., Wurst, M., and Engel, A.: Acidification increases microbial polysaccharide degradation in the ocean, Biogeosciences, 7, 1615–1624, doi:10.5194/bg-7-1615-2010, 2010.

Piontek, J., Borchard, C., Sperling, M., Schulz, K. G., Riebesell, U., and Engel, A.: Response of bacterioplankton activity in an Arctic fiord system to elevated pCO_2: results from a mesocosm perturbation study, Biogeosciences Discuss., accepted, 2012.

BGD
9, 15213–15235, 2012

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification in marine plankton in response to increased atmospheric CO_2, Nature, 407, 634–637, 2000.

Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Muche, R., and Schulz, K. G.: A mobile sea-going mesocosm system: New opportunities for ocean change research, Biogeosciences Discuss., in preparation, 2012.

Rivkin, R. B. and Legendre, L.: Biogenic carbon cycling in the upper ocean: Effects of microbial respiration, Science, 291, 2398–2400, 2001.

Robinson, C.: Heterotrophic bacterial respiration, in: Microbial ecology of the oceans second edition, edited by: Kirchman, D. L., Wiley, New York, 299–334, 2008.

Rochelle-Newall, E., Delille, B., Frankignoulle, M., Gattuso, J. P., Jacquet, S., Riebesell, U., Terbruggen, A., and Zondervan, I.: Chromophoric dissolved organic matter in experimental mesocosms maintained under different pCO_2 levels, Aquat. Microb. Ecol., 272, 25–31, 2004.

Schulz, K. G., Bellerby, R. G. J., Brussaard, C., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch Klavsen, S., Krug, S., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stuhr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences Discuss., in review, 2012

Shiah, F. K. and Ducklow, H. W.: Bacterioplankton growth responses to temperature and chlorophyll variations in estuaries measured by thymidine: leucine incorporation ratio, Aquat. Microb. Ecol., 13, 151–159, 1997.

Teira, E., Fernández, A., Álvarez-Salgado, X. A., García-Martín, E. E., Serret, P., and Sobrino, C.: Response of two marine bacterial isolates to high CO_2 concentration, Mar. Ecol. Prog. Ser., 453, 27–36, 2012.

Yamada, N. and Suzumura, M.: Effects of seawater acidification on hydrolytic enzyme activities, J. Oceanogr., 66, 233–241, 2010.

Yamada, N., Tsurushima, N., and Suzumura, M.: Effects of seawater acidification by ocean CO_2 sequestration on bathypelagic prokaryote activities, J. Oceanogr., 66, 571–580, 2010.

Zhang, R., Xia, X., Lau, S. C. K., Motegi, C., Weinbauer, M. G., and Jiao, N.: Response of bacterioplankton community structure to an artificial gradient of pCO_2 in the Arctic Ocean, Biogeosciences Discuss., accepted, 2012.

BGD
9, 15213–15235, 2012

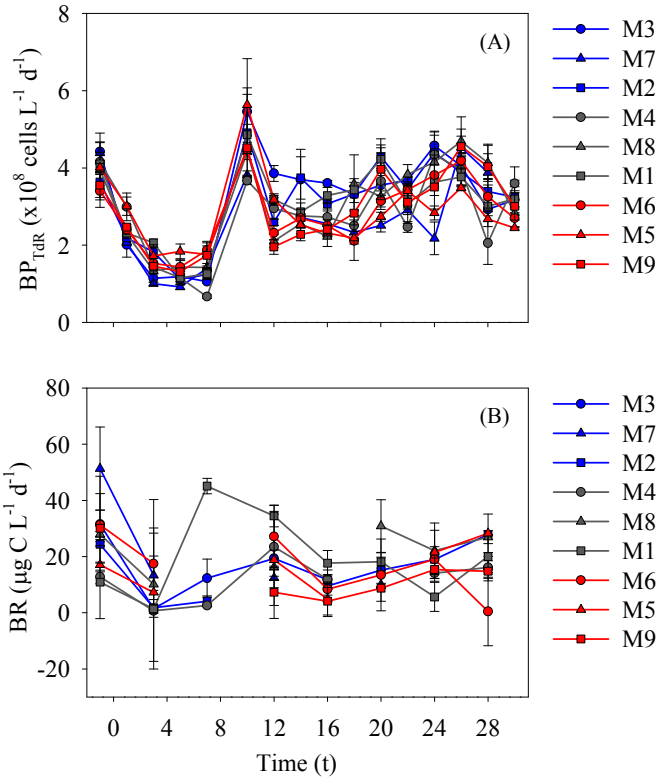

Fig. 1. Temporal variation of bacterial production estimated by TdR incorporation **(A)** and bacterial respiration **(B)**.

BGD
9, 15213–15235, 2012

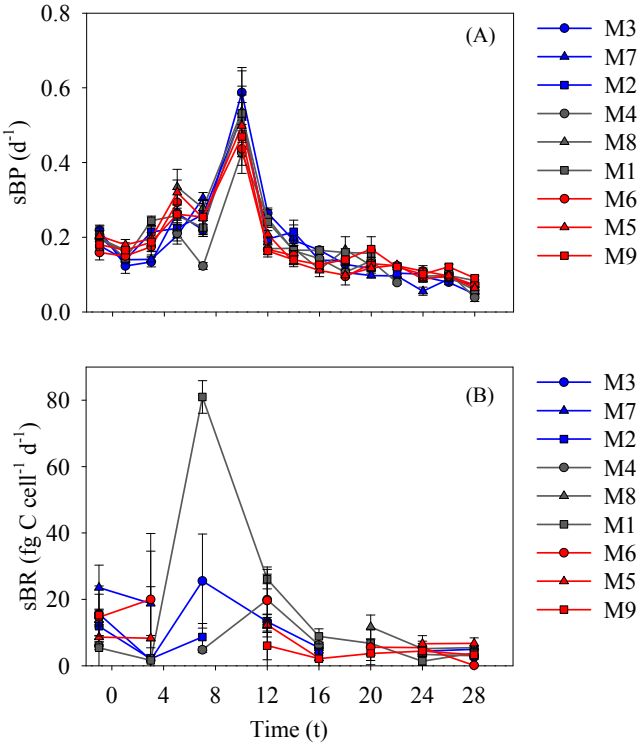

Fig. 2. Temporal variation of growth rate **(A)** and cell specific bacterial respiration **(B)**.

BGD
9, 15213–15235, 2012

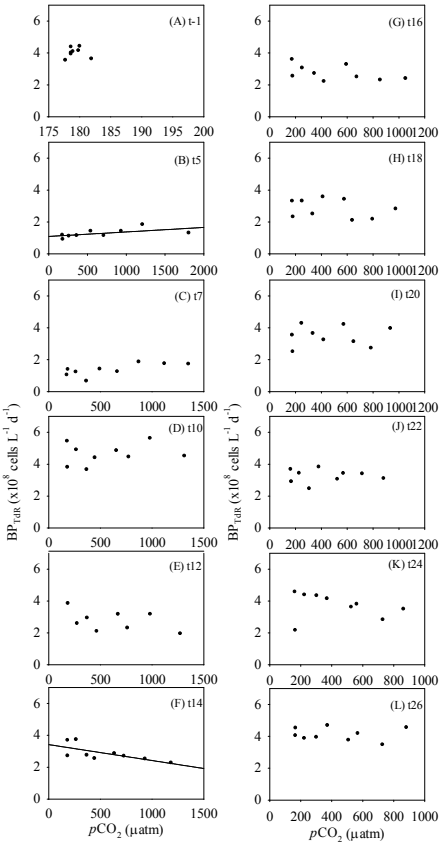

Fig. 3. Relationship between real pCO_2 value and the BP_{TdR} at t-1 **(A)**, t5 **(B)**, t7 **(C)**, t10 **(D)**, t12 **(E)**, t14 **(F)**, t16 **(G)**, t18 **(H)**, t20 **(I)**, t22 **(J)**, t24 **(K)** and t26 **(L)**.

BGD
9, 15213–15235, 2012

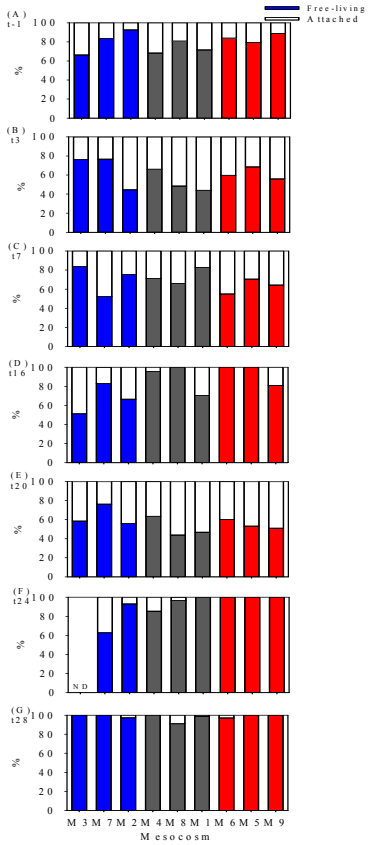

Fig. 4. Ratio of attached and free-living bacterial production at t-1 **(A)**, t3 **(B)**, t7 **(C)**, t16 **(D)**, t20 **(E)**, t24 **(F)** and t28 **(G)**.

BGD
9, 15213–15235, 2012

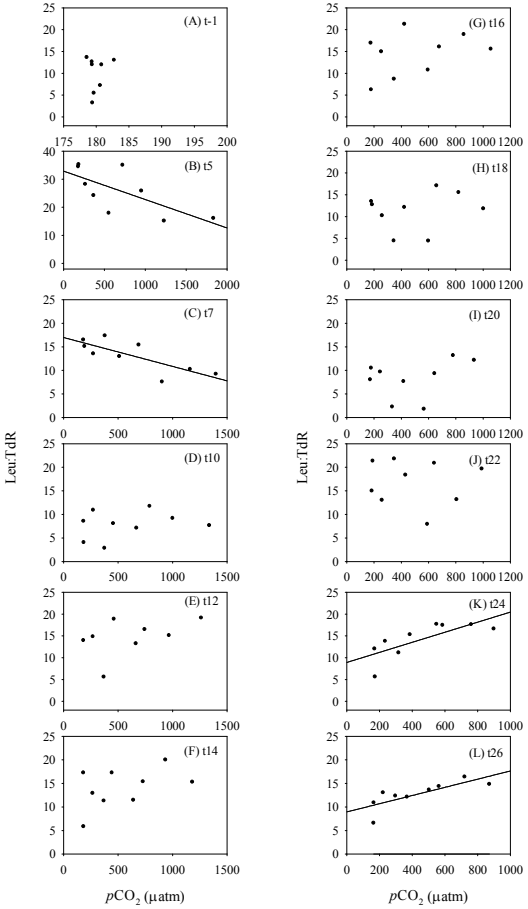

Fig. 5. Relationship between real pCO_2 value and the Leu : TdR ratio at t-1 **(A)**, t5 **(B)**, t7 **(C)**, t10 **(D)**, t12 **(E)**, t14 **(F)**, t16 **(G)**, t18 **(H)**, t20 **(I)**, t22 **(J)**, t24 **(K)** and t26 **(L)**.

BGD
9, 15213–15235, 2012

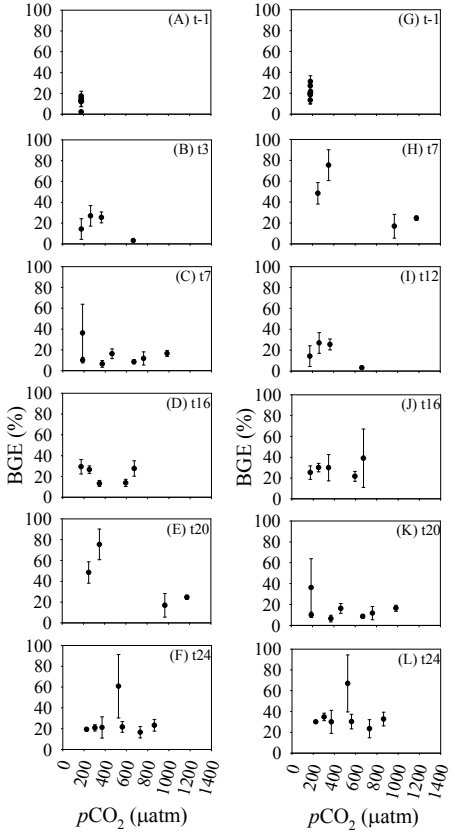

Fig. 6. Relationship between real pCO_2 value and BGE_{Leu} at t-1 **(A)**, t3 **(B)**, t7 **(C)**, t16 **(D)**, t20 **(E)** and t24 **(F)** and BGE_{TdR} at t-1 **(G)**, t7 **(H)**, t12 **(I)**, t16 **(J)**, t20 **(K)** and t24 **(L)**.

BGD
9, 15213–15235, 2012

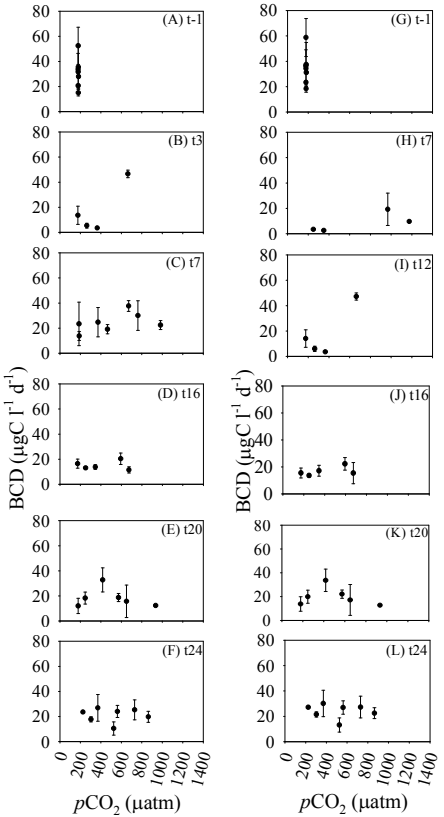

Fig. 7. Relationship between real pCO_2 value and BCD_{Leu} at t-1 **(A)**, t3 **(B)**, t7 **(C)**, t16 **(D)**, t20 **(E)** and t24 **(F)** and BCD_{TdR} at t-1 **(G)**, t7 **(H)**, t12 **(I)**, t16 **(J)**, t20 **(K)** and t24 **(L)**.

BGD
9, 15213–15235, 2012