Biogeosciences Discuss., doi:10.5194/bg-2016-56, 2016
Manuscript under review for journal Biogeosciences
Published: 7 March 2016

Biogeosciences Discussions
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Effects of ocean acidification on pelagic carbon fluxes in a mesocosm experiment

Kristian Spilling[1, 2], Kai G. Schulz[3], Allanah J. Paul[4], Tim Boxhammer[4], Eric P. Achterberg[4, 5], Thomas Hornick[6], Silke Lischka[4], Annegret Stuhr[4], Rafael Bermúdez[4, 7], Jan Czerny[4], Kate Crawfurd[8], Corina P. D. Brussaard[8, 9], Hans-Peter Grossart[6, 10], Ulf Riebesell[4]

[1] {Marine Research Centre, Finnish Environment Institute, P.O. Box 140, 00251 Helsinki, Finland}

[2] {Tvärminne Zoological Station, University of Helsinki, J. A. Palménin tie 260, 10900 Hanko, Finland}

[3] {Centre for Coastal Biogeochemistry, Southern Cross University, Military Road, East Lismore, NSW 2480, Australia}

[4] {GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany}

[5] {National Oceanography Centre Southampton, European Way, University of Southampton, Southampton, SO14 3ZH, UK}

[7] {Facultad de Ingeniería Marítima, Ciencias Biológicas, Oceánicas y Recursos Naturales. ESPOL, Escuela Superior Politécnica del Litoral, Guayaquil, Ecuador}

[6] {Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Experimental Limnology, 16775 Stechlin, Germany}

[8] {Department of Biological Oceanography, Royal Netherlands Institute for Sea Research (NIOZ), P. O. Box 59, 1790 AB Den Burg, Texel, The Netherlands}

[9] {Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, The Netherlands

[10] {Potsdam University, Institute for Biochemistry and Biology, 14469 Potsdam, Germany}

Correspondence to: K. Spilling (kristian.spilling@environment.fi)

Running title: Modified pelagic carbon fluxes

Key words: Carbon fluxes, carbon budget, gross primary production, respiration, bacterial production, sinking carbon flux, CO_2 exchange with atmosphere

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Abstract

About a quarter of anthropogenic CO_2 emissions are currently taken up by the oceans decreasing seawater pH. We performed a mesocosm experiment in the Baltic Sea in order to investigate the consequences of increasing CO_2 levels on pelagic carbon fluxes. A gradient of different CO_2 scenarios, ranging from ambient (~370 µatm) to high (~1200 µatm), were set up in mesocosm bags (~55 m^3). We determined standing stocks and temporal changes of total particulate carbon (TPC), dissolved organic (DOC), dissolved inorganic (DIC) and particulate organic carbon (POC) of specific plankton groups. We also measured carbon flux via CO_2 exchange with the atmosphere and sedimentation (export); and biological rate measurements of primary production, bacterial production and total respiration. The experiment lasted for 44 days and was divided into three different phases (I: *t0-t16*; II: *t17-t30*; III: *t31-t43*). Pools of TPC, DOC and DIC were approximately 420, 7200 and 25200 mmol C m^{-2} at the start of the experiment, and the initial CO_2 additions increased the DIC pool by ~7% in the highest CO_2 treatment. Overall, there was a decrease in TPC and increase of DOC over the course of the experiment. The decrease in TPC was lower, and increase in DOC higher, in treatments with added CO_2. During Phase I the estimated gross primary production (GPP) was ~100 mmol C fixed m^{-2} d^{-1}; from which 75-95% were respired, ~1% ended up in the TPC (including export) and 5-25% added to the DOC pool. During Phase II, the respiration loss increased to ~100% of GPP at the ambient CO_2 concentration, whereas respiration was lower (85-95% of GPP) in the highest CO_2 treatment. Bacterial production was ~30% lower, on average, at the highest CO_2 concentration compared with the controls during Phases II and III. This resulted in a higher accumulation DOC standing stock and lower reduction in TPC in the elevated CO_2 treatments at the end of Phase II extending throughout Phase III. The "extra" organic carbon at high CO_2 remained fixed in an increasing biomass of small-sized plankton and in the DOC pool, and did not transferred into large, sinking aggregates. Our results revealed a clear effect of increasing CO_2 on carbon production and mineralization, in particular under nutrient limited conditions. Lower carbon loss processes (respiration and bacterial remineralization) at elevated CO_2 levels resulted in higher TPC and DOC pools compared with the ambient CO_2 concentration. These results highlight the importance to address not only net changes in carbon standing stocks, but also carbon fluxes and budgets to better disentangle the effects of ocean acidification.

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1 Introduction

Combustion of fossil fuels and change in land use, have caused increasing atmospheric concentrations of carbon dioxide (CO_2). Ca. 25% of the anthropogenic CO_2 is absorbed by the oceans, thereby decreasing surface water pH, a process termed ocean acidification (Le Quéré et al., 2009). Ocean acidification and its alterations of aquatic ecosystems have received considerable attention during the past decade, but there are many open questions, in particular related to consequences for planktonic mediated carbon fluxes.

Some studies on ocean acidification have reported increased carbon fixation (Egge et al., 2009; Engel et al., 2013), bacterial production (Grossart et al., 2006) and bacterial degradation of polysaccharides (Piontek et al., 2010) at enhanced CO_2 levels, with potential consequences for carbon fluxes within pelagic ecosystems and export to the deep ocean, i.e. the biological carbon pump. Increasing carbon fixation in a high CO_2 environment can translate into an enhanced sequestration of carbon (Riebesell et al., 2007), but this depends on numerous environmental factors including phytoplankton community composition, aggregate formation and nutrient availability. For example, if the community shifts towards smaller cell sizes and/or enhanced cycling of organic matter carbon, export from the upper water layers may decrease (Czerny et al., 2013a).

The effect of ocean acidification has mostly been studied in marine ecosystems under high phytoplankton biomass. Brackish water has lower buffering capacity than ocean water and the pH fluctuates more. The limited number of studies of ocean acidification in brackish water and indications that ocean acidification effects are greatest under nutrient limitation (De Kluijver et al., 2010), motivated this mesocosm study in the Baltic Sea during low nutrient, summer months.

The Baltic Sea is functionally much like a large estuary, with a salinity gradient ranging from approximately 20 in the South-West to <3 in the Northernmost Bothnian Bay. It is an almost landlocked body of water with a large population in its vicinity (~80 million). Human activities (e.g. agriculture, shipping and fishing) cause a number of environmental problems such as eutrophication and pollution. As a coastal sea projected to change rapidly due to interaction of direct and indirect anthropogenic pressures, the Baltic Sea can be seen as a model ecosystem to study global change scenarios (Niiranen et al., 2013).

Most primary data from this experiment are published in several papers of this Special Issue (Riebesell et al., 2015). The aim of the present paper is to provide an overarching synthesis of

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all information related to carbon standing stocks and fluxes. This enabled us to calculate carbon budgets in relation to different CO_2 levels.

2 Materials and methods

2.1. Experimental set-up

Six Kiel Off-Shore Mesocosms for future Ocean Simulations (KOSMOS; with a volume of ca. 55 m^3) were moored at Storfjärden, on the south west coast of Finland (59° 51.5' N; 23° 15.5' E) on 12 June 2012. The mesocosms extended from the surface down to 19 m depth and had a conical bottom end, which enabled quantitative collection of the settling material. Different CO_2 levels in the bags were achieved by adding filtered (50 µm), CO_2-saturated seawater. The CO_2 enriched water was evenly distributed over the upper 17 m of the water columns and added in 4 consecutive time steps (*t0* – *t3*). Two controls and four treatments were used, and for the controls, filtered seawater (without additional CO_2 enrichment) was added. The CO_2 fugacity gradient after all additions ranged from ambient (average throughout the experiment: ~370 µatm fCO_2) in the two control mesocosms (M1 and M5), up to ~1200 µatm fCO_2 in the highest treatment (M8). We used the average fCO_2 throughout this experiment (from *t1* – *t43*) to denote the different treatments: 365 (M1), 368 (M5), 497 (M7), 821 (M6), 1007 (M3) and 1231 (M8) µatm fCO_2. On *t15*, additional CO_2–saturated seawater was added to the upper 7 m in the same manner as the initial enrichment, to counteract outgassing of CO_2.

We sampled the mesocosm every morning, but some variables were determined only every second day. Depth-integrated water samples (0 – 17 m) were taken by using integrating water samplers (IWS, HYDRO-BIOS, Kiel). The water was collected into plastic carboys (10 L) and taken to the laboratory for sub-sampling and subsequent determination of carbon stocks.

2.2. Primary variables

For more detailed descriptions of the primary variables and the different methods used during this CO_2 mesocosm campaign, we refer to other papers in this joint volume: i.e. total

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127 particulate carbon (TPC), dissolved organic carbon (DOC), and dissolved inorganic carbon
128 (DIC) are described by Paul et al. (2015b); micro and nanophytoplankton enumeration by
129 Bermúdez et al. (2016); picophytoplankton, heterotrophic prokaryotes and viruses by
130 Crawfurd et al. (2016); zooplankton community by Lischka et al. (2015); primary production
131 and respiration by Spilling et al. (2016); bacterial production (BP) by Hornick et al. (2016);
132 and sedimentation by Boxhammer et al. (2015); and Paul et al. (2015b).

133 Briefly, samples for TPC (500 mL) were GF/F filtered and determined using an elemental
134 analyser (EuroAE). DOC was measured using the high temperature combustion method
135 (Shimadzu TOC –VCPN) following Badr et al. (2003). DIC was determined by infrared
136 absorption (LI-COR LI-7000 on an AIRICA system). The DIC concentrations were
137 converted from µmol kg^{-1} to mmol m^{-2} using the average seawater density of 1.0038 kg L^{-1}
138 throughout the experiment. Settling particles were quantitatively collected every other day
139 from sediment traps at the bottom of the mesocosm units and the TPC determined as
140 described above.

141 Mesozooplankton was collected by net hauls (100 µm mesh size), fixed (ethanol) and
142 counted in a stereomicroscopy in combination with a Folsom plankton splitter. Zooplankton
143 carbon biomass (CB) was calculated using the displacement volume (DV) and the equation of
144 Wiebe (1988): (log DV + 1.429)/0.82 = log CB. Micro and nanoplankton (zoo- and
145 phytoplankton) CB was determined from microscopic counts of fixed (acidic Lugol's iodine
146 solution) samples, and the cellular bio-volumes were determined according to Olenina et al.
147 (2006) and converted to POC by the equations provided by Menden-Deuer and Lessard
148 (2000).

149 Picophytoplankton were counted using flow cytometry and converted to CB by size
150 fractionation (Veldhuis and Kraay, 2004) and cellular carbon conversion factors (0.2 pg C
151 $µm^{-3}$ (Waterbury et al., 1986). Prokaryotes and viruses were determined according to Marie et
152 al. (1999) and Brussaard (2004), respectively. All heterotrophic prokaryotes, hereafter termed
153 bacteria, and viruses were converted to CB assuming 12.5 fg C $cell^{-1}$ (Heinänen and
154 Kuparinen, 1991) and 0.055 fg C $virus^{-1}$ (Steward et al., 2007), respectively.

155 The respiration rate was calculated from the difference between the O_2 concentration
156 (measured with a Fibox 3, PreSens) before and after a 48 h incubation period in a dark,
157 climate controlled room set to the average temperature observed in the mesocosms.

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158 Bacterial protein production (BPP) was determined by ^{14}C-leucine (^{14}C-Leu) incorporation
159 (Simon and Azam, 1989) according to (Grossart et al., 2006). The amount of incorporated
160 ^{14}C-Leu was converted into BPP by using an intracellular isotope dilution factor of 2. A
161 conversion factor of 0.86 was used to convert the produced protein into carbon (Simon and
162 Azam, 1989).

163 Net primary production (NPP) was measured using radio-labeled $NaH^{14}CO_3$ (Steeman-
164 Nielsen, 1952). Samples were incubated for 24 h in duplicate, 8 ml vials moored on small
165 incubation platforms at 2, 4, 6, 8 and 10 m depth next to the mesocosms. The areal primary
166 production was calculated based on a simple linear model of the production measurements
167 from the different depths (Spilling et al., 2016).

168

169 **2.3. Gas exchange**

170 In order to calculate the CO_2 gas exchange with the atmosphere, we used N_2O as tracer gas,
171 and this was added to mesocosm M5 and M8 (control and high CO_2 treatment) according to
172 Czerny et al. (2013b). The N_2O concentration was determined every second day using gas
173 chromatography. Using the N_2O measurements, the fluxes across the water surface (F_{N2O})
174 was calculated according to:

175 $$F_{N2O} = I_{t1} - I_{t2} / (A * \Delta t) \quad (2)$$

176 where I_{t1} and I_{t2} is the bulk N_2O concentration at time: t_1 and t_2; A is the surface area and Δt
177 is the time difference between t_1 and t_2.

178 The flux velocity was then calculated by:

179 $$K_{N2O} = F_{N2O} / (C_{N2Ow} - (C_{N2O\,aw}) \quad (3)$$

180 where C_{N2Ow} is the bulk N_2O concentration in the water at a given time point, and C_{N2Oaw} is
181 the equilibrium concentration for N_2O (Weiss and Price, 1980).

182 The flux velocity for CO_2 was calculated from the flux velocity of N_2O according to:

183 $$k_{CO2} = k_{N2O} / (Sc_{CO2}/Sc_{N2O})^{0.5} \quad (4)$$

184 where Sc_{CO2} and Sc_{N2O} are the Schmidt numbers for CO_2 and N_2O, respectively. The CO_2flux
185 across the water surface was calculated according:

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186 $F_{CO2} = k_{CO2} (C_{CO2w} - C_{CO2aw})$ (5)

187 where C_{CO2w} is the water concentration of CO_2 and C_{CO2aw} is the equilibrium concentration of
188 CO_2. CO_2 is preferentially taken up by phytoplankton at the surface, where also the
189 atmospheric exchange takes place. For this reason, we used the calculated CO_2 concentration
190 (based on the integrated CO_2 concentration and pH in the surface) from the upper 5 m as the
191 input for equation 5.

192 In contrast to N_2O, the CO_2 flux can be chemically enhanced by hydration reactions of CO_2
193 with hydroxide ions and water molecules in the boundary layer (Wanninkhof and Knox,
194 1996). Using the method outlined in (Czerny et al., 2013b) we found an enhancement of up to
195 12% on warm days and this was included into our flux calculations.

196

197 **2.4. Data treatment**

198 The primary data generated in this study comprise of carbon standing stock measurements of
199 TPC, DOC, DIC, as well as carbon estimates of meso- and microzooplankton, micro-, nano-
200 and picophytoplankton, bacteria and viruses. Flux measurements of atmospheric CO_2
201 exchange and sedimentation of TPC, as well as the biological rates of net primary production
202 (NPP_{14C}), bacterial production (BP) and total respiration (TR) enabled us to make a closed
203 carbon budget.

204 NPP was measured directly and additionally estimated (NPP_e) from the total change in the
205 organic carbon pool plus the exported TPC (EXP_{TPC}) according to:

206 $NPP_e = EXP_{TPC} + \Delta TPC + \Delta DOC$ (6)

207 Comparing direct measurements using ^{14}C isotope incubations should in principal provide a
208 higher value than summing the difference in overall carbon balance (our NPP_e), as the latter
209 would incorporate total respiration and not only autotrophic respiration.

210 In order to close the budget we estimated gross primary production (GPP) and DOC
211 production (DOC_{prod}). GPP is defined as the photosynthetically fixed carbon without any loss
212 processes (i.e. NPP + autotrophic respiration). GPP can be estimated based on changes in
213 organic (GPP_o) or inorganic (GPP_i) carbon pools, and we used these two different approaches
214 providing a GPP range:

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$GPP_o = NPP_e + TR$ (7)

$GPP_i = TR + CO_{2flux} - \Delta DIC$ (8)

During Phase III, TR was not measured and we estimated TR based on the $NPP_e\ TR^{-1}$ and BP TR^{-1} ratios during Phase II. The minimum production of DOC (DOC_{minp}) in the system was calculated assuming bacterial carbon uptake was taken from the DOC pool according to:

$DOC_{minp} = \Delta DOC + BP$ (9)

However, this could underestimate DOC_{prod} as a fraction of bacterial DOC uptake is respired. Without direct measurement of (heterotrophic prokaryote) bacterial respiration, (BR), we estimated BR from TR. The share of active bacteria contributing to bacterial production is typically in the range of 10-30% of the total bacterial community (Lignell et al., 2013). We used the fraction of bacterial biomass (BB) of total biomass (TB) as the maximum limit of BR, and hence calculated max DOC production (DOC_{maxp}) according to:

$DOC_{maxp} = \Delta DOC + BP + (BB * TR / TB)$ (10)

We assumed that carbon synthesized by bacteria added to the TPC pool, thus aggregation of DOC equaled BP.

There are a number of uncertainties in these calculations, but this budgeting exercise provides an order-of-magnitude estimate of the flow of carbon within the system and enables comparison between the treatments. The average of the two controls (M1 and M5) and two highest CO_2 treatments (M3 and M8) were used to illustrate CO_2 effects. The three different phases of the experiments (I, II and III) were of different length (16, 14 and 13 day respectively). We used the average carbon pools from the whole period, but normalized fluxes and biological rates to day^{-1}. All error estimates were calculated as standard error (SE). The primary papers present detailed statistical analyses and we only refer to those here.

3. Results and discussion

3.1 Change in plankton community, from large to small forms over time

The overall size structure of the plankton community decreased over the course of the experiment. Fig 1 illustrates the carbon content in different plankton groups in the control

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mesocoms. During Phase I, the phytoplankton concentration increased at first in all treatments before starting to decrease at the end of Phase I (Paul et al., 2015b). At the start of Phase II (t17), the phytoplankton biomass was higher than at the start of the experiment (~130 mmol C m^{-2} in the controls) but decreased throughout Phase II and III. The fraction of picophytoplankton increased in all treatments, but some groups of picophytoplankton increased more in the high CO_2 treatments (Crawfurd et al., 2016).

Nitrogen was the limiting nutrient during the experiment (Paul et al., 2015b), and primary producers are generally N-limited in the main sub-basins of the Baltic Sea (Tamminen and Andersen, 2007). The surface : volume ratio increases with decreasing cell size, and consequently small cells have higher nutrient affinity, and are better competitors for scarce nutrient sources than large cells (Reynolds, 2006). The prevailing N-limitation was likely the reason for the decreasing size structure of the phytoplankton community.

Micro and mesozooplankton standing stock was approximately half of the phytoplankton biomass initially, but decreased rapidly in the control treatments during Phase I. In the CO_2 enriched treatments the zooplankton biomass also decreased but not to the same extent as in the control treatments (Spilling et al., 2016). Overall, smaller species benefitted from the extra CO_2 addition, but there was no significant negative effect of high CO_2 on the mesozooplankton community (Lischka et al., 2015).

Bacterial biomass was the main fraction of the plankton carbon throughout the experiment. The bacterial community was controlled by mineral nutrient limitation, bacterial grazing and viral lysis (Crawfurd et al., 2016). Bacterial growth is typically limited by N or a combination of N and C in the study area (Lignell et al., 2008; Lignell et al., 2013),

The bacterial carbon pool was higher than the measured TPC. Part of the bacteria must have passed the GFF filters (0.7 µm), and assuming pico- to mesoplankton was part of the TPC, >50% of the bacterial carbon was not contributing to the measured TPC. The conversion factor from cells to carbon is positively correlated cell size, and there is consequently uncertainty related to the absolute carbon content of the bacterial pool (we used a constant conversion factor). However, bacteria is known to be the dominating carbon share in the Baltic Sea during the N-limited summer months (Lignell et al., 2013), and its relative dominance is in line with this.

3.2. Biological rates: respiration

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Total respiration (TR) was lower in the CO_2 enriched treatments (Tables 1-3). The average TR was 83 mmol C m^{-2} d^{-1} during Phase I, and initially without any detectable treatment effect. The respiration rate started to be lower in the high CO_2 treatments, compared with the controls, in the beginning of Phase II. At the end of Phase II there was a significant difference ($p = 0.02$; Spilling et al 2016) between the treatments, and 40% lower respiration rate in the highest CO_2 treatment compared with the controls (Table 2).

Cytosol pH is close to neutral in most organisms, and reduced energetic cost for internal pH regulation at lower external pH levels could be one factor reducing respiration (Smith and Raven, 1979). Respiration rate in plant foliage decreases in high CO_2 environments, possibly affected by respiratory enzymes or other metabolic processes (Amthor, 1991; Puhe and Ulrich, 2012), and similar processes could take place in e.g. phytoplankton. Yet, previous studies of plankton has pointed at no effect or increased respiration at elevated CO_2 concentration (Li and Gao, 2012; Tanaka et al., 2013), and the metabolic changes behind reduced respiration, is an open question. However, there does seem to have been a connection between respiration and bacterial activity in the high CO_2 treatments.

3.3. Biological rates: bacterial production

Bacterial production (BP) became lower in the high CO_2 treatment in the latter part of the experiment. During Phase I, BP ranged from 27 to 46 mmol C m^{-2} d^{-1} (Table 1). The difference in BP between treatments became apparent in Phases II and III of the experiment. The average BP was 18% and 24% higher in the controls compared to the highest CO_2 treatments during Phases II and III, respectively (Tables 2 and 3). Statistical support ($p \geq 0.01$) for a treatment effect during parts of the experiment is presented in Hornick et al. (2016).

The lower bacterial production accounted for ~40% of the reduced respiration during Phase II, and this raises an interesting question: what was the mechanism behind the reduced bacterial activity in the high CO_2 treatment? There are examples of decreased bacterial production at high CO_2 concentration (Motegi et al 2013), but most previous studies have reported no change (Allgaier et al., 2008) or a higher bacterial production at elevated CO_2 concentration (Grossart et al., 2006; Piontek et al., 2010; Endres et al., 2014). The latter was also supported by the recent study of Bunse et al. (2016), describing up-regulation of bacterial genes related to respiration, membrane transport and protein metabolism at elevated

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307 CO_2 concentration; however, this effect was not evident when inorganic nutrients had been
308 added (high Chl *a* treatment).

309 In this study, the reason for the lower bacterial activity in the high CO_2 treatments could be
310 due to either limitation and/or inhibition of bacterial growth or driven by difference in loss
311 processes. Increased loss processes could also have affected BP. Bacterial grazing and viral
312 lysis was higher in the high CO_2 treatments during periods of the experiment (Crawfurd et al.,
313 2016), and would at least partly be the reason for the reduced bacterial production at high
314 CO_2 concentration.

315 N-limitation increased during the experiment (Paul et al., 2015b), and mineral nutrient
316 limitation of bacteria can lead to accumulation of DOC, i.e. reduced bacterial uptake
317 (Thingstad et al., 1997), similar to our results. Bacterial N limitation is common in the area
318 during summer (Lignell et al., 2013), however, this N-limitation was not apparently different
319 in the controls (Paul et al., 2015b), and CO_2 did not affect N-fixation (Paul et al., 2015a). In a
320 scenario where the competition for N is fierce, the balance between the bacteria and similar
321 sized picophytoplankton could be tilted in favor of phytoplankton if they gain an advantage
322 by having easier access to carbon, i.e. CO_2 (Hornick et al., 2016).

323 We have not found evidence in the literature that bacterial production will be suppressed in
324 the observed pH range inside the mesocosms, varying from approximately pH 8.1 in the
325 control to pH 7.6 in the highest fCO_2 treatment (Paul et al 2015), but enzymes seem to be
326 affected by moderate pH changes. For example, some studies report on an increase in protein
327 degrading enzyme leucine aminopeptidase activities at reduced pH (Grossart et al., 2006;
328 Piontek et al., 2010; Endres et al., 2014), whereas others indicate a reduced activity of this
329 enzyme (Yamada and Suzumura, 2010). A range of other factors affects this enzyme, for
330 example the nitrogen source and salinity (Stepanauskas et al., 1999), and any potential
331 interaction effects with decreasing pH are not yet resolved. Any pH-induced changes in
332 bacterial enzymatic activity could potentially affect bacterial production.

333

334 **3.4. Biological rates: primary production**

335 The estimated net primary production (NPP_e) indicated higher primary productivity during
336 Phase I than during the rest of the experiments. There was no consistent difference between
337 CO_2 treatments for NPP_{14C}, but NPP_e increased with increasing CO_2 enrichment during Phase
338 II. This was caused by the different development in the TPC and DOC pools. The pattern of

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339 gross primary production (GPP) was similar to NPP_e during Phases I and II. During Phase III
340 there were no respiration or NPP_{14C} measurements and the estimated GPP is more uncertain.
341 The NPP_e and GPP indicated a smaller difference between treatments during Phase III
342 compared with Phase II.

343 The two measures of NPP were of a similar magnitude (Tables 1-3). During Phase I, NPP_{14C}
344 < NPP_e (Table 1), this relationship reversed for most treatments during Phase II, with the
345 exception of the highest CO_2 levels (Table 2). Interestingly, an effect of the different CO_2
346 treatments was noticeable in the NPP_e but not in NPP_{14C}, suggesting that the effect of
347 elevated CO_2 concentration could refer to heterotrophic respiration. However, in terms of the
348 NPP_{14C} < NPP_e, the uncertainty seems to be higher than the potential signal of heterotrophic
349 respiration. This would also indicate that the NPP_{14C} during Phase I has been underestimated,
350 in particular for the control mesocosm M1. During Phase II, the NPP_{14C} was higher than
351 NPP_e, except for the two highest CO_2 treatments, more in line with our assumption of NPP_{14C}
352 > NPP_e. The systematic offset in NPP_{14C} during Phase I could be due to changed
353 parameterization during incubation in small volumes (8 mL, Spilling et al 2016), for example
354 increased loss due to grazing. Overall, however, the results suggest that the measured NPP_{14C}
355 and estimated NPP_e agree reasonably well.
356

357 **3.5. The DIC pool and atmospheric exchange of CO_2**

358 The DIC pool was the largest carbon pool: 3-4 fold higher than the DOC pool and roughly
359 60-fold higher than the TPC pool (Tables 1-3). After the addition of CO_2, the DIC pool was
360 ~7% higher in the highest CO_2 treatment compared to the control mesocosms (Table 1). The
361 gas exchange with the atmosphere was the most apparent flux affected by CO_2 addition
362 (Tables 1-3). Seawater in the mesocosms with added CO_2 were supersaturated, hence CO_2
363 outgassed throughout the experiment. The control mesocosms were initially undersaturated,
364 hence ingassing occurred during Phases I and II (Fig 2). In the first part of Phase III, the
365 control mesocosms reached equilibrium with the atmospheric fCO_2 (Fig. 2).

366 Using the direct flux measurements and the net change in the DIC pool, we calculated the net
367 uptake or release of carbon by biological activity. Comparing the controls to the mesocosm
368 with the highest CO_2 addition (Fig. 3), the CO_2 addition had an effect on the biologically
369 mediated carbon flux. In the mesocosm with an ambient CO_2 concentration, the flux

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370 measurements indicated net heterotrophy throughout the experiment. The opposite pattern,
371 net autotrophy, was indicated in the mesocosm with the highest CO_2 addition (Fig 3).

372

373 **3.6. The DOC pool, DOC production and remineralization**

374 The DOC pool increased throughout the experiment in all mesocosm bags, but more in the
375 treatments with elevated CO_2 concentration. The initial DOC standing stock in all treatments
376 was approximately 7200 mmol C m^{-2}. At the end of the experiment, the DOC pool was ~2%
377 higher in the high CO_2 treatments compared to the controls (Fig. 4), and there is statistical
378 support for these treatments being different (Phase III, p = 0.05) (Paul et al., 2015b).
379 Interestingly, the data does not point to a substantially higher release of DOC at high CO_2
380 (Fig 5). The bacterial production was notably lower during Phases II and III in the high CO_2
381 treatments (Hornick et al., 2016), and of similar magnitude as the rate of change in DOC pool
382 (Table 2 and 3), indicating reduced bacterial uptake and remineralization of DOC. The
383 combined results suggest that the increase in the DOC pool at high CO_2 was related to
384 reduced DOC loss (uptake by bacteria), rather than increased release of DOC by the plankton
385 community, at elevated CO_2 concentration.

386 The Baltic Sea is affected by large inflow of freshwater containing high concentrations of
387 refractory DOC such as humic substances, and the concentration in Gulf of Finland is
388 typically 400-500 µmol C L^{-1} (Hoikkala et al., 2015). The large pool of DOC and turn over
389 times of ~200 days (Tables 1-3) is most likely a reflection of the relatively low fraction of
390 labile DOC, but bacterial limitation of mineral nutrients can also increase turn over times
391 (Thingstad et al., 1997).

392 The DOC pool has been demonstrated to aggregate into transparent exopolymeric particles
393 (TEP) under certain circumstances, which can increase sedimentation at high CO_2 levels
394 (Riebesell et al., 2007). We did not have any direct measurements of TEP, but any CO_2 effect
395 on its formation is highly dependent on the plankton community and its physiological status
396 (MacGilchrist et al., 2014). No effect of CO_2 treatment on carbon export suggests that we did
397 not have a community where the TEP production was any different between the treatments
398 used.

399

400 **3.7 The TPC pool and export of carbon**

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There was a positive effect of elevated CO_2 on TPC relative to the controls. At the start of the experiment, the measured TPC concentration in the enclosed water columns was 400-500 mmol C m^{-2} (Table 1). The TPC pool decreased over time but less in the high CO_2 treatment and at the end of the experiment, the standing stock of TPC was ~6% higher (Phase III, $p = 0.01$; Paul et al. (2015b) in the high CO_2 treatment (Fig. 4).

The export of TPC was not dependent on the CO_2 concentration but varied temporally. The largest flux of TPC out of the mesocosms occurred during Phase I with ~6 mmol C m^{-2} d^{-1}. It decreased to ~3 mmol C m^{-2} d^{-1} during Phase II and was ~2 mmol C m^{-2} d^{-1} during Phase III (Table 1-3). The exported carbon as percent of average TPC standing stock similarly decreased from ~1.3% during Phase I to 0.3-0.5% during Phase III. The initial increase in the autotrophic biomass associated with relatively more of the carbon settling in the mesocosms. The decreasing carbon export was most likely caused by the shift towards a plankton community depending on recycled nitrogen, reducing the overall TPC and also the size structure of the plankton community.

3.8 Budget

A carbon budget for the two control mesocosms and two highest CO_2 additions is presented in Fig. 5. During Phase I the estimated gross primary production (GPP) was ~100 mmol C fixed m^{-2} d^{-1}; from which 75-95% were respired, ~1% ended up in the TPC (including export) and 5-25% added to the DOC pool. The main difference between CO_2 treatments became apparent during Phase II when the NPP_e was higher in the elevated CO_2 treatments. The respiration loss increased to ~100% of GPP at the ambient CO_2 concentration, whereas respiration was lower (85-95% of GPP) in the highest CO_2 treatment. Bacterial production was ~30% lower, on average, at the highest CO_2 concentration compared with the controls during. The share of NPP_e of GPP ranged from 2% to 20% and the minimum flux to the DOC pool was 11% to 18% of TPC.

The overall budget was calculated by using the direct measurements of changes in standing stocks and fluxes of export, respiration and bacterial production rates. The most robust data are the direct measurements of carbon standing stocks and their differences. These are based on well-established methods with relatively low standard error (SE) of the carbon pools. However, the dynamic nature of these pools made the relative SE for the rate of change much higher, reflecting that the rate of change varied considerably within the different phases.

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Manuscript under review for journal Biogeosciences
Published: 7 March 2016

433 The rate parameters, calculated based on conversion factors, have greater uncertainty,
434 although their SEs were relatively low, caused by uncertainty in the conversion steps. For
435 example, the respiratory quotient (RQ) was set to one, which is a good estimate for
436 carbohydrate oxidation. For lipids and proteins the RQ is close to 0.7, but in a natural
437 environment RQ is often >1 (Berggren et al., 2012), and is affected by physiological state e.g.
438 nutrient limitation (Romero-Kutzner et al., 2015). Any temporal variability in the conversion
439 factors would directly change the overall budget calculations, e.g. RQ affecting total
440 respiration and gross primary production estimates. However, the budget provides an order-
441 of-magnitude estimate of the carbon flow within the system. Some of the parameters such as
442 GPP was estimated using different approaches, providing a more robust comparison of the
443 different treatments.

444 The primary effect of increasing CO_2 concentration was the higher standing stocks of TPC
445 and DOC compared with ambient CO_2 concentration. The increasing DOC pool and
446 relatively higher TPC pool were driven by reduced respiration and bacterial production at
447 elevated CO_2 concentration. Decreasing respiration rate reduced the recycling of organic
448 carbon back to the DIC pool. The lower respiration and bacterial production also indicates
449 reduced remineralization of DOC. These two effects caused the higher TPC and DOC pools
450 in the elevated CO_2 treatments. The results highlight the importance of looking beyond net
451 changes in carbon standing stocks to understand how carbon fluxes are affected under
452 increasing ocean acidification.

453

454

455 **Acknowledgements**

456 We would like to thank all of the staff at Tvärminne Zoological station, for great help during
457 this experiment, and Michael Sswat for carrying out the TPC filtrations. We also gratefully
458 acknowledge the captain and crew of R/V ALKOR (AL394 and AL397) for their work
459 transporting, deploying and recovering the mesocosms. The collaborative mesocosm
460 campaign was funded by BMBF projects BIOACID II (FKZ 03F06550) and SOPRAN Phase
461 II (FKZ 03F0611). Additional financial support for this study came from Academy of Finland
462 (KS - Decisions no: 259164 and 263862) and Walter and Andrée de Nottbeck Foundation
463 (KS). TH and HPG were financially supported by SAW project TemBi of the Leibniz
464 Foundation. CPDB was financially supported by the Darwin project, the Royal Netherlands

Institute for Sea Research (NIOZ), and the EU project MESOAQUA (grant agreement number 228224).

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Manuscript under review for journal Biogeosciences
Published: 7 March 2016

Table 1. The standing stock of total particular carbon (TPC_{pool}), dissolved organic carbon (DOC_{pool}) and dissolved inorganic carbon (DIC_{pool}) at the start of Phase I in mmol C m^{-2} ± SE. The DOC_{pool} was missing some initial measurements and is the average for all mesocosms assuming that the DOC concentration was similar at the onset of the experiment. The net change in TPC (ΔTPC), DOC (ΔDOC) and DIC (ΔDIC) are average changes in the standing stocks during Phase I in mmol C m^{-2} d^{-1} ± SE. Flux measurements of atmospheric gas exchange (CO_{2flux}) and exported carbon (EXP_{TPC}) plus biological rates: total respiration (TR), bacterial production (BP), measured (NPP_{14C}) and estimated (NPP_e) net primary production, are all average for Phase I in mmol C m^{-2} d^{-1} ± SE. The NPP_e was calculated from the net change in carbon pools plus carbon export, whereas NPP_{14C} was measured carbon fixation using radiolabeled ^{14}C over a 24 h incubation period *in situ*. TR was measured as O_2 consumption and for comparison with carbon fixation we used a respiratory quotient (RQ) of 1. A total budget of carbon fluxes for ambient and high CO_2 treatments is presented in Fig 5.

Phase I (*t0-t16*)

CO_2 treatment (µatm fCO_2)	**365**	**368**	**497**	**821**	**1007**	**1231**
Mesocosm number	**M1**	**M5**	**M7**	**M6**	**M3**	**M8**
TPC_{pool}	417 ± 38	425 ± 39	472 ± 48	458 ± 38	431 ± 48	446 ± 57
DOC_{pool}	7172 ± 87	7172 ± 87	7172 ± 87	7172 ± 87	7172 ± 87	7172 ± 87
DIC_{pool}	25158 ± 9	25182 ± 10	25628 ± 8	26295 ± 22	26637 ± 36	26953 ± 48
ΔTPC	-4.6 ± 15	-5.2 ± 13	-8.3 ± 13	-8.2 ± 17	-7.0 ± 13	-6.3 ± 20
ΔDOC	15.5 ± 58	18.3 ± 30	18.5 ± 33	25.0 ± 36	18. 5 ± 73	18.1 ± 63
ΔDIC	5.5 ± 5.2	6.9 ± 9.2	-6.1 ± 11	-24 ± 14	-32 ± 20	-49 ± 42
CO_{2flux}	4.4 ± 0.2	4.8 ± 0.3	-0.8 ± 0.5	-11 ± 1.0	-17 ± 1.4	-23 ± 2.0
EXP_{TPC}	6.6 ± 0.10	5.6 ± 0.04	5.4 ± 0.07	6.0 ± 0.07	5.6 ± 0.06	6.0 ± 0.05
TR	107 ± 9	82 ± 7	81 ± 6	80 ± 8	75 ± 8	74 ± 8
BP	27 ± 8	41 ± 6	43 ± 8	41 ± 4	36 ± 5	46 ± 9
NPP_{14c}	4.8 ± 0.8	11.4 ± 2.1	14.9 ± 3.6	12.3 ± 2.3	11.3 ± 2.4	14.5 ± 2.7
NPP_e	17.4 ± 33	18.7 ± 20	15.6 ± 30	22.8 ± 28	17.1 ± 25	17.8 ± 28

Biogeosciences Discuss., doi:10.5194/bg-2016-56, 2016
Manuscript under review for journal Biogeosciences
Published: 7 March 2016

Table 2. The standing stock of total particular carbon (TPC_{pool}), dissolved organic carbon (DOC_{pool}) and dissolved inorganic carbon (DIC_{pool}) at the start of Phase II in mmol C m^{-2} ± SE. The net change in TPC (ΔTPC), DOC (ΔDOC) and DIC (ΔDIC) are average changes in the standing stocks during Phase II in mmol C m^{-2} d^{-1} ± SE. Flux measurements of atmospheric gas exchange (CO_{2flux}) and exported carbon (EXP_{TPC}) plus biological rates: total respiration (TR), bacterial production (BP), measured (NPP_{14C}) and estimated (NPP_e) net primary production, are all average for Phase II in mmol C m^{-2} d^{-1} ± SE. See Table 1 legend for further details.

Phase II (*t17-t30*)

CO_2 treatment (µatm *f*CO_2)	**365**	**368**	**497**	**821**	**1007**	**1231**
Mesocosm number	**M1**	**M5**	**M7**	**M6**	**M3**	**M8**
TPC_{pool}	339 ± 14	337 ± 20	331 ± 22	318 ± 9	312 ± 12	339 ± 23
DOC_{pool}	7435 ± 38	7483 ± 37	7487 ± 43	7597 ± 37	7487 ± 61	7479 ± 37
DIC_{pool}	25247 ± 34	25269 ± 34	25639 ± 8	26177 ± 25	26413 ± 28	26757 ± 45
ΔTPC	-2.4 ± 5	-2.3 ± 8	-1.6 ± 14	0.3 ± 6	2.8 ± 4	3.2 ± 8
ΔDOC	-0.6 ± 39	2.4 ± 30	3.6 ± 40	8.4 ± 31	11.3 ± 58	9.1 ± 36
ΔDIC	22.4 ± 12	17.6 ± 8.1	-0.4 ± 4.5	-10.5 ± 16	-14.2 ± 10	-23.1 ± 13
CO_{2flux}	1.7 ± 0.3	1.2 ± 0.3	-2.6 ± 0.3	-10 ± 0.5	-14 ± 0.6	-19 ± 1.0
EXP_{TPC}	3.3 ± 0.08	2.6 ± 0.06	2.5 ± 0.08	2.6 ± 0.06	2.8 ± 0.07	2.9 ± 0.06
TR	140 ± 7	127 ± 5	103 ± 3	103 ± 4	101 ± 5	86 ± 4
BP	66 ± 17	57 ± 8	61 ± 7	57 ± 7	43 ± 6	47 ± 6
NPP_{14c}	3.8 ± 0.6	11.2 ± 1.9	10.8 ± 2.0	14.3 ± 2.8	10.4 ± 2.1	12.0 ± 2.5
NPP_e	0.3 ± 20	2.7 ± 15	4.5 ± 22	11.4 ± 16	16.9 ± 19	15.2 ± 16

Biogeosciences Discuss., doi:10.5194/bg-2016-56, 2016
Manuscript under review for journal Biogeosciences
Published: 7 March 2016

Table 3. The standing stock of total particular carbon (TPC_{pool}), dissolved organic carbon (DOC_{pool}) and dissolved inorganic carbon (DIC_{pool}) at the start of Phase III in mmol C m^{-2} ± SE. The net change in TPC (ΔTPC), DOC (ΔDOC) and DIC (ΔDIC) are average changes in the standing stocks during Phase III in mmol C m^{-2} d^{-1} ± SE. Flux measurements of atmospheric gas exchange (CO_{2flux}) and exported carbon (EXP_{TPC}) plus biological rates: total respiration (TR), bacterial production (BP), measured (NPP_{14C}) and estimated (NPP_e) net primary production, are all average for Phase III in mmol C m^{-2} d^{-1} ± SE. See Table 1 legend for further details. During Phase III we did not have direct measurements of net primary production (NPP_{14C}) or total respiration (TR).

Phase III (*t31-t43*)						
CO_2 treatment (µatm *f*CO_2)	**365**	**368**	**497**	**821**	**1007**	**1231**
Mesocosm number	**M1**	**M5**	**M7**	**M6**	**M3**	**M8**
TPC_{pool}	306 ± 12	304 ± 20	309 ± 20	323 ± 2	351 ± 13	384 ± 16
DOC_{pool}	7426 ± 16	7469 ± 20	7485 ± 92	7553 ± 20	7593 ± 30	7562 ± 38
DIC_{pool}	25557 ± 9	25545 ± 10	25648 ± 13	26030 ± 19	26197 ± 31	26371 ± 32
ΔTPC	-3.8 ± 10	0.3 ± 7	3.3 ± 14	3.3 ± 10	-1.4 ± 8	-4.8 ± 8
ΔDOC	9.8 ± 5	8.8 ± 7	8.9 ± 43	9.2 ± 10	5.7 ± 17	16.3 ± 20
ΔDIC	4.3 ± 3.9	5.5 ± 8.7	6.2 ± 11	-12.3 ± 7.2	-16.3 ± 14	-20.1 ± 14
CO_{2flux}	-0.3 ± 0.7	-0.8 ± 0.6	-3.0 ± 0.5	-7.3 ± 0.5	-9.4 ± 0.6	-13 ± 0.6
EXP_{TPC}	1.5 ± 0.07	1.4 ± 0.05	0.4 ± 0.07	1.9 ± 0.05	1.6 ± 0.04	1.7 ± 0.05
BP	31 ± 6.8	37 ± 1.4	38 ± 1.4	27 ± 2.1	17 ± 3.8	28 ± 2.3
NPP_e	7.6 ± 16	10.5 ± 13	12.7 ± 20	14.3 ± 13	6.0 ± 10	13.2 ± 14

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Figure legends

Fig. 1. The different fractions of carbon in the control mesocosms (M1 and M5) at the start of Phase I (t0), II (t17) and III (t31) in mmol C m^{-2} ± SE. The differences between the controls and elevated CO_2 concentration are discussed in the text. The size of the boxes indicates the relative size of the carbon standing stocks.

Fig 2. The calculated exchange of CO_2 between the mesocosms and the atmosphere. Positive values indicate net influx (ingassing) and negative values net outflux (outgassing) from the mesocosms. The flux was based on measurements of N_2O as a tracer gas and calculated using equations 2-5.

Fig 3. Change in dissolved inorganic carbon (DIC) pool and the atmospheric CO_2 exchange (Fig. 2). All values are average mmol C m^{-2} d^{-1} ± SE for the three different phases. Black, solid arrows indicated measured fluxes. Grey, dashed arrows are estimated by closing the budget, and indicate biological uptake or release of CO_2.

Fig 4. Standing stocks of total particulate carbon (TPC) and dissolved carbon (DOC) at the last day of the experiment (*t43*), plus the sum of exported TPC throughout the experiment; all values are in mmol C m^{-2} ± SE. The values are averages of the two controls (M1 and M5) and the two highest CO_2 treatments (M3 and M8). Red circles indicate statistically significant higher standing stocks in the high CO_2 treatments (further details in text). The size of the boxes indicates the relative size of the carbon standing stocks and export.

Fig 5. Average carbon standing stocks and flow in the control mesocosms (M1 + M5) and high CO_2 mesocosms (M3 + M8) during the three phases of the experiment. All carbon stocks (squares): dissolved inorganic carbon (DIC), total particulate carbon (TPC) and dissolved organic carbon (DOC), are average from the start of the period in mmol C m^{-2} ± SE. Fluxes (arrows) and net changes (Δ) are averages for the whole phase in mmol C m^{-2} d^{-1} ± SE. Black, solid arrows indicated measured fluxes (Tables 1-3): total respiration (TR), bacterial production (BP), exported TPC (EXP_{TPC}). Grey, dashed arrows are estimated by closing the budget: gross primary production (GPP) using equations 7 and 8; DOC production (DOC_{prod}) using equations 9 and 10. Bacterial respiration was calculated using equation 10 and is a share of TR (indicated by the parenthesis). Aggregation was assumed to

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1 equal BP. Red circles indicate statistically higher values compared with the other CO_2
2 treatment. The size of the boxes indicates the relative size of the carbon standing stocks.

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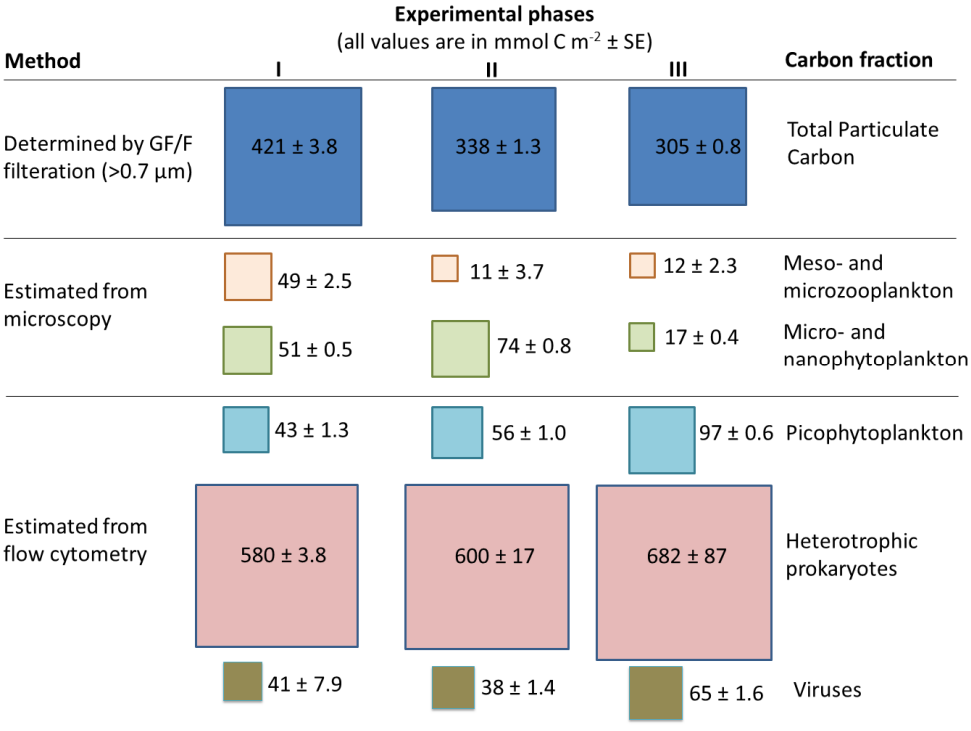

Fig 1

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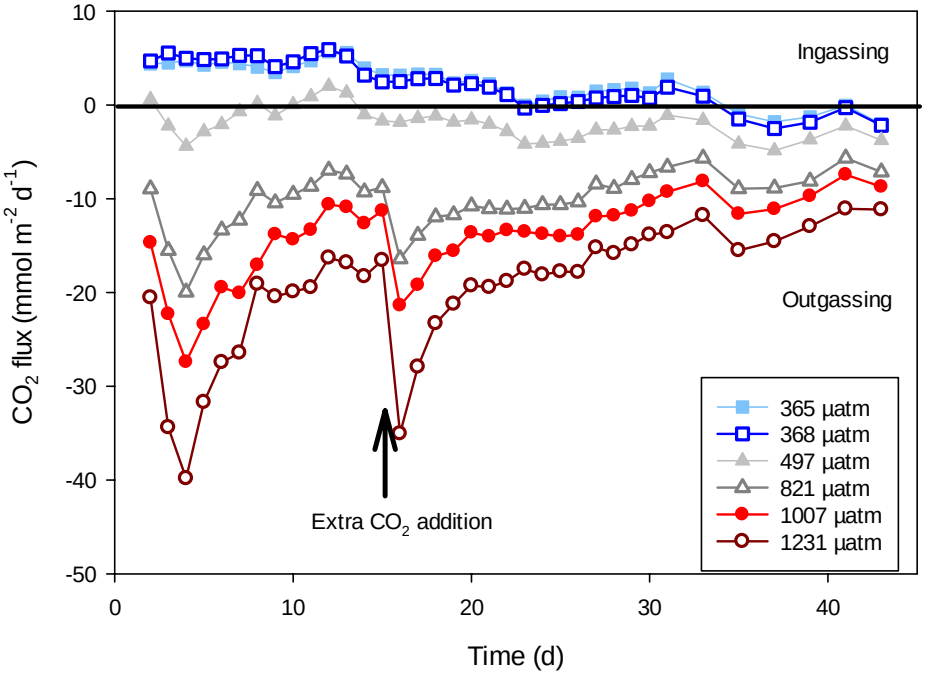

Fig 2

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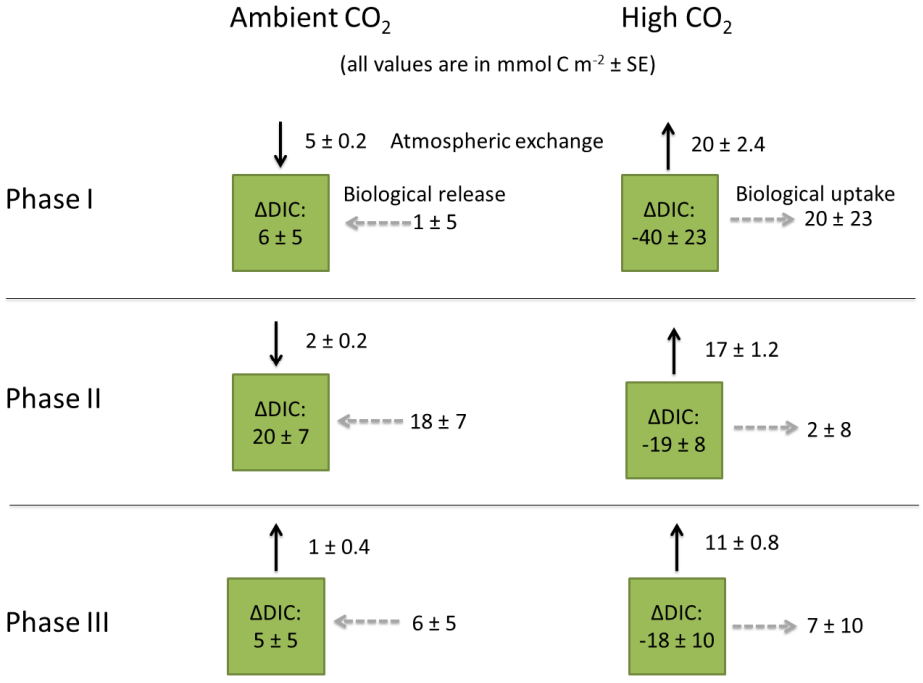

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3 **Fig 3**

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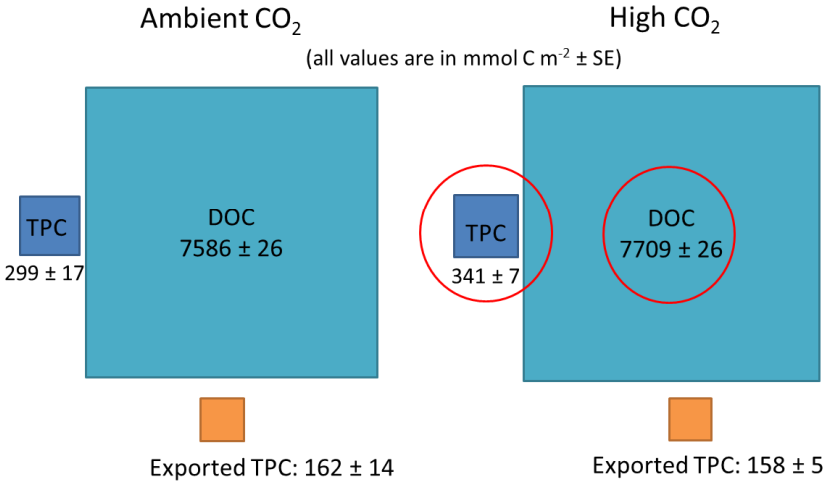

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3 **Fig 4**

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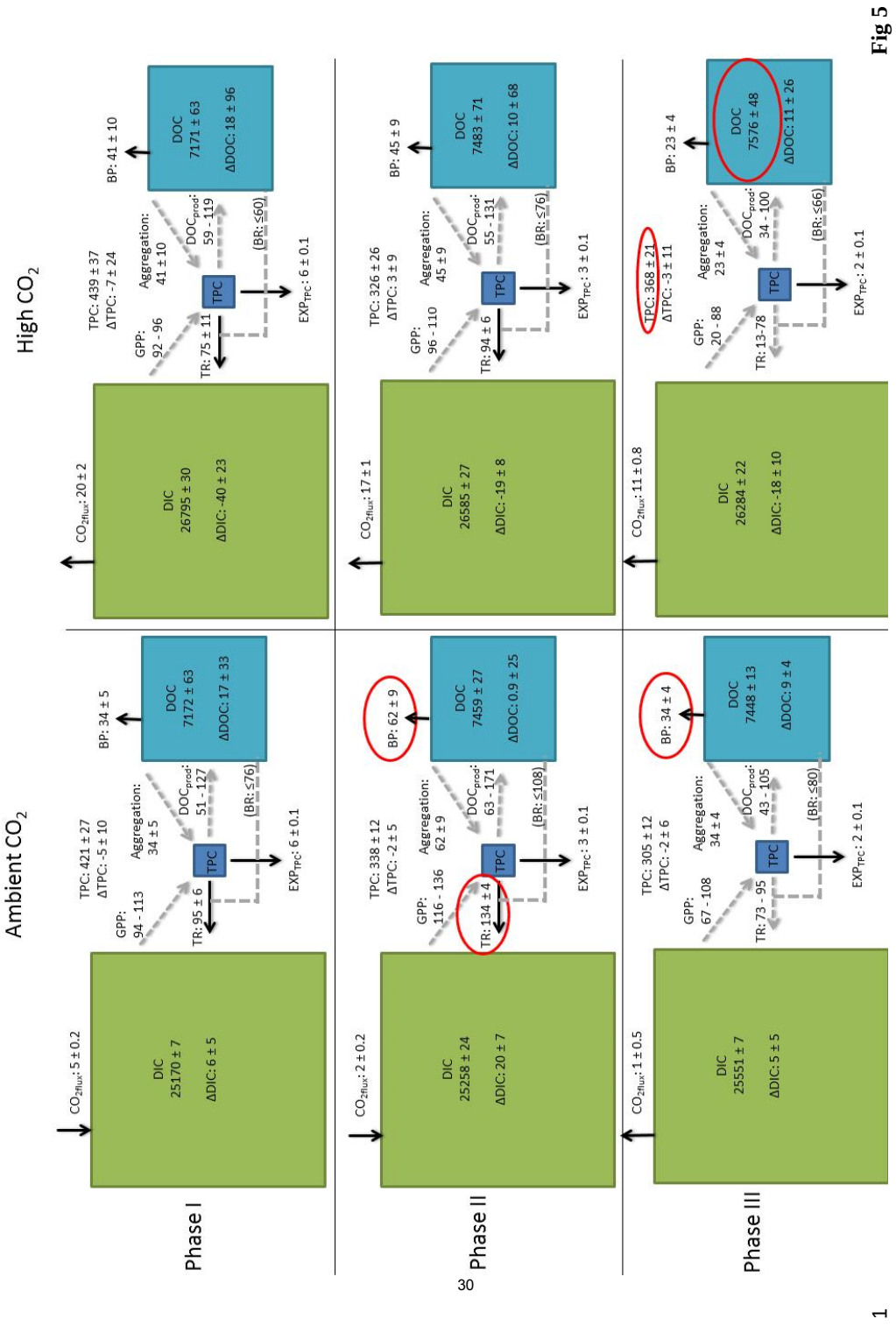

Fig 5