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Effect of ocean acidification on microbial diversity and on microbe-driven biogeochemistry and ecosystem functioning

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ABSTRACT: The ocean absorbs about 25% of anthropogenic CO_2 emissions, which alters its chemistry. Among the changes of the carbonate system are an increase in the partial pressure of CO_2 (pCO_2) and a decline of pH; hence, the whole process is often referred to as 'ocean acidification'. Many microbial processes can be affected either directly or indirectly via a cascade of effects through the response of non-microbial groups and/or through changes in seawater chemistry. We briefly review the current understanding of the impact of ocean acidification on microbial diversity and processes, and highlight the gaps that need to be addressed in future research. The focus is on *Bacteria, Archaea,* viruses and protistan grazers but also includes total primary production of phytoplankton as well as species composition of eukaryotic phytoplankton. Some species and communities exhibit increased primary production at elevated pCO_2. In contrast to their heterocystous counterparts, nitrogen fixation by non-heterocystous cyanobacteria is stimulated by elevated pCO_2. The experimental data on the response of prokaryotic production to ocean acidification are not consistent. Very few other microbial processes have been investigated at environmentally relevant pH levels. The potential for microbes to adapt to ocean acidification, at either the species level by genetic change or at the community level through the replacement of sensitive species or groups by non- or less sensitive ones, is completely unknown. Consequently, the impact of ocean acidification on keystone species and microbial diversity needs to be elucidated. Most experiments used a short-term perturbation approach by using cultured organisms; few were conducted in mesocosms and none *in situ*. There is likely a lot to be learned from observations in areas naturally enriched with CO_2, such as vents, upwelling and near-shore areas.

KEY WORDS: Ocean acidification · Microbial diversity · Microbe · *Bacteria* · Phytoplankton · Viruses · Biogeochemistry · Meta-analysis

INTRODUCTION

The partial pressure of carbon dioxide (pCO_2) increases in the atmosphere due to the anthropogenic input of CO_2 through the burning of fossil fuel, cement production and land-use change. It has increased by 32% between 1880 and 2000 (280 to 379 µatm; Solomon et al. 2007), leading to changes in the Earth's climate and the functioning of terrestrial ecosystems. Over the past 250 yr, the world's oceans have absorbed about one-third of the anthropogenic CO_2, which is now distributed from the surface to depths ranging from a few hundred to a few thousand metres (Sabine et al. 2004). The uptake of anthropogenic CO_2 profoundly affects the parameters of the carbonate chemistry (e.g. Gattuso & Lavigne 2009), leading to an increase of pCO_2 and of the concentrations of dissolved inorganic carbon (DIC) and bicarbonate ions (HCO_3^-), as well as to a decrease of pH and of the concentration of carbonate ions (CO_3^{2-}). The term 'ocean acidifica-

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tion' (Caldeira & Wickett 2003) relates to the decrease in pH but does not imply that the pH of ocean surface waters will become acidic (i.e. below 7) any time soon.

If the current trends in CO_2 emissions continue to increase, the pH of the global surface ocean could decrease by about 0.4 units by the end of the century compared to pre-industrial times. Changes will be more pronounced in areas such as the Southern Ocean, which will become undersaturated with respect to aragonite in 2050 (Orr et al. 2005), and the Arctic Ocean where aragonite undersaturation will occur even sooner (Steinacher et al. 2009). This change in the chemistry of the oceans is quantifiable and predictable for a given level of atmospheric pCO_2. Observations at several time-series stations, even though all of them relatively short (<20 yr), confirm the predicted changes in the carbonate chemistry (e.g. Santana-Casiano et al. 2007).

The biological, ecological and biogeochemical responses of marine organisms and communities have only been actively studied in the past few years, and there is still a high level of uncertainty and debate on the significance and magnitude of those responses (Hendriks et al. 2010, Dupont et al. 2010). A meta-analysis of the response of marine organisms to ocean acidification recently performed by Hendriks et al. (2010) only partly covers microbial groups and microbial biogeochemistry. Marine microbes, here considered as single-celled organisms (i.e. prokaryotes and protists) and viruses, are very diverse, as they thrive in a large range of habitats and perform a wide range of functions. They are therefore involved in virtually all geochemical reactions occurring in the oceans (Kirchman 2008). Some of these functions are even exclusively performed by prokaryotes. Some prokaryotes are able to withstand extreme pH values with optimal pH for growth ranging from 0.7 to >10 (Cavicchioli 2002).

The microbial group that has been investigated most thoroughly with respect to the effect of ocean acidification is eukaryotic phytoplankton (Riebesell 2004, Giordano et al. 2005, Beardall et al. 2009). In contrast, other groups such as viruses, *Archaea*, *Bacteria* and heterotrophic protists have received considerably less attention. In a recent 'perspective' paper, Joint et al. (in press) asked 'Will ocean acidification affect marine microbes?' In a narrative (sensu Gates 2002) review, Joint et al. (in press) looked at some of the relevant literature and came to the conclusion that 'perhaps the most appropriate null hypothesis to test is that marine microbes possess the flexibility to accommodate pH change and there will be no catastrophic changes in marine biogeochemical processes that are driven by phytoplankton, bacteria and archaea' and recognised that calcification and photosynthesis could be affected. Narrative reviews have the potential for serious bias, which could lead to misleading conclusions (Gates 2002). Meta-analysis was developed to overcome most biases of narrative reviews. It statistically combines the results (effect size) of several studies that address a shared research hypothesis.

Here we used a meta-analytic approach to comprehensively review the current understanding of the effect of ocean acidification on microbes and microbial processes, and to highlight the gaps that need to be addressed in future research.

METHODS

Data were collected from the EPOCA/EUR-OCEANS database (Nisumaa et al. 2010), the tables or text of papers, or interpolated from figures. Only papers reporting the effect of elevated pCO_2 or decreased pH in the range of values expected during the period spanning the last glacial maximum and the year 2100 were selected. Unless mentioned otherwise, pH values are reported on the total scale (Dickson 2010). pCO_2 levels were calculated from pH and other ancillary data with the R software package seacarb (Lavigne & Gattuso 2010) as described by Nisumaa et al. (2010). Data permitting, 2 effect sizes were calculated for each variable: H:C, the value at high pCO_2 versus the value at control pCO_2; and C:L, the value at control pCO_2 versus the value at low pCO_2. The ranges of high (H), control (C) and low (L) pCO_2 were 450 to 1500, 300 to 450 and 100 to 300 µatm, respectively. These levels roughly correspond to 'future', 'present' and 'glacial' conditions. For mesocosm experiments, in which pCO_2 was drifting, the data were categorised into the 'low', 'control' and 'high' pCO_2 categories according to the pCO_2 values at the beginning of the time period considered. Only the data collected during the pre-bloom phase of mesocosm experiments were used because the termination of the blooms may have distinct causes in addition to nutrient exhaustion (e.g. viral lysis): Days 5 to 14 for PeECE I (Engel et al. 2005), Days 0 to 14 for PeECE II (Grossart et al. 2006a) and Days 6 to 9 for PeECE III (Riebesell et al. 2008). There are 2 exceptions. The community respiration data reported by Egge et al. (2009) exhibit a large day-to-day variability and only Day 8 was considered. Tanaka et al. (2008) did not report alkaline phosphatase activity data for Days 6 to 9; hence data from Days 13 to 19 were used. The effect size is shown in Fig. 1 (see Fig. 1 in 'Results'. It is important to note that the effect is monotonous when H:C and L:C are both above or below 1, whereas the effect exhibits an extreme when one of the effects is above 1 and the other below 1.

Meta-analyses were performed with the R package meta 1.1.8 (Schwarzer 2010) to test the significance of the effect sizes H:C and L:C. The results are shown in Tables 1 & 2. Fixed and random effects models can be

used in a meta-analysis (Borenstein et al. 2009). The fixed effect model generally assumes that all studies shared a single effect, whereas the effect could be different from study to study in the random effects model. There is no reason to assume that ocean acidification has the same effect on a variable measured in different species and/or under different experimental conditions. However, some have argued that the fixed effect method is valid without assuming a common true effect size (Borenstein et al. 2009); hence, both models were used. Inverse variance weighting was used for pooling and the DerSimonian-Laird estimate was used in the random effects model. Hedges' adjusted g was applied to calculate the standardised mean difference (SMD). The heterogeneity of responses with a random effects model was assessed using the Q statistics as well as with the H and I^2 indices (Borenstein et al. 2009). I^2 is 0 when all variability in effect size estimates is due to sampling error within studies; I^2 values of 25, 50 and 75% correspond to mean low, medium and high heterogeneity, respectively. When $H < 1.2$, there is no heterogeneity within the studies, while there are obvious differences between studies when $H > 1.5$.

pH REGULATION

Marine microbes have an optimum pH range that varies between species, and many physiological processes are pH-dependent. It is therefore critical that

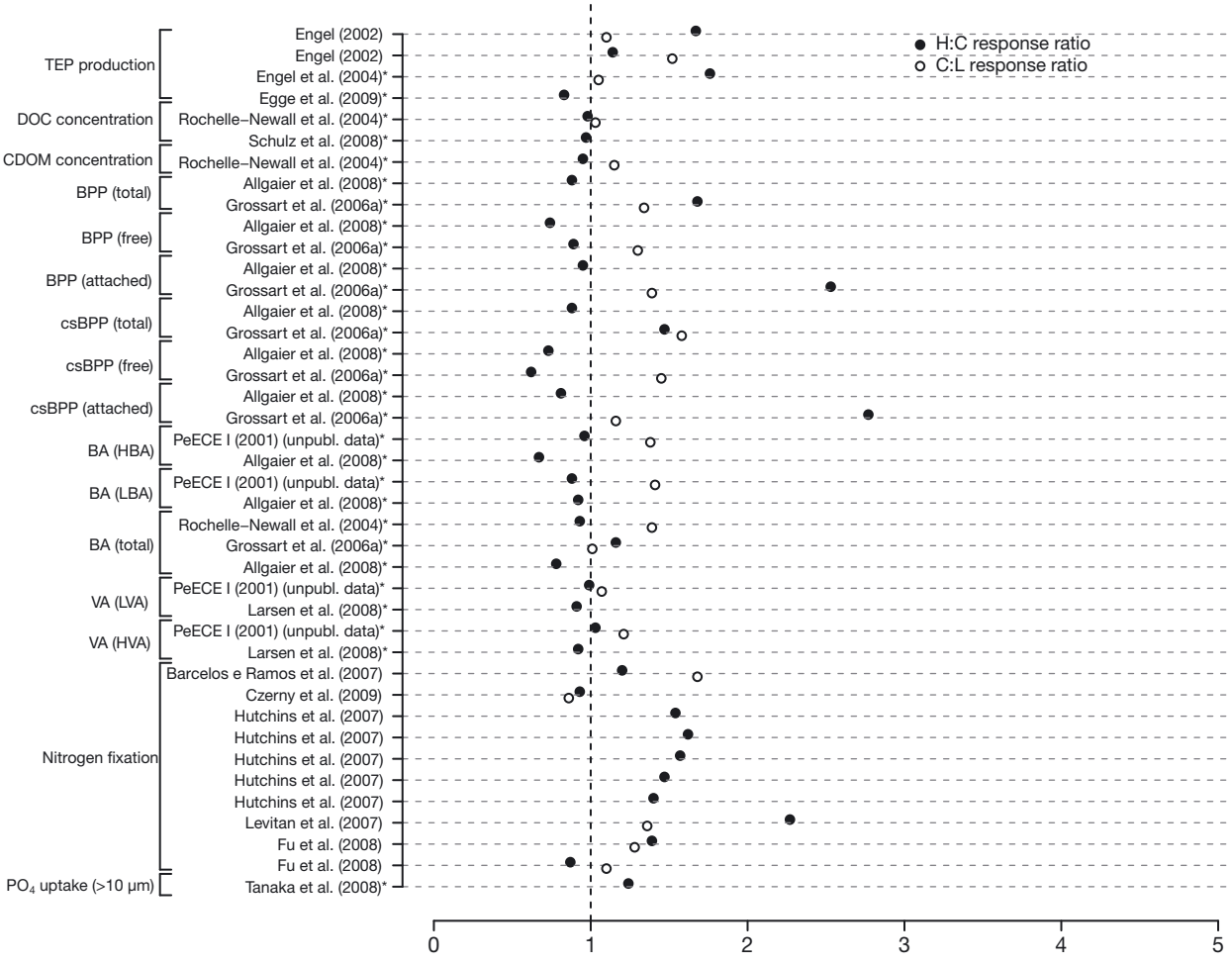

Fig. 1. Impact of ocean acidification on microbial processes and on the parameters involved. The H:C ratios, i.e. the values at high partial pressure of CO_2 (pCO_2) versus the values at control pCO_2, are shown as filled circles, whereas the C:L ratios, i.e. the values at control pCO_2 versus the values at low pCO_2, are shown as open circles. The nitrogen fixation rate reported by Hutchins et al. (2007) at low pCO_2 was 0; hence the C:L ratios could not be calculated. Asterisks (*) indicate data collected in a mesocosm experiment during which pCO_2 was drifting (see 'Methods'). TEP: transparent exopolymer particles; DOC: dissolved organic carbon; CDOM: chromophoric or coloured dissolved organic matter; BPP: bacterial protein production; attached and free: *Bacteria* attached on particles or free; csBPP: cell-specific bacterial protein production; BA: bacterial abundance; HBA and LBA: abundances of high- and low-fluorescence *Bacteria*; VA: viral abundance; HVA and LVA: abundances of high- and low-fluorescence viruses; APA: alkaline phosphatase activity

Fig. 1 (continued on next page)

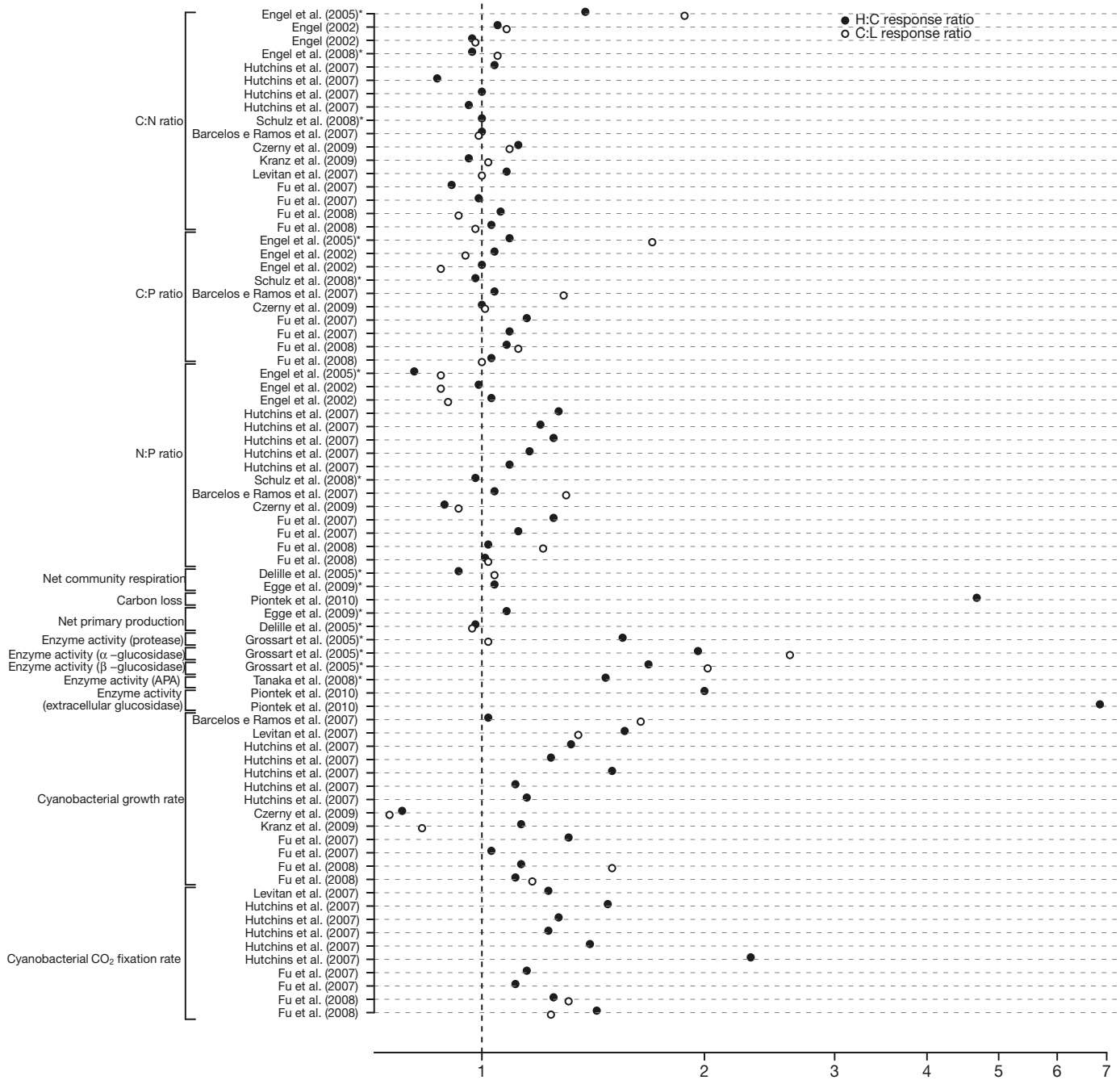

Fig. 1 (continued)

intracellular pH (pH_i) is maintained by a pH homeostatic system (Booth 1985). pH_i depends on the external pH, cytoplasmic buffers, the intracellular generation of acids and bases, and an active transport of H^+ (or OH^-). On short time scales, micro-organisms are often able to buffer external changes in pH, thereby preventing damage to internal processes and functioning. Few studies on pH control were performed on marine microbes, and all were carried out in experimental conditions that are not environmentally relevant (Takeuchi et al. 1997, Labare et al. 2010). Cultures were done in highly enriched media and the context of CO_2 disposal, hence at CO_2 and pH levels that are not relevant to 'natural' ocean acidification. For example, Labare et al. (2010) reported morphological changes and a temporary inhibition of growth in the marine bacterium *Vibrio* sp. grown at pH (scale not mentioned) 5.2. The recovery of growth after 6 h indicates

an efficient pH regulation system. It is unknown how long such compensation mechanisms can withstand an environmentally relevant decline of pH, and whether adaptation may occur over longer time scales.

TEP, DOM and CDOM

Transparent exopolymer particles (TEP) are major components of marine aggregates that are enriched in carbon relative to nitrogen or phosphorus, contribute to carbon export and provide habitats and sites for attachment of *Bacteria* (Passow 2002). Bacterial degradation can be an important pathway of TEP loss (Passow 2002). The effect size is above 1 (Fig. 1), suggesting a monotonous effect of elevated pCO_2 that stimulates TEP production. However, the SMD of TEP production between high and control pCO_2 is significantly different from 0 with the fixed effect model but it is not significantly different from 0 with the random effect model (Table 1). The SMD between the control and low pCO_2 is not significant with any of the models.

Engel (2002) reported that the production of TEP by natural phytoplankton communities increases with increasing pCO_2, but that at present-day pCO_2, TEP production is already saturated. Higher pCO_2 levels, as predicted for the future, would not lead to higher TEP production even though primary production might be stimulated (see below). In another mesocosm experiment, TEP production normalised to the abundance of *Emiliania huxleyi* was significantly higher in the high CO_2 treatment 'future' (~710 µatm) than in the 'present' (~410 µatm) and 'glacial' (~190 µatm) treatments (Engel et al. 2004). This indicates a possible direct effect of pCO_2 on polysaccharide exudation. However, the enhanced DIC level did not cause an increase in the particulate organic carbon (POC) concentrations, possibly due to an increased TEP production, which stimulated particle aggregation and accelerated sedimentation as previously observed by Logan et al. (1995) and Engel (2000). Mari (2008) also investigated the effect of seawater acidification on aggregation and sedimentation of TEP. Their results were similar to those of Engel et al. (2004) and suggested that a decrease of seawater pH would lead to a significant increase of the TEP pool. The most recent study available to date did not find a significant effect of elevated pCO_2 on the TEP concentration (Egge et al. 2009). In contrast to other studies, Mari (2008) found that the buoyancy of TEP is pH-dependent, and increases in pH cause TEP to ascend in the water column.

Chromophoric or coloured dissolved organic matter (CDOM) is the fraction of the dissolved organic matter (DOM) that absorbs light in both the ultraviolet and visible ranges. CDOM diminishes light and is degraded by UVA and UVB light and thus likely plays an important optical and ecological role in surface waters. There is a consensus that heterotrophic *Bacteria* produce CDOM (Nelson et al. 1998, Rochelle-Newall et al. 1999). However, how ocean acidification will affect heterotrophic *Bacteria* and CDOM release is still poorly understood. Only 1 study reported the effect of elevated pCO_2 on the concentration of CDOM, preventing the use of meta-analysis. The effect size on the dissolved organic carbon (DOC) concentration is around 1 (Fig. 1) and the SMD of TEP production between high and control pCO_2 is significantly different from 0 with both the fixed and random effect model (Table 1), suggesting an overall significant impact of elevated pCO_2 on the DOC concentration. Too few data are available to test the significance of the SMD between the control and low pCO_2.

This result contrasts with the conclusions drawn from the 2 individual studies available to date. In a mesocosm experiment carried out under different initial pCO_2 (190, 414 and 714 µatm), no impact of pCO_2 was observed on the concentrations of CDOM and DOC (Rochelle-Newall et al. 2004). In the same experiment, DOC was neither related to the abundance of *Emiliania huxleyi* nor to TEP concentration (Engel et al. 2004). No statistically significant effect of the CO_2 treatment on DOC concentration was found, although during the course of the bloom, the DOC concentration increased in 2 of the 3 'future' mesocosms and in 1 of the 'present' mesocosms, but in none of the 'glacial' mesocosms (initial pCO_2 of 714, 414 and 190 µatm, respectively). Engel et al. (2004) suggested that the different response of TEP (discussed above) and DOC may be due to differences in their bioavailability, which could have generated a rapid response of the microbial food web, possibly obscuring the effect of pCO_2 on the DOC production of autotrophic cells. No statistically significant CO_2 treatment effects on the concentration of DOC were detected in other PeECE experiments (Rochelle-Newall et al. 2004, Grossart et al. 2006a, Schulz et al. 2008) or in a mesocosm experiment with similar CO_2 treatments (Kim et al. 2006).

CARBON CYCLE

Primary production

A relatively large number of studies have investigated the effect of elevated pCO_2 on photosynthesis at the organismal level, especially to clarify the physiological and molecular mechanisms governing the carbon-concentrating mechanisms (CCMs). DIC is mostly fixed via the enzyme ribulose biphosphate carboxylase (RUBISCO), which has a relatively low affinity for CO_2.

Table 1. Summary standardised mean difference (SMD ± SEM) for each parameter calculated using fixed-effect and random-effect models. N: sample size; na: no data or the data do not meet the requirements for meta-analysis; p: probability. Other abbreviations as in the legend of Fig. 1

Parameters	High vs. control pCO_2 (H:C) SMD		Low vs. control pCO_2 (L:C) SMD	
	Fixed effect	Random effects	Fixed effect	Random effects
TEP production	0.83 ± 0.18 (p < 0.001)	1.06 ± 1.29 (p = 0.41)	−0.26 ± 0.18 (p = 0.15)	−0.26 ± 0.18 (p = 0.15)
DOC concentration	−0.69 ± 0.22 (p = 0.002)	−0.73 ± 0.28 (p = 0.009)	na	na
BPP (total)	−0.12 ± 0.21 (p = 0.58)	−0.13 ± 0.78 (p = 0.86)	na	na
BPP (free)	−0.70 ± 0.22 (p = 0.001)	−0.73 ± 0.59 (p = 0.21)	na	na
BPP (attached)	0.25 ± 0.21 (p = 0.24)	0.3 ± 0.6 (p = 0.62)	na	na
csBPP (total)	−0.22 ± 0.21 (p = 0.28)	−0.24 ± 0.55 (p = 0.66)	na	na
csBPP (free)	−0.82 ± 0.21 (p < 0.001)	−0.84 ± 0.38 (p = 0.027)	na	na
csBPP (attached)	0.10 ± 0.23 (p = 0.65)	0.21 ± 1.15 (p = 0.85)	na	na
BA (HBA)	−0.43 ± 0.18 (p = 0.013)	−1.48 ± 1.34 (p = 0.27)	na	na
BA (LBA)	−0.63 ± 0.17 (p < 0.001)	−0.7 ± 0.26 (p = 0.008)	na	na
BA (total)	0.06 ± 0.1 (p = 0.53)	−0.67 ± 0.50 (p = 0.18)	−0.38 ± 0.10 (p < 0.001)	−0.79 ± 0.78 (p = 0.32)
VA (LVA)	−0.42 ± 0.23 (p = 0.06)	−1.02 ± 1.22 (p = 0.406)	na	na
VA (HVA)	−0.61 ± 0.22 (p = 0.006)	−1.13 ± 1.09 (p = 0.3)	na	na
Nitrogen fixation	1.06 ± 0.26 (p < 0.001)	1.95 ± 0.68 (p = 0.004)	−0.76 ± 0.27 (p = 0.005)	−0.66 ± 0.46 (p = 0.15)
C:N ratio	0.05 ± 0.14 (p = 0.72)	−0.01 ± 0.51 (p = 0.98)	−0.84 ± 0.15 (p < 0.001)	−0.68 ± 0.53 (*N* = 130, p =0.20)
C:P ratio	0.26 ± 0.17 (p = 0.14)	0.27 ± 0.26 (p = 0.3)	−1.76 ± 0.23 (p < 0.001)	−1.44 ± 0.52 (p =0.005)
N:P ratio	−0.48 ± 0.19 (p = 0.01)	0.69 ± 0.59 (p = 0.24)	0.49 ± 0.27 (p = 0.08)	−0.17 ± 1.56 (p =0.92)
Community respiration	−0.78 ± 0.22 (p = 0.001)	−0.55 ± 0.50 (p =0.28)	na	na
Net primary production	−0.21 ± 0.30 (p = 0.48)	0.22 ± 0.81 (p = 0.78)	na	na
Cyanobacterial growth rate	0.68 ± 0.21 (N = 69, p = 0.001)	1.06 ± 0.36 (N = 69, p = 0.003)	−1.41 ± 0.33 (N = 33, p < 0.001)	−0.68 ± 1.11 (N = 33, p = 0.54)
Cyanobacterial CO_2 fixation rate	1.21 ± 0.33 (N = 43, p < 0.001)	1.58 ± 0.49 (N = 43, p = 0.001)	0.44 ± 0.60 (N = 12, p = 0.46)	−3.30 ± 3.02 (N = 12, p = 0.27)

In most species, RUBISCO is less than half saturated at present CO_2 levels (Giordano et al. 2005). Hence, autotrophs that only rely on diffusive entry of CO_2 have poor photosynthetic efficiency. However, all cyanobacteria and most algae have developed CCMs to elevate the concentration of CO_2 at the site of carboxylation. It is beyond the scope of this paper to cover this literature extensively, as several excellent reviews are available (Riebesell 2004, Giordano et al. 2005, Beardall et al. 2009). We will focus on cyanobacteria and community primary production below and simply wish to point out that large differences in the CO_2-sensitivity between the major groups of eukaryotic phytoplankton exist (Riebesell 2004).

Cyanobacteria are the largest and the most widely distributed group of photosynthetic prokaryotes (Burns et al. 2005). This group has a major impact on the global carbon cycle and contributes up to 50% of the

Table 2. Estimates of the heterogeneity of the effect size (Q, H and I^2; see 'Methods'). df: degrees of freedom; na: no data or the data do not meet the requirements for meta-analysis; p: probability; other abbreviations as in the legends to Table 1 and Fig. 1

Parameters	High vs. control pCO_2					Low vs. control pCO_2				
	Q	df	p	H	I^2 (%)	Q	df	p	H	I^2 (%)
TEP production	45.7	3	<0.001	3.9	93	1.5	2	0.46	1	0
DOC concentration	1.4	1	<0.001	1.2	30	na	na	na	na	na
BPP (total)	13.4	1	<0.001	3.7	93	na	na	na	na	na
BPP (free)	7.2	1	0.01	2.7	86	na	na	na	na	na
BPP (attached)	7.9	1	0.01	2.8	87	na	na	na	na	na
csBPP (total)	6.8	1	0.009	2.6	85	na	na	na	na	na
csBPP (free)	3.1	1	0.07	1.8	68	na	na	na	na	na
csBPP (attached)	26	1	<0.001	5.1	96	na	na	na	na	na
BA (HBA)	18.9	1	<0.001	4.4	95	na	na	na	na	na
BA (LBA)	1.5	1	0.22	1.2	34	na	na	na	na	na
BA (total)	32.8	2	<0.001	4.1	94	42.8	1	<0.001	6.5	97
VA (LVA)	22	1	<0.001	4.7	95	na	na	na	na	na
VA (HVA)	17.4	1	<0.001	4.2	94	na	na	na	na	na
Nitrogen fixation	33.72	9	<0.001	1.9	73	8	4	0.09	1.4	50
C:N ratio	119.2	12	<0.001	3.2	90	61.4	6	<0.001	3.2	90
C:P ratio	11.3	7	0.13	1.3	38	13.5	4	0.01	1.8	71
N:P ratio	89	12	<0.001	2.7	87	105.7	4	<0.001	5.1	96
Community respiration	2.1	1	0.15	1.5	53	na	na	na	na	na
Net primary production	3.7	1	0.06	1.9	73	na	na	na	na	na
Cyanobacterial growth rate	27.7	12	0.01	1.5	57	17.1	3	<0.001	2.4	82
Cyanobacterial CO_2 fixation rate	13.5	8	0.09	1.3	41	9.4	2	0.01	2.2	79

fixed carbon in marine systems (Partensky et al. 1999). Cyanobacteria are known to utilise CCMs, such as the active transport of HCO_3^- and CO_2, to facilitate CO_2 fixation and maintain rapid growth at low external DIC concentrations (Badger & Price 2003, Badger et al. 2006). It is reasonable to assume that increased CO_2 availability will reduce the need for CCM activity and hence reduces the allocation of energy or nutrients for carbon acquisition (Burkhardt et al. 2001, Beardall & Giordano 2002). This may further affect the photosynthesis, growth rate and other activities.

The effect of elevated pCO_2 on the growth rate and/or photosynthesis of the widespread cyanobacterium *Trichodesmium* spp. was investigated by Barcelos e Ramos et al. (2007), Levitan et al. (2007), Hutchins et al. (2007) and Kranz et al. (2009). Four other cyanobacterial species were investigated: *Synechococcus* sp. and *Prochlorococcus* sp. (Fu et al. 2007), *Crocosphaera watsonii* (Fu et al. 2008) and *Nodularia spumigena* (Czerny et al. 2009).

Overall, the effect size on the growth rate is above 1 both for the H:C and C:L ratios (Fig. 1), suggesting a monotonous increase in growth rate as a function of increasing pCO_2. One notable exception, with effect sizes below 1, is *Nodularia spumigena*, which exhibits a monotonous decrease in growth rate as a function of increasing pCO_2. The SMD of cyanobacterial growth rate between high and control pCO_2 is significantly different from 0 both with the fixed effect and random effects models (Table 1). The SMD between the control and low pCO_2 is significantly different from 0 only with the fixed effect model. An additional demonstration of species specificity is the result that *Synechococcus* sp. exhibits a much greater response to elevated pCO_2 (750 versus 380 µatm) and temperature (4°C increase) than *Prochlorococcus* sp. (Fu et al. 2007). There is some evidence that the growth rate of *Crocosphaera* increases at elevated pCO_2 but only under Fe-replete conditions (Fu et al. 2008).

The effect size on the cyanobacterial photosynthesis (i.e. CO_2 fixation rate) is above 1 both for the H:C and C:L ratios (Fig. 1), suggesting a monotonous increase in the rate of photosynthesis with increasing pCO_2. There is only 1 exception in the study of Levitan et al. (2007), where photosynthesis was higher at low and high pCO_2 than in the control. The SMD of cyanobacterial photosynthesis between high and control pCO_2 was significantly different from 0 both with the fixed effect and random effects models, but the SMD between the control and low pCO_2 was not significantly different from 0 (Table 1).

The stimulation of net photosynthesis at elevated pCO_2 is predominantly attributed to changes in cell division (Hutchins et al. 2007, Levitan et al. 2007) but also to altered elemental ratios of carbon to nitrogen (Levitan et al. 2007, Kranz et al. 2009) or nitrogen to phosphorus (Barcelos e Ramos et al. 2007). Fu et al. (2007) reported that the photosynthetic parameters of *Synechococcus* sp. significantly changed at elevated pCO_2 but only when combined with elevated temperature.

There are several reports of increased community primary production of phytoplanktonic assemblages (some not microbial) at elevated pCO_2. Hein & Sand-Jensen (1997) indicated that elevated CO_2 will stimulate primary production in the North Atlantic. The PeECE mesocosm experiments also investigated the effect of elevated pCO_2 on net primary production. No conspicuous change was observed in the PeECE I (Delille et al. 2005) and II (J. Egge unpubl. data) experiments, but a significant effect was found in the PeECE III experiment. Riebesell et al. (2007) reported a 27 and 39% increase in net primary production at 2× and 3× ambient pCO_2, and Egge et al. (2009) found a higher cumulative primary production based on the ^{14}C-incorporations at higher pCO_2 towards the end of the experiment. However, other studies had reported that increased pCO_2 resulted in no significant increase in primary production (Tortell et al. 2002).

Bacterial abundance, production and enzyme activity

Bacteria are the main group of organisms able to use DOC. Since they can be grazed by flagellates, some of the DOC which would otherwise be lost from the food web, can be cycled back via grazing (microbial loop; Azam et al. 1983). Inagaki et al. (2006) reported that the pH values of the deep-sea sediments overlying a CO_2 lake ranged from 4.0 to 6.6 units, in contrast to a pH (presumably on the National Bureau of Standards, NBS, scale) of 7.3 outside of the CO_2-hydrate zone. In their study, a strong decline in cell numbers and abundance of specific lipid biomarkers toward the liquid CO_2 interface on a scale of decimetres was observed: along this gradient high abundances ($>10^9$ cm^{-3}) of microbial cells found in sediment pavements above the CO_2 lake decreased to strikingly low cell numbers (10^7 cm^{-3}) at the liquid CO_2/CO_2-hydrate interface. In other studies performed at pCO_2 levels relevant to future surface ocean acidification (190 to 1050 µatm), the total abundance of *Bacteria* varied considerably in phytoplankton blooms triggered in pelagic mesocosms, but pCO_2 had little or no effect on bacterial abundance (BA; Rochelle-Newall et al. 2004, Grossart et al. 2006a, Allgaier et al. 2008). Note, however, that a significant impact was observed during the decline of the bloom in 1 of the experiments together with a higher growth rate and abundance of attached prokaryotes at the highest pCO_2 level (700 µatm; Grossart et al. 2006a). Yamada et al. (2008) also found no significant effect of pCO_2 values up to 10 000 µatm.

The H:C effect sizes of the abundance of high DNA *Bacteria* (HBA) and low DNA *Bacteria* (LBA) are lower than 1, suggesting an inhibition of bacterial abundance under high pCO_2, but the C:L ratios are higher than 1, indicating a minimum abundance at control pCO_2 and larger abundances at low and high pCO_2 (Fig. 1). Only 2 SMDs are significantly different from 0: the high DNA *Bacteria* H:C ratio and the total BA L:C ratio, both with a fixed model.

Diverse responses of bacterial production (BPP) to elevated pCO_2 were found, partly depending on the community considered (attached versus free *Bacteria*) and on the normalisation used (total BPP or cell-specific BPP, csBPP). The effect sizes suggest a monotonous response in 4 of the data sets available at low, control and high pCO_2 (Fig. 1). The H:C effect size is most often much higher than the L:C effect size. In the other 3 data sets, the response does not appear to be monotonous. Only a few SMDs of bacterial production between high and control pCO_2 are significantly different from 0 with the fixed effect model (total and csBPP of free *Bacteria*) and the random effect model (csBPP of free *Bacteria*; Table 1). Too few data are available to test the significance of the SMD between the control and low pCO_2.

Grossart et al. (2006a) reported that the total BPP (estimated using tritiated leucine incorporation) as well as the csBPP of total and attached *Bacteria* are higher at elevated pCO_2. They also found a higher protease activity at elevated pCO_2, whereas the activities of α- and β-glucosidase remained unchanged. The preferential stimulation of the abundance and activity of attached *Bacteria* may result from an increased production of TEP, which would provide surfaces for *Bacteria* and favour aggregation (Grossart et al. 2006b). Allgaier et al. (2008) suggested that there was no difference in BPP among pCO_2 treatments. However, linear regressions between BPP of free-living *Bacteria*, BPP of attached *Bacteria* or csBPP of attached *Bacteria* and C:N ratio of suspended matter were significantly different between pCO_2 levels. Yamada et al. (2008) also reported an increase in bacterial production, but they used very high pCO_2 levels ranging between 2000 and 10 000 µatm.

Although there is some evidence that elevated CO_2 affects some microbial processes such as bacterial production and degradation, the understanding of the mechanisms involved is still poor. Extracellular enzymes are vital for microbial metabolism, and their activity could provide clues on the mechanisms involved. Grossart et al. (2006a) found that the activity of total protease as well as α- and β-glucosidase was highest at elevated pCO_2 levels, but this effect was statistically significant only for protease activity. Similar results were reported by Piontek et al. (2010), with higher rates of extracellular glucosidases at lower pH. Tanaka et al. (2008) reported that the specific glucose affinity of *Bacteria* at 3 pCO_2 levels was similar.

Kranz et al. (2009) investigated the external carbonic anhydrase (eCA) activities of the cyanobacterium *Tri-*

chodesmium erythraeum in response to pCO_2 levels of 150, 370 and 1000 µatm. eCA is an enzyme which promotes the conversion of HCO_3^- ions to CO_2. They reported low activities of eCA, which did not change as a function of pCO_2, indicating a minor role of eCA in the carbon acquisition of this species.

Among the factors which determine the consequences of bacterial DOC consumption are the rate at which biomass is produced (bacterial carbon assimilation) and the rate at which DOC is converted into CO_2 (bacterial respiration). However, bacterial respiration and growth efficiency have not yet been studied with respect to ocean acidification. Also, there are no data on bacterial production rates estimated as cell division rates using incorporation of tritiated thymidine. Thus, it is not known whether pCO_2 changes result in differences of the 2 main methods used for estimating bacterial production, i.e. leucine and thymidine incorporation. Such differences are known under stress situations such as UVB exposure.

Organic carbon consumption and loss

Piontek et al. (2010) recently tested the effect of ocean acidification on the degradation activity of marine *Bacteria* in a pH perturbation experiment. Higher loss of polysaccharides (up to 32%) and POC were found under lowered pH conditions, which suggested that ocean acidification could affect the cycling of organic carbon in the future ocean by weakening the biological carbon pump and by increasing the respiratory production of CO_2. Riebesell et al. (2007) reported that the community consumed up to 39% more DIC at higher pCO_2, whereas nutrient uptake remained the same. This excess carbon consumption was associated with higher loss of organic carbon from the upper layer of the mesocosm. This has an implication on a variety of marine biological and biogeochemical processes. In the same mesocosm experiment, community respiration did not reveal any clear response to pCO_2, neither in terms of the timing nor of the level of cumulative consumption for the 24 d of the experiment (Egge et al. 2009).

NUTRIENT CYCLES

Inorganic nutrients such as nitrate and phosphate are vital for microbial growth. Almost all published studies have investigated the nitrogen cycle. Tanaka et al. (2008) investigated the availability of phosphate for phytoplankton and *Bacteria* at 3 pCO_2 levels (350 µatm: 1×CO_2; 700 µatm: 2×CO_2; 1050 µatm: 3×CO_2). Its response was similar to that of the total particulate phosphorus concentration and phosphate turnover time. The phosphate transferred to the >10 µm fraction was greater in the 3×CO_2 mesocosm during the first 6 to 10 d when the phosphate concentration was still high. Also, the lower availability of inorganic nutrients after the phytoplankton bloom reduced the bacterial capacity to consume labile DOC. The specific alkaline phosphatase activity (APA) of *Bacteria* tended to be higher at 3×CO_2 than at 2× and 1×CO_2 during the phosphate depletion period.

Nitrogen fixation

Diazotrophic cyanobacteria affect marine ecosystems by providing reactive nitrogen to otherwise nitrogen-limited regions. Most effect sizes for heterocystous cyanobacteria are above 1 (Fig. 1; Barcelos e Ramos et al. 2007, Hutchins et al. 2007, Levitan et al. 2007, Fu et al. 2008, Kranz et al. 2009), whereas they are below 1 for the non-heterocystous species *Nodularia spumigena* (Czerny et al. 2009). The SMDs of nitrogen fixation between high and control pCO_2 are significantly different from 0 with the fixed effect model and the random effect model (Table 1). The SMD between control and low pCO_2 is significantly different from 0 only with the fixed effect model.

The filamentous non-heterocystous cyanobacterium *Trichodesmium* spp. thrives in oligotrophic areas of tropical and subtropical seas. This group contributes about half of all marine N_2 fixation (Mahaffey et al. 2005). The effect of pCO_2 levels ranging from 140 to 850 µatm revealed that rates of N_2 fixation per unit of phosphorus utilisation more than doubled at high CO_2 (Barcelos e Ramos et al. 2007). In 2 other studies, very similar results were obtained (Hutchins et al. 2007, Levitan et al. 2007). Relative to ambient or low pCO_2, high pCO_2 levels enhanced N_2 fixation as well as the filament length and biomass of *Trichodesmium* (Levitan et al. 2007). For example, N_2 fixation increased by 121% between 400 and 900 µatm. Hutchins et al. (2007) reported a significant increase of N_2 fixation and growth rate between 380 and 1500 µatm, with an increase of 63% at a pCO_2 of 750 µatm. Kranz et al. (2009) also reported a stimulation in nitrogen fixation of *T. erythraeum* by almost 40% at a pCO_2 of 1000 µatm relative to 370 µatm. Only 1 study is available in a cyanobacterium other than *Trichodesmium*. The unicellular diazotroph *Crocosphaera watsonii* revealed N_2 fixation rates that were enhanced by 40% at 750 µatm relative to that at 380 µatm (Fu et al. 2008).

It seems clear that CO_2 is limiting nitrogen fixation and that ocean acidification could substantially increase, at least in short-term experiments, the fixation of N_2 (and CO_2 as reviewed above) of *Trichodesmium*. Hutchins et al. (2009) pointed out that the magnitude

of the response of nitrogen and carbon fixation to elevated pCO_2 is the largest physiological response yet reported for marine microbes. This could fundamentally alter the N and C cycles. It is important to note that the rate of N_2 fixation did not continue to rise as pCO_2 levels were further elevated above 750 µatm. This suggests that the observed increase of N_2 fixation by *Trichodesmium* might level off by the end of the century (Hutchins et al. 2009).

In contrast to the non-heterocystous cyanobacteria mentioned above, the heterocystous, bloom-forming diazotroph *Nodularia spumigena* showed a slight decrease of N_2 fixation rate at increasing pCO_2 levels (Czerny et al. 2009).

Nitrification

Nitrification, another important process in the nitrogen cycle, is the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates. Huesemann et al. (2002) investigated the effects of CO_2-induced pH changes on marine nitrification in the context of deep-sea CO_2 disposal. They found that the rate of nitrification drops drastically with decreasing pH. Relative to the rates at pH 8 (presumably on the NBS scale), nitrification decreased by ca. 50% at pH 7 and by more than 90% at pH 6.5, while it was completely inhibited at pH 6.0.

ELEMENTAL RATIOS

Since the growth efficiency of heterotrophic microbes is controlled by the quality of food, any change in the elemental composition of particulate or dissolved organic matter (POM or DOM) could directly or indirectly affect processes such as growth rate, respiration and nutrient recycling (Engel et al. 2005). The effect sizes on the C:N ratio are distributed around 1 for all studies but 1 (Fig. 1). Nevertheless, the SMD is significantly different from 0 with the fixed effect model between the high and control pCO_2 and between the control and low pCO_2 (Table 1). The effect sizes on the C:P ratio do not seem to follow a consistent pattern (Fig. 1). The SMD between the high and control pCO_2 is not significantly different from 0 with both the fixed and random effect models but is statistically different from 0 between the control and low pCO_2 (Table 1). Finally, the effect size of the N:P ratio also does not seem to follow a coherent pattern in the 5 studies available to date (Fig. 1), but the SMD is nevertheless statistically significant with the fixed effect model between the high and control pCO_2 and between the control and low pCO_2 (Table 1).

Significant changes in the consumption ratio of various inorganic nutrients in response to increasing pCO_2 were reported by Tortell et al. (2002). They reported that a pelagic community of the Equatorial Pacific consumed NO_3^- and H_2SiO_3 in a ratio close to 1:1 (range from 1.0 to 1.55) in the high CO_2 (750 µatm) treatment, while the consumption ratio was much higher (2.16 to 2.71) at a low pCO_2 of 150 µatm. In contrast, the assimilation of nitrate and phosphate was similar in the 3 CO_2 treatments investigated by Engel et al. (2005) in a mesocosm experiment. The concentration of particulate constituents was highly variable among the replicate mesocosms, likely disguising direct CO_2-related effects.

Changes in the C:N:P ratios have been shown in cultures of several eukaryotic phytoplankton groups (Riebesell 2004). Differential effects were observed on the 2 main cyanobacterial groups: *Synechococcus* and *Prochlorococcus*. Fu et al. (2007) reported that *Synechococcus* sp. had a slight decrease in C:N and an increase in C:P and N:P at the higher CO_2 concentration, while there was no significant difference in *Prochlorococcus* sp. As mentioned in the previous section, pCO_2 has an effect on the N_2 uptake and C fixation rates of some nitrogen-fixing cyanobacteria. This could significantly affect the C:N and N:P ratios, but the effect found in the various experiments is not consistent. Elevated pCO_2 increased the C:N ratio in 3 studies (Levitan et al. 2007, Czerny et al. 2009, Kranz et al. 2009) but had no effect in another study (Barcelos e Ramos et al. 2007). The N:P ratio of *Trichodesmium* sp. increases at elevated pCO_2 (Barcelos e Ramos et al. 2007), whereas the N:P ratio of *Nodularia spumigena* decreases (Czerny et al. 2009).

Changes in elemental ratios were also reported at the community level and could have a considerable impact on the strength of the biological pump. For example, in a mesocosm experiment, Riebesell et al. (2007) found that the stoichiometry of C:N drawdown increased from 6 at low CO_2 to 8 at high CO_2, thus exceeding the Redfield C:N ratio of 6.6 in today's ocean. These authors speculated that this could translate to an excess CO_2 sequestration potential, through the biological carbon pump, of 116 Pg C until 2100. Increasing C:N ratios would also lower the nutritional value of primary-produced organic matter, which may affect the efficiency of bacterial degradation and zooplankton reproduction. In the same mesocosm experiment, Bellerby et al. (2008) also found that the cumulative C:N and C:P ratios of organic production until the height of the bloom decreased with increasingpCO_2. The C:N:P ratios were 1:6.3:121 at 350 µatm, 1:7.1:144 at 700 µatm and 1:8.25:168 at 1050 µatm. Other studies also reported species-dependent changes of phytoplankton C:N:P ratios at elevated

pCO_2 (Burkhardt & Riebesell 1997, Burkhardt et al. 1999, Tortell et al. 2000).

MORTALITY

Viral lysis and grazing are the 2 main factors of microbial mortality. Lysis transfers lysis products such as the cell content (including viruses) and cell debris into the DOM pool. This viral shunt (Wilhelm & Suttle 1999) increases bacterial production and respiration and enhances nutrient recycling. As mentioned above, grazing on *Bacteria* transfers organic matter back to the food web, whereas grazing on phytoplankton is the first step in the grazing food chain, which also plays a key role in carbon cycling. Thus, the relative contribution of viral lysis and grazing is an important factor for shaping the fate of primary production and bacterial production. Not much is known about how changes in pCO_2 could influence mortality.

Rochelle-Newall et al. (2004) reported that elevated pCO_2 had no effect on total viral abundance (VA), thus suggesting that viral lysis was not influenced strongly. Viral lysis rates have not yet been measured in combination with pCO_2 changes. For grazing, more information is available. Riebesell et al. (2007) noted that the DIC consumption increased with increasing pCO_2, whereas the nutrient uptake remained stable. This leads to an offset in the Redfield ratios, and possibly causes a deterioration of the food quality. Veloza et al. (2006) reported that some microzooplankton groups, e.g. some dinoflagellates, may have the capacity to use low quality prey. However, it is still unclear how and to what extent this takes place. Until now, only 2 studies investigated the response of microzooplankton grazing to increasing pCO_2 levels. In the PeECE III experiment, grazing was highly dynamic over time, and no effect of CO_2 on microzooplankton grazing was found (Suffrian et al. 2008). Rose et al. (2009) investigated the potential effects of climate change variables (temperature and pCO_2) on the trophic dynamics using a shipboard continuous culture system. They observed increases in both the abundance and grazing rates of microzooplankton in the high pCO_2 treatments (690 µatm) relative to ambient pCO_2 treatments (390 µatm).

COMMUNITY COMPOSITION AND DIVERSITY

Changes in community composition in the sense of differences in the relative abundance of large plankton groups have been documented for pCO_2 manipulation experiments and are basically the result of differences in the time developments of different groups. Changes in diversity are considered in the following as changes of the species composition.

Phytoplankton

Phytoplanktonic diversity was profoundly affected by elevated pCO_2 in some but not all perturbation experiments performed at the community level (Allgaier et al. 2008, Paulino et al. 2008). Changes in phytoplankton diversity also led to changes in bacterial community structure and subsequently in bacterial activities.

Tortell et al. (2002) provided direct evidence that CO_2 concentrations can influence the species composition of a marine phytoplankton assemblage. The phytoplankton assemblage exposed to pCO_2 levels of 150 and 750 µatm was dominated by diatoms and *Phaeocystis* sp. by the end of the experiment, but the abundance of diatoms decreased by ~50% at low pCO_2 relative to high pCO_2 levels, while the abundance of *Phaeocystis* increased by about 60%. This shift associated with a higher ratio of nitrate:silicate (N:Si) and N:P consumption at low pCO_2 also suggested that CO_2 concentrations could potentially influence competition among marine phytoplankton taxa and affect oceanic nutrient cycling.

Prokaryotes

Mühling et al. (2006) did not find any changes in the diversity of *Bacteria* subject to different pCO_2 levels in a CO_2 perturbation experiment carried out in mesocosms. Vega Thurber et al. (2009) reported changes in the diversity of coral-associated microbiota, which shifts from a healthy-associated community to a community often found on diseased corals (e.g. *Bacterioidetes*, *Fusobacteria* and Fungi) after a strong decline in pH (8.1 to 6.7). Additionally, decreased pH as well as other stressors, such as elevated temperature, nutrients and DOC, led to an increased abundance of genes involved in virulence, stress resistance and production of secondary metabolites.

The diversity of free-living *Bacteria* of pelagic mesocosms assessed by community fingerprinting changes with pCO_2, whereas that of attached *Bacteria* seems to be independent of pCO_2 and coupled to the development of the phytoplankton bloom (Allgaier et al. 2008).

Viruses and grazers

Larsen et al. (2008) investigated how the virioplankton community responded to increased levels of CO_2 during the PeECE III mesocosm experiment. Some

viral populations detected and enumerated by flow cytometry did not respond to altered CO_2 levels. No clear effect was found in the 'low-fluorescence viruses' (LFV), 'medium-fluorescence viruses' (MFV) and 'putative large viruses' (PLV). However, the 'high-fluorescence viruses' (HFV) exhibited a higher maximum abundance in the 1×CO_2 than in the 2× and 3×CO_2 mesocosms, thus suggesting changes in viral diversity. The abundance of *Emiliania huxleyi* virus (EhV) and an unidentified double-stranded DNA (dsDNA) virus decreased with increasing CO_2 levels. Only 1 study investigated the effect of elevated pCO_2 on the community composition of viruses (Larsen et al. 2008). In their study, 2 specific large dsDNA viruses (EhV and CeV, infecting the haptophytes *E. huxleyi* and *Crysochromulina ericina*) were identified. Their results indicate that the change in parameters of the carbonate chemistry might affect the marine pelagic food web at the viral diversity level. It also demonstrated that in order to unravel ecological problems as to how pCO_2 and nutrients affect the relationship between marine algal viruses and their hosts, an effort to develop molecular markers used to identify both hosts and viruses is needed. Nothing is known about the effect of pCO_2 on the diversity of grazers.

DISCUSSION AND CONCLUSIONS

Microbial processes play an important role in the functioning and the biogeochemical cycles of marine ecosystems. Although some obvious effects of ocean acidification on microbes were detected, their response is not always consistent and our understanding remains poor. There are many gaps and challenges for future research.

Most data on the effect of ocean acidification on microbial processes and diversity were gathered in perturbation experiments carried out in the laboratory and in mesocosms. Few studies investigated the simultaneous effects of ocean acidification and other perturbations. Yet, it is well established that microbial processes are greatly affected by changes in temperature and light, as well as changes in the supply of inorganic nutrients and organic matter (e.g. Kirchman et al. 2009). It is therefore critical that the interactions between the carbonate chemistry and other parameters are investigated. Equally critical is the need to use pCO_2 gradients rather than only 2 pCO_2 levels to determine critical threshold levels for parameterising biogeochemical models. Open water CO_2 fertilisation experiments still seem unrealistic (Lance 2009), but it is potentially rewarding to take advantage of systems naturally enriched with CO_2 such as shallow-water CO_2 vents (Hall-Spencer et al. 2008), deep-sea vents (Inagaki et al. 2006), cold-eddies and upwelling systems (Feely et al. 2008), which have lower pH and high pCO_2 levels compared to ambient water and are thus highly suitable to study effects of ocean acidification, although the data interpretation is challenging due to factors such as advection or migrations.

There is evidence for acclimation to the pH_i regulation by a *Vibrio* strain (Labare et al. 2010). Adaptation, i.e. adjustment to environmental change by genetic change, is likely faster in microbes than in multi-cellular marine organisms. This is due to their short generation time of a few days, which allows for thousands of generations by 2100, hence increasing the accumulation of mutations, and, at least for prokaryotes, due to more efficient lateral gene transfer. Most experiments have been conducted over short periods (days to weeks), and there is a strong need to carry out longer-term experiments to detect if adaptation or acclimation occurs. Genomics, transcriptomics, proteomics and assessment of the expression of specific marker genes for crucial functions are among the most promising methods that are or soon will be available to tackle these problems.

So far, most studies have investigated individual species. More research is needed at multi-species and community scales. Losses of diversity in the sense of extinction of species are unlikely for free-living microorganisms. However, a large body of research supports the idea that free-living microbial taxa exhibit biogeographic patterns (Martiny et al. 2006). Recently, the so-called 'rare biosphere' was detected, i.e. bacterial phylotypes which only occur in low abundance (Sogin et al. 2006) and may serve as seed banks available for adaptation to environmental changes (such as increasing pCO_2) at the species level. Such questions can be addressed by large-scale sequence approaches such as high-throughput DNA sequencing. The decrease in costs is making this approach affordable.

Until now, no standard protocols have been available to manipulate the carbonate chemistry during perturbation experiments and no guidelines have been available for data reporting. Consequently, some of the experiments published to date are difficult to interpret, for example due to inadequate pCO_2 levels or the lack of information in the data reporting, which seriously hampers comparative studies and meta-analyses. The publication of the 'Guide for best practices on ocean acidification research and data reporting' (Riebesell et al. 2010) will hopefully lead to better experimental set ups and reporting in future publications.

Joint et al. (in press) pointed out that ocean pH is variable on short time scales in surface waters and also as a function of depth. They noted that microbial processes continue at depths where pH reaches values projected in the surface ocean in 2100, asked whether

'microbial assemblages will continue to function at the lower pH values that are projected for the near future', and suggested a null hypothesis that biogeochemical processes other than calcification will not be fundamentally different in a high-CO_2 ocean. Meta-analysis is the right tool to test the null hypothesis that ocean acidification will have no effect on microbial processes. Although it has not proven to be of great use for many variables because of the low sample sizes, notable exceptions are nitrogen fixation, cyanobacterial photosynthesis and, to a lesser extent, elemental ratios. This review and analysis therefore suggests that it is unlikely that any microbial process will cease to function due to ocean acidification and that the null hypothesis of Joint et al. (in press) can be rejected. The rates of several processes will be affected by ocean acidification, some positively, others negatively.

Another outcome of our meta-analysis is that the response of almost all parameters to ocean acidification is heterogenous among studies, suggesting the occurrence of confounding effects. There is no doubt that the launch of major national and international projects on ocean acidification will considerably increase the number of datasets available over the next few years and will lead to more solid conclusions on the effect of ocean acidification on microbial processes.

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