A $^{13}$C labelling study on carbon fluxes in Arctic plankton communities under elevated CO$_2$ levels

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Abstract. The effect of CO$_2$ on carbon fluxes (production, consumption, and export) in Arctic plankton communities was investigated during the 2010 EPOCA (European project on Ocean Acidification) mesocosm study off Ny Ålesund, Svalbard. $^{13}$C labelled bicarbonate was added to nine mesocosms with a range in $p$CO$_2$ ($185$ to $1420$ µatm) to follow the transfer of carbon from dissolved inorganic carbon (DIC) into phytoplankton, bacterial and zooplankton consumers, and export. A nutrient–phytoplankton–zooplankton–detritus model amended with $^{13}$C dynamics was constructed and fitted to the data to quantify uptake rates and carbon fluxes in the plankton community. The plankton community structure was characteristic for a post-bloom situation and retention food web and showed high bacterial production (~31% of primary production), high abundance of mixotrophic phytoplankton, low mesozooplankton grazing (~6% of primary production) and low export (~7% of primary production). Zooplankton grazing and export of detritus were sensitive to CO$_2$: grazing decreased and export increased with increasing $p$CO$_2$. Nutrient addition halfway through the experiment increased the export, but not the production rates. Although mixotrophs showed initially higher production rates with increasing CO$_2$, the overall production of POC (particulate organic carbon) after nutrient addition decreased with increasing CO$_2$. Interestingly, and contrary to the low nutrient situation, much more material settled down in the sediment traps at low CO$_2$. The observed CO$_2$ related effects potentially alter future organic carbon flows and export, with possible consequences for the efficiency of the biological pump.

1 Introduction

About 30% of anthropogenic CO$_2$ has accumulated in the oceans, causing the modification of the ocean’s chemistry. The most important impacts of anthropogenic CO$_2$ on marine carbonate chemistry are higher concentrations of CO$_2$ and a concurrent drop in pH, collectively referred to as ocean acidification. The CO$_2$ uptake capacity of the oceans is influenced by the plankton organisms that live in the surface waters. The flux of CO$_2$ from atmosphere to oceans is largely controlled by three biological processes: primary production, community respiration, and export (biological pump). Primary production and subsequent sinking of organic matter (OM) to depth increases the ocean’s uptake capacity for CO$_2$. Community respiration in the upper ocean, dominated by heterotrophic bacteria, converts organic carbon back into CO$_2$ and thus decreases the ocean’s CO$_2$ uptake capacity (Rivkin and Legendre, 2001). Understanding the effects of increasing CO$_2$ levels on these three processes is central to predicting the ocean’s response to rising atmospheric $p$CO$_2$. Particularly, production and export showed to be potentially sensitive to changes in CO$_2$ (Riebesell et al., 2009).

The high latitude oceans are especially vulnerable for anthropogenic CO$_2$ disturbances because of lower temperatures. The solubility of CO$_2$ increases with decreasing temperatures, so that polar oceans contain naturally high CO$_2$ and low carbonate ion concentrations. With a lower buffer capacity, pH changes are considerably larger in the polar regions than at lower latitudes for future climate scenarios (Steinacher et al., 2009). Our knowledge about the potential effects of ocean acidification on plankton...
communities in polar regions is limited, but plankton community studies have been done in mid-latitude regions. In a mesocosm experiment in a Norwegian fjord (Bergen, 2005), an increased inorganic carbon consumption relative to nutrient (N, P) uptake was observed at higher CO$_2$ levels in natural plankton communities (Riebesell et al., 2007; Bellerby et al., 2008). The enhanced uptake was not reflected in increased organic matter production (Schulz et al., 2008; de Kluijver et al., 2010) nor in increased bacterial activity (Algaier et al., 2008; de Kluijver et al., 2010) so enhanced export was the suggested sink for the extra carbon consumed at elevated pCO$_2$ (Riebesell et al., 2007). A proposed mechanism is that CO$_2$ induced carbon overconsumption is exuded by phytoplankton as dissolved organic matter (DOM), which aggregates with other particles and increases export (Engel et al., 2004a). In another mesocosm experiment (Bergen, 2001) no CO$_2$ effects on primary production (DeLille et al., 2005) were recorded, but a stimulating effect of CO$_2$ on bacterial activity was observed (Engel et al., 2004b; Grossart et al., 2006). In the mesocosm studies mentioned above, nutrients were added to stimulate phytoplankton production at the start of the experiments, so CO$_2$ effects on a eutrophic, blooming community were observed. However, throughout most of the year, plankton communities exist under low nutrient conditions dominated by regenerated production, rather than new production (Legendre and Rassoulzadegan, 1995).

This mesocosm study is the first to investigate the effects of elevated CO$_2$ on high-latitude plankton communities and on plankton communities in a post-bloom, nutrient regenerating state. In summer 2010, nine mesocosms were set up in Kongsfjorden, Svalbard, with a range of CO$_2$ levels and monitored for changes in plankton community functioning. To study the uptake of carbon by phytoplankton (primary production) and subsequent transfer to bacteria and zooplankton (community respiration) and settling material (export), $^{13}$C-DIC (dissolved inorganic carbon) was added as a tracer.

The $^{13}$C labelling dynamics of phytoplankton and bacteria were determined by compound specific isotope analyses of polar lipid fatty acid (PLFA) biomarkers. Groups of phytoplankton and bacteria produce characteristic fatty acids, so the abundance and enrichment of these fatty acids can be used as proxies for biomass and label incorporation in these groups, respectively (Boschker and Middelburg, 2002). Because PLFA are membrane fatty acids, which degrade rapidly after cell death, they are more suitable as a proxy for total biomass than, for example, storage lipids (Boschker and Middelburg, 2002). The technique has been successfully applied in the previous CO$_2$ enrichment mesocosm experiment (Bergen, 2005) to study the interactions between phytoplankton and bacteria (de Kluijver et al., 2010). In addition to the previous mesocosm experiment (Bergen, 2005), $^{13}$C POC and zooplankton analyses as well as quantitative sediment trap samples were included in this mesocosm study. A nutrient-phytoplankton-zooplankton-detritus model was constructed to quantify uptake and loss parameters and carbon flows in the mesocosms. The obtained parameters and fluxes were tested for CO$_2$ sensitivity.

2 Materials and methods

2.1 Experimental setup and sampling

The mesocosm experiment was carried out in Kongsfjorden, Svalbard (78°56,2' N, 11°53,6' E), in June-July 2010 as part of the 2010 EPOCA (European project on Ocean Acidification) Arctic campaign. The experimental setup and mesocosm characteristics are described in detail in Riebesell et al., 2012; Czerny et al., 2012a. Briefly, 9 mesocosms of ~50 m$^3$ were deployed in the Kongsfjorden, about a mile off Ny Ålesund, on 28 May 2010. While lowering to ~15 m depth, the bags filled with nutrient-poor, post-bloom fjord water. A 3 mm mesh size net was used to exclude large organisms. The bags were closed on 31 May 2010, defined as time $t_0$ and time steps ($t$) continued per day. The CO$_2$ manipulation was done in steps over 5 days, from $t_{-1}$ to $t_4$, by adding calculated amounts of CO$_2$ enriched seawater to each mesocosm. The main additions were done from $t_{-1}$ to $t_2$ and a final adjustment was done on $t_4$. A range of initial pCO$_2$ levels of ~185-1420 pmatm was achieved (exact CO$_2$ levels are provided in Bellerby et al., 2012). Due to gas exchange and photoautotrophic uptake, pCO$_2$ levels declined in the mesocosms, especially in the high CO$_2$ treatments, to a final pCO$_2$ range from ~160-855 pmatm at the end of the experiment. $^{13}$C-bicarbonate (10 μg per mesocosm), corresponding to ~0.1% of DIC, was added to the mesocosms together with the first CO$_2$ addition ($t_{-1}$), increasing the $^{13}$C signature of DIC by ~100% to stimulate phytoplankton production. The total added concentrations were 5 μM nitrate, 0.32 μM phosphate, and 2.5 μM silicate. The experiment was terminated at $t_30$. The experimental period was divided into three phases based on the applied perturbations and Chl a dynamics. Phase 1 was before nutrient addition ($t_{4-13}$). Phase 2 was after nutrient addition until the 2nd Chl $a$ minimum ($t_{14-21}$) and phase 3 was from the 2nd Chl $a$ minimum until the end of the experiment ($t_{22-29}$) (Schulz et al., 2012). In this manuscript we only consider two phases, phase 1 before nutrient addition ($t_{0-12}$) and phase 2 after nutrient addition ($t_{13-29}$).

Depth integrated samples (0-12 m) were taken each morning (9-11 h), with an integrating water sampler (IWS; Hydros, Kiel, Germany), for most parameters, including nutrients, chlorophyll, particulate organic carbon, phosphate, and nitrogen (POC, POP, PON), dissolved organic carbon, phosphate, and nitrogen (DOC, DOP, DON), dissolved inorganic carbon (DIC), and $^{13}$C content of carbon pools (DIC, DOC, POC, biomarkers). Daily samples for $^{13}$C DIC and $^{13}$C DOC were taken directly from the IWS and stored in dark, gas-tight glass bottles. The sediment traps were emptied every other day before daily routine sampling and processed as
described in (Czerny et al., 2012a). Zooplankton samples were taken weekly, in the afternoon, by vertical 55 μm mesh size Apstein net hauls over the upper 12 m.

Daily 13C-polar lipid fatty acid (PLFA) samples were collected on pre-combusted 47 mm GF/F filters by filtering ~3-4 L and filters were stored at ~80 °C. Daily 13C POC samples were collected on pre-weighted and pre-combusted 25 mm GF/F filters by filtering ~0.5 L, filters were subsequently stored at ~20 °C and freeze-dried afterwards. From the gas-tight water samples, headspace vials (20 mL) were filled using an overflow method and sealed with gas-tight caps for DIC isotope analyses. Mercury chloride was added for preservation and the samples were stored upside down at room temperature. Samples for dissolved organic carbon (DOC) were GF/F filtered and stored frozen (~20 °C) in clean (HCl and MQ rinsed) vials until further analyses. Zooplankton were transferred to filtered seawater and kept there for a minimum of 3 h to empty their guts. On average, 7 (range 3–30) individuals of Calanus sp. and 30 (range 16–35) individuals of Cirtipedia larvae were handpicked and transferred to pre-combusted tin cups (200 °C, 12 h), which were subsequently freeze-dried. Zooplankton samples were analyzed for organic 13C content. Subsamples of freeze-dried and homogenized sediment trap material were analyzed for total organic 13C. Sediment trap material of the last 8 days (t2–30) was additionally analyzed for 13C-PLFA to characterize the nature of settling material.

2.2 Laboratory analyses

POC, sediment trap material and zooplankton samples were analyzed for organic carbon content and isotope ratios on a Thermo Electron Flash EA 1112 analyser (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS). For DIC isotope analyses, a helium headspace was added to the headspace vials and samples were acidified with H3PO4 solution. After equilibration, the CO2 concentration and isotope ratio in the headspace was measured on EA-IRMS. PLFA were extracted using a modified Bligh and Dyer method (Bligh and Dyer, 1959; Middelburg et al., 2000). The lipids were fractionated in different polarity classes by column separation on a heat activated silicic acid column and subsequent elution with chloroform, acetone and methanol. The methanol fractions, containing most of the polar lipid fatty acids were collected and derivatized to fatty acid methyl esters (FAME). The standards 12:0 and 19:0 were used as internal standards. Concentrations and δ13C of individual PLFA were measured using gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS) (Middelburg et al., 2000; de Kluijver et al., 2010).

2.3 Data analyses

Carbon stable isotope ratios are expressed in the delta notation relative to Vienna Pee Dee Belemnite (VPDB) standard (δ13C). Relative (13C) incorporation in carbon samples is presented as $\Delta^{13}C_{\text{sample}} - \Delta^{13}C_{\text{background}}$. Absolute label incorporation was calculated as 13C concentration = $\Delta^{13}C_x$ concentration (μmol C L$^{-1}$), with $\Delta^{13}P$ being $\Delta^{13}C_{\text{sample}} - \Delta^{13}C_{\text{background}}$ and $\Delta^{13}F$ being the 13C fraction ($\Delta^{13}C/(\Delta^{12}C + \Delta^{13}C)$) derived from the delta notation. δ13Cbackground and $\Delta^{13}F_{\text{background}}$ are the natural abundance isotope ratios, which were sampled before label addition. To compare 13C concentrations of organic carbon pools between mesocosms, we used a correction factor for gas exchange according to Czerny et al. (2012b). The δ13C of CO2 [aq] was calculated according to Zhang et al. (1995) and the δ13C of atmospheric CO2 was assumed as −8‰.

$\Delta^{13}C_{\text{PLFA}}$ of phytoplankton showed 2 responses of 13C incorporation: rapid label incorporation and more gradual label incorporation. Phytoplankton were therefore separated into 2 groups (phytoplankton and mixotrophs) (Fig. 1a). The rapidly incorporating PLFA were 18:3ω6, 18:4ω3, 18:5ω3 (12–15), 18:5ω3 (12–16), and 16:4ω3 and their weighted average (Δ)$δ^{13}C$ was used to determine (Δ)$δ^{13}C$ of autotrophic phytoplankton, hereafter phytoplankton. The PLFA with delayed incorporation were 20:5ω3, 22:6ω3, and 16:4ω1 and their weighted average (Δ)$δ^{13}C$ was used to determine (Δ)$δ^{13}C$ of mixotrophic phytoplankton, hereafter mixotrophs. PLFA present in phytoplankton is characteristic for green algae, prymnesiophytes (haptophytes), cryptophytes, and autotrophic dinoflagellates. PLFA of mixotrophs is characteristic for diatoms and (heterotrophic) dinoflagellates (Dijkman et al., 2009). It was possible to distinguish between autotrophic dinoflagellates and total dinoflagellates, because 18:5ω3 is considered a chloroplast fatty acid, while 22:6ω3 is a cell membrane lipid (Adolf et al., 2007). The branched fatty acids 11:0ω0, 11:0ω0, and 11:0ω0 were used to characterize heterotrophic bacteria. These fatty acids occur primarily in gram-positive bacteria (Kaneda, 1991), although they are found in gram-negative bacteria as well (Zelles et al. 1999). The last step involved conversion from PLFA biomass to total organic carbon (OC) concentration for each group. The conversion factor for phytoplankton was calculated as 0.06 (sum PLFA:OC) and 0.05 (sum PLFA:OC) for mixotrophs, based on phytoplankton culture and literature values (Dijkman et al., 2006). The conversion factor for bacterial carbon was 0.01 (sum PLFA:OC) (van den Meersche et al., 2004). The conversion factors were kept constant during the experiment.
Group specific daily growth rates \( \mu (\text{days}^{-1}) \) were calculated according to Dijkman et al. (2009) as

\[
\mu (\text{days}^{-1}) = \ln \left( \frac{13C_{\text{concentration, initial}} - 13C_{\text{concentration, final}}}{\Delta t \times \text{cf}} \right)
\]

(1)

\[
\text{cf} = \text{mean} \left( \frac{\Delta 13C_{\text{organism}}}{\Delta 13C_{\text{DIC}}} \right)_{t \rightarrow t + \Delta t}
\]

(2)

The correction factor (cf) is necessary to correct for label saturation and represents the difference between organism (phytoplankton, mixotrophs and bacteria) and DIC labelling \( \Delta 13C \) relative to the \( \Delta 13C \) of DIC averaged over the considered growth period for each mesocosm. Production rates were calculated as

\[
P \left( \mu \text{mol C L}^{-1} \text{days}^{-1} \right) = \frac{\Delta 13C_{\text{producer}}}{\Delta 13C_{\text{DIC}}} \times \frac{C_{\text{producer}}}{t}
\]

(3)

### 2.4 Model

A nutrient–phytoplankton–zooplankton–detritus (NPZD) model, amended with isotope values, was constructed to quantify carbon fluxes within the plankton food web. The model is based on that of de Kluijver et al. (2010) and Van den Meersche et al. (2011). A detailed article about the model is in preparation (Van Engeland et al., 2012). The model equations are also found in the supplementary material, where phytoplankton is named phyto I and mixotrophs are named phyto II. The model code is incorporated in an R package, which is available upon request (R Core Team, 2012). The concentrations of both \(^{12}C\) and \(^{13}C\) were modelled separately for the following carbon pools: phytoplankton, mixotrophs, labile DOC (LDOC), bacteria, zooplankton, detritus, and sedimented OM. The nitrogen pools explicitly described in the model were DIN and DON. Nitrogen fluxes relating to other pools were calculated from carbon fluxes with a fixed Redfield stoichiometry. POC and PON were calculated in the model equations are also found in the supplementary material, where phytoplankton is named phyto I and mixotrophs are named phyto II. The model code is incorporated in an R package, which is available upon request (R Core Team, 2012). The concentrations of both \(^{12}C\) and \(^{13}C\) were modelled separately for the following carbon pools: phytoplankton, mixotrophs, labile DOC (LDOC), bacteria, zooplankton, detritus, and sedimented OM. The nitrogen pools explicitly described in the model were DIN and DON. Nitrogen fluxes relating to other pools were calculated from carbon fluxes with a fixed Redfield stoichiometry. POC and PON were calculated in the model.

The correction factor (cf) is necessary to correct for label saturation and represents the difference between organism (phytoplankton, mixotrophs and bacteria) and DIC labelling \( \Delta 13C \) relative to the \( \Delta 13C \) of DIC averaged over the considered growth period for each mesocosm. Production rates were calculated as

\[
P \left( \mu \text{mol C L}^{-1} \text{days}^{-1} \right) = \frac{\Delta 13C_{\text{producer}}}{\Delta 13C_{\text{DIC}}} \times \frac{C_{\text{producer}}}{t}
\]

(3)

#### 2.5 Statistics

Results are presented as average ± standard deviation (SD) over all mesocosms \( n = 9 \). Simple Pearson correlation tests were used to test the effect of CO\(_2\) on growth rates (Eq. 1).
production rates (Eq. 3), linear increase in $^{13}$C concentrations, and parameters and fluxes derived from the model. The results were tested and plotted against the average $pCO_2$ level in the corresponding phase. All statistical analyses were done in the software R.

3 Results

3.1 $^{13}$C-DIC dynamics

Addition of $^{13}$C bicarbonate together with the first CO$_2$ addition on $t_{-1}$ caused an increase in $\delta^{13}$C of DIC of $117 \pm 6\%$ in all mesocosms (Fig. 1a). The decrease in $\Delta\delta^{13}$C-DIC in perturbed mesocosms during the first 4 days ($t_{0-4}$) can be largely explained by exchange with the dead volume, which was the space between the sediment traps and the bottom of the mesocosms and comprised $\sim 10\%$ of total mesocosm volume (Schulz et al., 2012). Other processes that contributed to the initial label decrease were the subsequent (unlabelled) CO$_2$ additions, which diluted the $^{13}$C-DIC pool and respiration of unlabelled organic material. The loss of $^{13}$C-DIC due to air–sea exchange was low ($< 0.15\%$). From day 7 onwards, the $\Delta\delta^{13}$C of DIC remained quite stable (Fig. 1a). The labelled DIC concentrations were
Table 1. Growth ($\mu$) and production ($P$) rates based on Eqs. (1) and (3), respectively, for each phase. Values are presented as average of all mesocosms ± standard deviation ($n = 9$).

<table>
<thead>
<tr>
<th>Phase 1</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 1</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate ($\mu$, d$^{-1}$)</td>
<td>Production rate ($P$, $\mu$mol C L$^{-1}$ d$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>0.85 ± 0.06</td>
<td>0.19 ± 0.08</td>
<td>-</td>
<td>0.65 ± 0.08</td>
<td>0.19 ± 0.08</td>
</tr>
<tr>
<td>Mixotrophs</td>
<td>0.48 ± 0.04</td>
<td>0.23 ± 0.02</td>
<td>0.22 ± 0.06</td>
<td>0.55 ± 0.06</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>Bac</td>
<td>0.68 ± 0.11</td>
<td>0.33 ± 0.02</td>
<td>0.13 ± 0.04</td>
<td>0.58 ± 0.05</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>POC</td>
<td>0.80 ± 0.13</td>
<td>0.75 ± 0.22</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Fig. 3. Production rates vs. average $pCO_2$ levels of each phase based on data (Eq. 3) of (A) phytoplankton; (B) mixotrophs; and (C) sum phytoplankton and mixotrophs production rates ($\mu$mol C L$^{-1}$ d$^{-1}$) in phase 1 for the build-up ($t_{0-6}$), the build-up and decline ($t_{0-12}$), and the production loss during decline (difference) denoted with ($\Delta$); (D) mixotroph production rates ($\mu$mol C L$^{-1}$ d$^{-1}$) after nutrient addition for initial phase 2 ($t_{14-22}$) and total phase 2 ($t_{14-29}$).

2.6 ± 0.1 $\mu$mol $^{13}$C L$^{-1}$ at $t_0$ and decreased during the first 9 days to 2.2 ± 0.2 $\mu$mol $^{13}$C L$^{-1}$ at $t_9$ and did not show large changes afterwards (Fig. 2a).

3.2 Phytoplankton and POC dynamics

After the enclosure of post-bloom water, a phytoplankton bloom developed even though inorganic nutrient concentrations were low (0.64 and 0.05 $\mu$mol L$^{-1}$ DIN and phosphate, respectively). Phytoplankton rapidly incorporated $^{13}$C; on $t_7$ the whole phytoplankton community had been turned-over, as indicated by the plateau (Fig. 1a), although phytoplankton never reached the $\Delta$8$^{13}$C of DIC. Mixotrophs showed clearly slower enrichment and never became saturated with $^{13}$C (Fig. 1a). Phytoplankton initially had low biomass (1.2 ± 0.05 $\mu$mol C L$^{-1}$, ~6% of POC) compared to mixotrophs (8.3 ± 1.2 $\mu$mol C L$^{-1}$, ~40% of POC) (Fig. 1b). A comparison with Chl a as a proxy for autotrophic biomass, and after subtraction of phytoplankton, indicated that >65% of mixotroph biomass in phase 1 belonged to heterotrophs (Schulz et al., 2012, Czerny et al., 2012a). Both groups contributed to the bloom during phase 1 in biomass and reached a bloom peak at $t_9$ and declined afterwards (Fig. 1b). The development of $^{13}$C labelled biomass showed that the bloom build-up and decline were more pronounced for phytoplankton compared to mixotrophs (Fig. 2b, c). This was also reflected in higher growth rates of phytoplankton ($\mu_{phyt}$) compared to mixotrophs ($\mu_{mix}$) during bloom build up ($t_{0-6}$) (Table 1). Bloom peak height, as well as growth rates of phytoplankton and mixotrophs were independent of $CO_2$. 
Production of phytoplankton and mixotrophs during the build-up ($t_{0-6}$) averaged $1.20 \pm 0.11 \mu\text{mol C L}^{-1} \text{d}^{-1}$. Production rates in overall phase 1, averaged over build-up and decline ($t_{0-12}$), were only $0.48 \pm 0.13 \mu\text{mol C L}^{-1} \text{d}^{-1}$, due to the bloom decline after $t_6$ (Table 1, Fig. 3). Phytoplankton production during the build-up ($t_{0-6}$) was independent of CO$_2$, but the overall production ($t_{0-12}$) increased with increasing pCO$_2$ (Fig. 3a, $r = 0.81$, $p < 0.01$). Production rates of mixotrophs showed a different response to CO$_2$: the production rates during the build-up ($t_{0-6}$) were lower at higher pCO$_2$ (Fig. 3b, $r = -0.79$, $p < 0.05$) and overall production rates ($t_{0-12}$) were independent of CO$_2$. Despite contrasting responses to pCO$_2$, both phytoplankton groups had a loss in (particulate) production during the bloom collapse ($t_{12-29}$), which was CO$_2$ dependent (Fig. 3a, b). As a consequence, total production rates of phytoplankton (sum of phytoplankton and mixotrophs) were independent of pCO$_2$, but the loss in production during the bloom collapse ($\Delta P$) was significantly higher at low pCO$_2$ than at high pCO$_2$ ($r = -0.70$, $p < 0.05$, Fig. 3c).

The production of phytoplankton and mixotrophs was reflected in the build-up of $^{13}$C enriched POC with a peak on $t_{8-11}$ and a subsequent decline (Fig. 2d). POC production averaged $0.80 \pm 0.13 \mu\text{mol C L}^{-1} \text{d}^{-1}$ (Table 1). POC production was independent of CO$_2$ in phase 1, in agreement with the dynamics of the sum of phytoplankton (Fig. 3c).

After nutrient addition, phytoplankton and mixotrophs increased again in biomass, but there was more variation between mesocosms. Bloom peaks of phytoplankton were reached on $t_{18-29}$, depending on the mesocosm, but not on CO$_2$ (Fig. 2b). Bloom peaks of mixotrophs were reached on $t_{22-29}$ and were also independent of CO$_2$ (Fig. 2c). Although $^{13}$C biomass of mixotrophs kept increasing, total biomass, growth and production rates of mixotrophs after nutrient addition remained similar to phase 1 (Fig. 1b, Table 1). Production rates of mixotrophs were initially higher in the high CO$_2$ treatments ($t_{14-22}$, $r = 0.72$, $p < 0.05$, Fig. 3d). However, overall production rates in phase 2 ($t_{14-29}$) showed an optimum around current CO$_2$ levels (Fig. 3d). Because of label saturation (Fig. 1a), growth and production rates could not be determined for phytoplankton after nutrient addition. Also, POC production rates before and after nutrient addition were similar (Table 1, Fig. 4a). The average production rate of POC after nutrient addition ($t_{14-29}$) decreased with increasing CO$_2$ ($r = -0.87$, $p < 0.01$, Fig. 4a).

### 3.3 $^{13}$C labelling of bacteria and zooplankton consumers

Heterotrophic bacteria followed the labelling pattern of POC (Fig. 1a). Initial bacterial biomass was $4.6 \pm 0.6 \mu\text{mol C L}^{-1}$ ($\sim 19$ % of POC) and stayed constant during phase 1.
Table 3. Carbon fluxes (µmol C L⁻¹ d⁻¹) in phase 1 (t₀–12) derived from the model between the major carbon pools, shown as arrows in Fig. 7. The values present the average ± standard deviation of all mesocosms (n = 9).

<table>
<thead>
<tr>
<th>Processes</th>
<th>Flux (µmol C L⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total primary production</td>
<td>1.78 ± 0.17</td>
</tr>
<tr>
<td>Phytoplankton production</td>
<td>1.17 ± 0.10</td>
</tr>
<tr>
<td>Production of mixotrophs</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>Phytoplankton exudation</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>Exudation by mixotrophs</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Bacterial production</td>
<td>0.60 ± 0.062</td>
</tr>
<tr>
<td>Zooplankton production</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>Faeces production</td>
<td>0.028 ± 0.007</td>
</tr>
<tr>
<td>Phytoplankton mortality</td>
<td>0.60 ± 0.062</td>
</tr>
<tr>
<td>Mortality of mixotrophs</td>
<td>0.21 ± 0.11</td>
</tr>
<tr>
<td>Mortality to DOC</td>
<td>0.044 ± 0.029</td>
</tr>
<tr>
<td>Respired mortality</td>
<td>0.47 ± 0.093</td>
</tr>
<tr>
<td>Mortality to detritus</td>
<td>0.30 ± 0.074</td>
</tr>
<tr>
<td>Export of detritus</td>
<td>0.021 ± 0.003</td>
</tr>
<tr>
<td>Total export</td>
<td>0.13 ± 0.018</td>
</tr>
</tbody>
</table>

(Fig. 1b). Due to label incorporation, the ¹³C-enriched bacteria biomass increased in the first phase and peaked on t₀–8 (Fig. 2e). Bacterial production in phase 1 started with 0.58 ± 0.05 µmol C L⁻¹ d⁻¹ (t₀–8), but declined with the bloom collapse to 0.47 µmol C L⁻¹ d⁻¹, a production rate similar to primary production. Bacterial ¹³C biomass increased again after nutrient addition until the end of the experiment. Both growth and production of bacteria were twice as high before rather than after nutrient addition (Table 1). Bacteria growth and production were independent of CO₂ levels.

Zooplankton (Calanus sp. and Cirripedia) incorporated ¹³C in a similar way and the incorporation of tracer into copepods was used as representative for the mesozooplankton community. The ¹³C incorporation into zooplankton was low (Fig. 1a). With a constant biomass of ~5 µmol C L⁻¹ (Niehoff et al., 2012), the ¹³C showed a negative correlation with CO₂ (r = −0.92, p < 0.001, Figs. 2f, 4b). From day 24 onwards, the variance in ¹³C biomass increased and the CO₂ effect disappeared (Fig. 2f).

3.4 ¹³C labelling of sedimented organic material

The label enrichment in sediment trap organic matter in the first 7 days was low, indicating that little freshly produced material was sinking into the traps (Fig. 1a). After day 7, the material became more enriched, probably because of the bloom collapse and after day 20, the Δδ¹³C of sediment trap POC increased rapidly (Fig. 1a). After day 25, the Δδ¹³C of sediment POC was higher than of water column POC, showing that there was preferential sinking of freshly produced material. The cumulative ¹³C of sediment trap POC is shown in Fig. 2g. The settling of ¹³C enriched POC in the traps was very low in the first phase and increased with increasing CO₂ (r = 0.75, p < 0.05, Fig. 4c). After nutrient addition, the sinking of ¹³C POC was much higher and the effect of CO₂ on sedimentation was reversed compared to phase 1 (Figs. 2g, 4c): sedimentation of freshly labeled (¹³C enriched) POC decreased with increasing CO₂ (r = −0.78, p < 0.05, Fig. 4c). The ¹³C increase in POC in the water column and sediment traps showed a non-linear response to CO₂ in phase 2, which indicates a step-wise rather than a gradual CO₂ effect (Fig. 4a, c). Mesocosms with CO₂ levels below 340 µatm had high POC production and sedimentation rates, while mesocosms with CO₂ above 400 µatm had low POC production and sedimentation rates after nutrient addition (Fig. 4a, c). The exception was at 395 µatm (average pCO₂ in phase 2) in the mesocosm where there was high production and low sedimentation (Fig. 2d, f). The fatty acid
composition of settling material in phase 3 revealed that all groups were present, but there were more mixotrophs' markers than phytoplankton markers in the sediment traps.

### 3.5 Model results: parameters and carbon fluxes

The construction of a model and subsequent fitting to the data provides the possibility to study the community as a whole, instead of studying carbon production in each carbon pool separately as done above. Fits for phase 1 of one mesocosm (M4, 375 μatm) are shown in Fig. 5 and the fits for the other mesocosms can be found in the supplementary material A. The set of parameters that were selected during the MCMC analysis was used to calculate average carbon fluxes over phase 1 ($t_{0-12}$).

The bloom of phytoplankton in phase 1 caused a decrease in DIN and DON concentrations (Fig. 5). Phytoplankton had high growth rates ($μ_{\text{phy}}$, Table 2) resulting in a large flux of DIC to phytoplankton (Table 3). Mixotrophs had lower growth rates ($μ_{\text{mix}}$, Table 2) and lower primary production rates (Table 3). To reach the high biomass of phytoplankton, mortality was set to 0 during the first six days. Large parts from gross phytoplankton production were exuded as DOC: exudation averaged over all mesocosms 30.7 ± 1.2% of total primary production (for both phytoplankton and mixotrophs), which was subsequently used by bacteria. Bacteria had high growth rates ($μ_{\text{Bac}}$) and were the primary consumers, consuming 33.8 ± 3.2% of total primary production. Mesozooplankton had low grazing rates ($μ_{\text{Zoop}}$) and consumed only 10.5 ± 2.5%, on average, of total primary production in all mesocosms. The loss for bacteria was assumed to be respiration, while zooplankton loss was not only due to
respiration (35%), but primarily because of settling (65%; \( \xi_{\text{zoo}} \)).

Mortality after day 6 was higher for phytoplankton than for mixotrophs (Table 2). The mortality carbon flow was 51.3 ± 7.0 % of phytoplankton production and 36.2 ± 19.8 % of mixotroph production (Table 3). The largest fraction of plankton mortality was respiration, 37.0 ± 5.0 % went into detritus \(( f_{\text{det}} \) and 5.6 ± 3.7 % was channeled into DOC \(( f_{\text{DOM}} \) (Table 2). The sinking rate (as fraction) of detritus \(( r_{\text{sink}} \) was low \((0.0082 ± 0.0048 \text{ d}^{-1} \text{ in all mesocosms})\) and also mineralisation \((\rho)\) showed low rates (Table 2). Consequently, the export of detritus was low (Table 3). With the contribution of zooplankton to the sediment traps, the total export was 7.1 ± 1.4 % of total primary production averaged over all mesocosms.

Two of the twelve model parameters potentially sensitive to CO\(_2\) showed to be indeed affected by CO\(_2\) treatments. Grazing rates \((\mu_p)\) decreased with increasing CO\(_2\) (Fig. 6a, \( r = -0.79, p < 0.05 \)). Sinking rates \((r_{\text{sink}})\) showed a positive correlation with \text{pCO}_2 \(( r = 0.81, p < 0.01 \), Fig. 6b). The sinking was 5 times higher at high CO\(_2\) \((0.016 ± 0.0034 \text{ d}^{-1})\) compared to lower CO\(_2\) \((0.0020 ± 0.0014 \text{ d}^{-1})\). For validation of the parameters, the model was also tested with \(\xi_{\text{zoo}}\) included as a CO\(_2\) sensitive parameter, \(\xi_{\text{zoo}}\) is the part of zooplankton carbon gain that ended in the sediment traps. \(\xi_{\text{zoo}}\) was found to be CO\(_2\) independent. The amount of zooplankters that ended in the traps were also independent of CO\(_2\) levels (Niehoff et al., 2012).

The fluxes are graphically presented in Fig. 7, showing that the largest fluxes went from DIC to phytoplankton and subsequently bacteria. Because grazing rates and sinking rates were CO\(_2\) sensitive (Fig. 5), the carbon flows from phytoplankton to zooplankton and detritus to sediment traps were also CO\(_2\) sensitive as indicated by the dashed lines (Fig. 7).

4 Discussion

4.1 Plankton carbon flows under low nutrients

While most of the CO\(_2\) enrichment mesocosm experiments involved inorganic nutrient addition and focussed on production and export food chains, this study investigated ocean acidification in a nutrient regenerating food chain, at least during phase 1 of the experiment. The low nutrient concentrations, low Chl \( a\), and high heterotrophic biomass in Kongsfjorden waters were characteristic for a post-bloom situation (Rokkan-Iversen and Seuthe, 2011).

Although nutrient concentrations were low, a small phytoplankton bloom started right after enclosure, probably fuelled by efficient recycling of nutrients accompanied with remineralisation of DON, which decreased after the start of the experiment (Fig. 5, Schulz et al., 2012). Total net primary production rates in our experiment \((21 \text{ mmol C m}^{-2} \text{ d}^{-1})\), average of all mesocosms integrated over the 12 m sampling depth) were similar to the median particulate primary production of \(20 \text{ mmol C m}^{-2} \text{ d}^{-1}\) in Arctic regions (synthesis by Kirchman et al., 2009a). However, net particulate primary production in this study was lower, \( \sim 14 \text{ mmol C m}^{-2} \text{ d}^{-1}\) (integrated over the 12 m sampling depth), suggesting nutrient limitation in our study. Primary production during the bloom was dominated by phytoplankton as indicated by their high growth and production rates (Tables 1, 2). Despite their low biomass, they were responsible for two thirds of the primary production in phase 1 (Tables 1, 3, Fig. 7).

According to flow cytometry, the productive phytoplankton consisted of nanophytoplankton during this time (Brussaard et al., 2013) and pigment analyses indicated that haptophytes were the main autotrophs (Schulz et al., 2012). The other third of primary production was contributed by the mixotrophs. Mixotrophs dominated in terms of biomass (Fig. 1b) and microscopy showed that they were mainly heterotrophic dinoflagellates and probably chrysophytes (Schulz et al., 2012). Regardless their high biomass, they had lower growth and production rates (Tables 1, 2, 3), as expected due to the mixotrophic character of the group.

The difference in model-based net primary production and data-based particulate primary production is the dissolved primary production: the release of organic matter. Two thirds of NPP was used for net particulate primary production \((1.2 \mu\text{mol C L}^{-1} \text{ d}^{-1}\) Table 1) and the other one third was exuded as dissolved primary production to fuel bacterial production. Bacteria were an important component of...
the pelagic food web and a rapid consumer of primary production, as indicated by rapid transfer of label from phytoplankton to bacteria (Fig. 1a). Bacteria production amounted to a third of total phytoplankton production (34%) (Table 3, Fig. 7). A remarkably similar average BP:PP ratio (34%) was observed in Arctic transect studies by Kirchman et al. (2009b), although their absolute production rates were much lower.

The bacterial growth efficiency (BGE) during phase 1 was estimated to be ~ 15% (Motegi et al., 2012), indicating that a large part of bacterial production was respired. High community respiration was also observed by Tanaka et al. (2012), who found respiration close to or sometimes exceeding primary production during phase 1. The net bacterial production under nutrient limitation was in the range measured with 3H-thymidine (Table 2, Motegi et al., 2012). BP:PP ratios from our analyses were higher than those measured with 14C during the same study (Engel et al., 2012). The discrepancy can be largely explained by their higher measured PP rates (Engel et al., 2012, and discussed therein). Bacterial growth rates in phase 1 (0.33–0.36 d−1) were rather similar to those measured with 14C leucine: 0.24–0.37 d−1 (Piontek et al., 2012).

Despite the high growth rates, the biomass of bacteria did not increase (Fig. 1b), indicating a strong removal pressure (top–down control) on bacteria, e.g. by viruses or microzooplankton (heterotrophic dinoflagellates) grazing, which were both important during phase 1 (Brussaard et al., 2013; Schulz et al., 2012). Even an initial decline in bacterial numbers until t6 was determined with flow cytometry, although this was not seen in PLFA (Fig. 1b).

Although mesozooplankton were largely present (Niehoff et al., 2012), their grazing rates on primary production were very low, as indicated by maximum daily grazing rates of 0.022 d−1 on phytoplankton biomass. In phase 1, only 11% of primary production was consumed by mesozooplankton (Table 3, Fig. 7).

In summary, the high BP:PP, high microzooplankton abundance, and low mesozooplankton grazing indicate that the microbial food web was more important in this study than a herbivorous food web (Legendre and Razoulzagane, 1995). Our results on plankton food web structure fit very well with the previously described post bloom (May–July) situation in Kongsfjorden (Rokkan Iversen and Seuthe, 2011), with high BP:PP production and a prominent role for the microbial food web. However, they suggested a control of phytoplankton biomass by mesozooplankton grazing, because of low phytoplankton biomass, high primary production, and high zooplankton biomass, which is not supported by our findings.

Viral infection together with microzooplankton grazing likely caused the bloom to collapse after t6, since phytoplankton decline coincided with high microzooplankton grazing and increased virus abundance (Brussaard et al., 2013). Mortality affected phytoplankton much more than mixotrophs, consistent with virus–host specificity. Phytoplankton mortality rates of up to 0.3 d−1, as observed for phytoplankton, have been recorded during bloom declines as well as in oligotrophic systems (reviewed in Brussaard, 2004). When phytoplankton cells die, the cells lyse and a large portion is released as DOM, which can be subsequently used by bacteria (reviewed in Brussaard, 2004). In our study, phytoplankton mortality did not stimulate bacterial production per se, since bacterial production declined after day 6 as well (Table 2, but some DOC accumulation was observed (Czerny et al., 2012; Engel et al., 2012). Possible explanations for the decline in bacterial production are concurrent viral infections or a shift from microzooplankton grazers from phytoplankton to bacteria.

Although it was difficult to constrain, we estimated that approximately one third of dying phytoplankton (phytoplankton mortality) ended up as detritus. Detritus formed only a small part of total POC produced (10%) and was mainly formed of dead algae. The sedimentation losses of detritus were low (0.008 d−1) and in phase 1, sinking detritus comprised only 1% of primary production (Table 3, Fig. 7). In phase 1, zooplankton contributed substantially to sedimented organic material (Niehoff et al., 2012). Together with zooplankton settling in the traps, the average export corresponded to ~ about 7% of primary production. In contrast, the calculated export in a previous mesocosm experiment with nutrient addition was ~ 24 times higher than the export rate in this experiment (Riebesell et al., 2007).

4.2 Plankton carbon flows after nutrient addition

The addition of nutrients did not increase total phytoplankton and bacterial biomass in the mesocosms (Fig. 1b). However, Chl a increased after nutrient addition (Schulz et al., 2012), indicating that the community shifted away from mixotrophy more towards autotrophy. Pigment and microscopy analyses indicated a shift in the autotrophic community towards dinoflagellates (Schulz et al., 2012), which are also part of the mixotrophs. Even though phytoplankton production increased the 13C biomass (Fig. 2b, c), the total amount of phytoplankton carbon showed little increase (Fig. 1b). High grazing rates and viral lyses were factors that kept phytoplankton biomass low (Brussaard et al., 2013).

Interestingly, bacterial production and growth rates decreased after nutrient addition (Table 1), contrary to the generally observed positive relation between nutrient concentrations and growth efficiency (del Giorgo and Cole, 1998). Bacteria in phase 2 could have been limited by substrate (DOC) availability, since extra cellular release decreased after nutrient addition (Engel et al., 2012). In agreement with our findings, a similar decrease in bacterial growth from day 8 onwards was found with radioactive leucine incorporation during the experiment (Piontek et al., 2012).

The largest change in phase 2 compared to phase 1 was an increase in sedimentation. Large sedimentation of (freshly produced) organic matter occurred after day 24, when chain-forming diatoms started to grow in the mesocosms (Czerny
et al., 2012a). The diatoms probably formed aggregates that facilitated sinking of organic matter. The higher isotopic enrichment of sedimented organic matter compared to the water column (Fig. 1a) showed that the aggregates were formed of freshly produced organic matter and the dominance of diatoms was confirmed by the high presence of mixotroph markers in the sediment trap material.

4.3 Methodological considerations and assumptions

$^{13}$C labelling combined with modelling has been used successfully in previous mesocosm studies, allowing quantifying carbon flows and interactions in plankton food webs (Van den Meersche et al., 2004, 2011; de Kluijver et al., 2010). However, there are some assumptions and potential errors that need attention. A main advantage of using a $^{13}$C tracer is that production can be measured in situ, in contrast to other methods like radioactive tracers that require side incubations with perturbed environmental (e.g. light) conditions. Using PLFA biomarkers, phytoplankton and bacteria group specific primary production can be estimated in addition to total POC production (Dijkman et al., 2009). A comparison of community production measurements performed during the experiment with different methods (DIC, oxygen, $^{13}$C) is presented in Tanaka et al., 2012. There was a good correlation between $^{13}$C POC and DIC-based NCP, as we expected, since they were both measured in situ.

Although PLFAs can be used as taxonomic markers, the majority of PLFA markers do not allow distinction between heterotrophic and autotrophic (phyto)plankton, such as mixotrophic dinoflagellates, and therefore we had to consider them together as mixotrophs. To separate autotrophic and mixotrophic phytoplankton, additional methods are needed, such as fluorescence activated cell sorting combined with PLFA analysis (Pel et al., 2004). Because fatty acids are often shared among taxonomic groups, we choose a conservative approach to consider only two phytoplankton groups based on their $^{13}$C uptake patterns (phytoplankton and mixotrophs). However, temporal changes in total fatty acid composition were observed by Leu et al. (2012), indicating shifts in community composition within the two groups. An assumption, potentially introducing errors, was the application of a single conversion factor for PLFA:OC. Because we lacked (1) detailed species composition, (2) single-species biomarkers and (3) specific PLFA:OC ratios for each species, grouping phytoplankton and applying a single conversion factor seemed the most appropriate approach. Another assumption was that branched fatty acids are representative for the whole bacterial community, even though they primarily occur in gram-positive bacteria (Kaneda, 1991). Part of the (gram-negative) bacteria might have been overlooked, resulting in a potential underestimation of bacterial biomass and production, although the PLFA-based growth and production rates were in the range reported by Motegi et al. (2012) and Piontek et al. (2012).

The $^{13}$C incorporation method is limited when phytoplankton is saturated with tracer, i.e. it has taken the signature of the source corrected for fractionation, in which case uptake of substrate will not cause further changes in $^{13}$C. Saturation was observed in phytoplankton after the first six days precluding growth estimates after this period and precluding model application for phase 2. For future experiments an additional $^{13}$C spike with nutrient addition is recommended. The other carbon pools did not get saturated with tracer (Fig. 1a) and bacteria never reached the isotope labelling of phytoplankton (Fig. 1a). Assuming that phytoplankton derived matter is the only carbon source for bacteria, this implies a senescent or dormant pool of bacteria that did not grow during the experiment.
Zooplankton never reached label enrichment of any carbon pool (Fig. 1a). Mesozooplankton has a slow turnover in response to dietary changes, contributing to low labelling patterns. A study on carbon turnover in Arctic crustaceans showed low turnover in stable isotopes with a half life of 14 days (Kaufman et al., 2008). For simplicity, a uniform grazing rate on total phytoplankton was assumed in the model, but there was probably selective grazing on different phytoplankton groups. Due to the labelling differences between phytoplankton and mixotrophs, grazing rates would decrease if zooplankton primarily grazes on phytoplankton and increase if zooplankton primarily grazes on mixotrophs.

Another assumption was the application of a fixed Redfield stoichiometry in the model to fit the nitrogen fluxes (Table 2), although there was variability in this ratio (Schulz et al., 2012). Sensitivity of the fitted parameters to variable stoichiometry was tested and a variable stoichiometry showed little effect on parameter fitting (Van Engeland et al., personal communication). Potential changes in stoichiometry are a primary interest in ocean acidification research (e.g. Riebesell et al., 2007), but changes in stoichiometry seemed independent of CO2 in this study (Schulz et al., 2012).

Production processes are relatively easy to determine with 13C incorporation, but it is more challenging to quantify and allocate loss processes. The partitioning of carbon from phytoplankton mortality was difficult to constrain (Van Engeland et al., 2012). The partitioning in the particulate fraction was relatively easy to determine, because of direct POC measurements, but partitioning into dissolved material was more difficult, because of lack of accurate 13C-DOC measurements. In our study, the amount of tracer added was insufficient to measure 13C enrichment in DOC, due to the high background pool of DOC. For sufficient 13C enrichment in DOC, the amount of added tracer should be > 10 times higher.

The data from the sediment trap samples have to be considered with care. The sediment traps were positioned only ~ 15 m deep, so the material in the sediment traps cannot be quantitatively considered to be exported compared to studies where traps were placed below the euphotic zone. The sediment traps were also within the daily migration zone of zooplankton and there were a large number of Cirripedia settling in the sediment traps. Zooplankton can contribute largely to settling material, especially in shallow traps, and contributions of 14-90% of zooplankton to POC in traps were reported by Buesseler et al. (2007). In the model, an 82% contribution of zooplankton to sediment trap material was necessary to achieve the low labelling of sediment material in phase 1. Preferential settling of old, unlabelled material in the traps could have contributed to the low labelling as well, but this was not considered in the model.

Although the above processes can cause potential errors in the estimated carbon fluxes, they do not explain the observed CO2 effects, since they are expected to occur in all mesocosms.

### 4.4 CO2 effects

In this study, we aimed to increase our understanding of CO2 effects on primary production, community respiration, and export in Arctic communities by looking at individual uptake and loss rates and by quantifying the interactions between food web compartments with a food web model. Some of the CO2 effects in phase 1 that were observed in individual fluxes (grey arrows in Fig. 7) were not shown in the integrated food web model, so we consider them with care.

Although it was not captured by the model, the data suggest that reduction in phytoplankton production due to phytoplankton mortality can be CO2 sensitive. When the bloom collapsed (after t6), the loss in particulate primary production was significantly lower at higher CO2 levels (Fig. 3c). A similar CO2 effect on production losses in nanophytoplankton was seen, where production loss was twice as much at low CO2 compared to high CO2 (Brusbaar et al., 2013). Reduced grazing by mesozooplankton at high CO2 (Fig. 5b) can partly explain the reduced loss at high CO2. However, grazing fluxes were too low (Table 3) to cover the differences in loss. Another explanation is the presence of CO2 effects on the partitioning of phytoplankton mortality in phase 1. Both simple regression (Fig. 4c) and model output (Fig. 6b) showed that sedimentation of fresh organic matter increased with increasing CO2 in phase 1. Since mortality rates were not sensitive to CO2 and viral numbers were not CO2 dependent (Brusbaar et al., 2013), we speculate that there were CO2 effects on the partitioning of dry phytoplankton in particulate and dissolved organic matter fractions. The organic material released at high CO2 could be of a more sticky nature, serving as a precursor of transparent exopolymer particles (TEP), or less degradable (Engel et al., 2002; Czerny et al., 2012; Engel et al., 2012). When more dead phytoplankton ends in aggregates or particles, it could lead to enhanced sinking at high CO2, as observed in phase 1.

Both simple regression (Fig. 4b) and model output (Fig. 6a), showed reduced zooplankton grazing in phase 1 with increasing CO2. There was no CO2 effect found on zooplankton numbers (Niehoff et al., 2012) and we can only speculate about the mechanisms. Reduced grazing could result from the reduced initial production of mixotrophs at higher CO2 (Fig. 3b). Another possible explanation for reduced grazing could be CO2 induced changes in food quality, i.e. the production of less essential fatty acids. Organic matter at high CO2 contained less 22:6ω3 (Leu et al., 2012). 22:6ω3 is an essential fatty acid for zooplankton and can be growth limiting (Anderson and Pond, 2000). A hampering CO2 effect on Cirripedia development to the next stage was observed (Niehoff et al., 2012), but whether this was related to lower grazing, needs to be further addressed.

In this study, no CO2 effect on bacterial growth and production were observed. There was also no CO2 effect on carbon exudation by phytoplankton as source for bacteria, although this process is considered potentially CO2 sensitive.
It has been hypothesized that increasing CO₂ could stimulate carbon overconsumption and subsequent extracellular release, but most studies done so far showed no effects on DOC production in community-level CO₂ enrichment (e.g., Engel et al., 2004b). Previous mesocosm studies focused on nutrient replete situations and it was suggested that CO₂ effects on extracellular release would be more pronounced under nutrient limitation (Thingstad et al., 2008; de Kluijver et al., 2010). The results here show that bacterial production on phytoplankton exudation is also not enhanced with CO₂ in a post bloom situation. However, a lack of bacterial response does not necessary mean that there was no stimulation of extracellular release by phytoplankton. Exudates are also important players in formation of TEP, marine snow and subsequent export (Engel et al., 2004a).

After nutrient addition, phytoplankton production rates (mixotrophs) were initially stimulated by higher CO₂ (t14-22). The positive effect of CO₂ acted mainly on (autotrophic) dinoflagellates, shown by pigment analyses and microscopy (Schulz et al., 2012) and a relative fatty acid composition (Leu et al., 2012). Another group that benefitted from increased CO₂ were prasinophytes, which were part of phytoplankton (Schulz et al., 2012). The higher production of phytoplankton at high CO₂ in phase 1 (Fig. 3a) could have initialized this trend. Unfortunately, we could not measure production rates of phytoplankton after nutrient addition.

Mixotroph production showed an optimum around current CO₂ levels of 340 µatm over the whole phase after nutrient addition (t14-28; Fig. 3c). The response of mixotrophs was likely an indirect effect of CO₂ due to competition with other phytoplankton groups. The proposed mechanism (based on pigments and flow cytometry) is that increasing CO₂ stimulated picoplankton directly after nutrient addition, leaving less dissolved inorganic nutrients for larger phytoplankton, like diatoms, in the final stage of the experiment (Schulz et al., 2012). The response to CO₂ after nutrient addition was also not gradual for POC production and sedimentation rates. POC production rates after nutrient addition showed a stepwise response to CO₂ with a transition point around current CO₂ levels (Fig. 4a). Production rates were lower at CO₂ levels above 400 µatm and because of the large export in phase 3, the CO₂ effect on POC production was directly reflected in settling material (Fig. 4c). Our findings suggest that CO₂ effects on some processes are stepwise rather than gradual, which can be of interest for future research.

5 Conclusions

This mesocosm study is the first to study ocean acidification effects on Arctic plankton communities in a system dominated by regenerated production. Before nutrient addition (phase 1), the pelagic food web was characterized by high BP:PF, high micro-zooplankton abundance, low mesozooplankton grazing and low export. Comparable production rates, but increased export were observed after nutrient addition (phase 2). CO₂ effects were subtle and different for each phase. We observed a stimulating effect of CO₂ on export and a hampering effect on community (mesozooplankton) respiration in phase 1 and a hampering effect of CO₂ on production and export in phase 2. The observed CO₂ related effects potentially alter future organic carbon flows and export, with possible consequences for the efficiency of the biological pump.

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