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Arctic microbial community dynamics influenced by elevated CO$_2$ levels

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Abstract. The Arctic Ocean ecosystem is particularly vulnerable to ocean acidification (OA) related alterations due to the relatively high CO$_2$ solubility and low carbonate saturation states of its cold surface waters. Thus far, however, there is only little known about the consequences of OA on the base of the food web. In a mesocosm CO$_2$-enrichment experiment (overall CO$_2$ levels ranged from ~ 180 to 1100 µatm) in Kongsfjorden off Svalbard, we studied the consequences of OA on a natural pelagic microbial community. OA distinctly affected the composition and growth of the Arctic phytoplankton community, i.e. the picoeukaryotic photautotrophs and to a lesser extent the nanophytoplankton thrived. A shift towards the smallest phytoplankton as a result of OA will have direct consequences for the structure and functioning of the pelagic food web and thus for the biogeochemical cycles. Besides being grazed, the dominant pico- and nanophytoplankton groups were found prone to viral lysis, thereby shunting the carbon accumulation in living organisms into the dissolved pools of organic carbon and subsequently affecting the efficiency of the biological pump in these Arctic waters.

1 Introduction

The increase of $p$CO$_2$ in the surface ocean (ocean acidification, OA) profoundly affects the seawater carbonate system through well-known chemical reactions, lowering the pH, increasing the concentration of bicarbonate ions, decreasing the availability of carbonate ions and lowering the saturation state of the major shell-forming carbonate minerals. Whereas surface water pH values were still 8.2 just prior to the industrial era, they are 8.11 at present and anticipated to reach 7.8 in the year 2100 (The Royal Society, 2005). This corresponds to CO$_2$ levels of 380 µatm at present to a projected high of at least 750 µatm by the end of this century.

Changes in carbonate chemistry can be expected to directly affect phytoplankton photosynthesis and subsequently growth because of their dependence on CO$_2$ supply. Not all algal groups will be equally affected, as certain groups (most notably diatoms) have developed CO$_2$-concentrating mechanisms (CCMs). However, algal species differ in CCM efficiency (Rost et al., 2008). Non-calculifying phytoplankton show, as expected, a range of responses, varying from no effect on growth to stimulating or adverse effect on growth or primary production (Riebesell and Tortell, 2011). Only a few studies have reported on OA-induced changes of phytoplankton community composition (Tortell et al., 2002, Engel et al., 2008; Meakin and Wyman, 2011; Feng et al., 2009), and the ecological consequences of OA on natural phytoplankton dynamics are still understudied.

Furthermore, a major gap in our understanding concerns the transfer of responses from the organism to the community and ecosystem levels. Rose et al. (2009) recently showed that climate change variables (temperature and $p$CO$_2$) did affect trophic dynamics during a North Atlantic spring bloom. As a result, predicting the impact of ocean acidification on marine ecosystem dynamics, and consequently biogeochemically cycling, is presently still limited. As pointed out by The Royal Society (2005), marine ecosystems are likely to become less robust as a result of ocean acidification and will be more vulnerable to other environmental changes.
(e.g. temperature increase, light availability, nutrient limitation). Potential restructuring of the phytoplankton community (classes, species and cell size; Falkowski et al., 1998; Boyd and Doney, 2002) as a result of ocean acidification will have direct consequences for grazer communities and organic carbon flow. It may also influence the dominance of grazing over other loss processes such as viral lysis, and consequently the cycling of energy and biogeochemically relevant elements, the ratio of production and respiration of the ocean and the efficiency of the biological pump (Brussaard et al., 1996, 2008; Ruardij et al., 2005; Suttle, 2007). Phytoplankton that are consumed by grazers are channeled to higher trophic levels, whereas viral lysis directly forces the food web towards a more regenerative pathway (Brussaard et al., 2005; Suttle, 2007).

OA and other global climate change-related impacts are most striking in both polar regions, where temperatures and acidities are changing at more than twice the global average (Hoegh-Guldberg and Bruno, 2010). The cold Arctic surface waters allow relatively high CO$_2$ solubility, making this area particularly vulnerable to OA. The present study is part of a collaborative mesocosm CO$_2$-enrichment experiment performed in Kongsfjorden off Svalbard, summer 2010, within the framework of the European Project on Ocean Acidification (EPOCA). We present here the microbial community dynamics under the influence of elevated pCO$_2$ levels and discuss the consequences for the functioning of the pelagic food web.

2 Material and methods

2.1 Study site and experimental set-up

The study was conducted in Kongsfjorden (78°56’2”N, 11°53’6”E), near Ny-Ålesund, Svalbard, between 31 May and 7 July 2010 (day t = 7 to t30, where t0 is the day after the first CO$_2$ addition). Nine large mesocosms of approximately 50 m$^3$ (15 m below sea level) were moored in the fjord in sets of 3. The experimental set-up, carbon chemistry dynamics and nutrient concentrations throughout the experiment are described in detail by Riebesell et al. (2013), Bellerby et al. (2013) and Schulz et al. (2013). In short, the mesocosms were filled with fjord water (inorganic nutrient concentrations < 0.1, 0.09, 0.2 and 0.7 µM for nitrate, phosphate, silicate and ammonium, respectively) and screened through a 3 mm mesh to eliminate larger organisms on the 1 of June (t = 6). The mesocosms were open to the atmosphere but covered with transparent hoods to minimize input of rain and bird droppings. Two of the mesocosms received no CO$_2$ addition but were treated otherwise the same, representing the present day carbonate chemistry of the fjord (∼ 185 µatm at the start of the experiment). The addition of CO$_2$ was gradual between day t = 1 and t4 by pumping CO$_2$-enriched seawater through a dispersal device which was lowered to about 13 m depth and gently pulled up several times, resulting in an even distribution throughout the water column. The other 7 mesocosms were enriched with CO$_2$ over a period of several days (t = 1 to t4) in varying amounts, resulting in a range of initial pCO$_2$ levels from ∼ 270 to ∼ 1420 µatm (corresponding to pH$_T$ values of 8.18 to 7.51). Two weeks into the experiment (t13), inorganic nutrients were added to the originally nutrient-poor water in order to stimulate primary production (5 µM nitrate, 0.3 µM phosphate and 2.5 µM silicate for all mesocosms, to simulate the upwelling of deeper, nutrient-rich water to the nutrient-depleted surface water). The one month experiment showed 4 phases: phase 0 represents the period from closing of the mesocosms to the end of the CO$_2$ manipulations (t = 7 to t3), phase 1 corresponds to the period after CO$_2$ manipulation until the addition of inorganic nutrients (t4 to t12), phase 2 stands for the period after nutrient addition and until the second chlorophyll minimum (t13 to t21; Schulz et al., 2013), and phase 3 includes the final period until the end of the experiment (t22 to t30). Throughout this study the data are presented using 3 colors (blue, grey and red), representing low, intermediate and high pCO$_2$ additions. The low pCO$_2$ addition group contains mesocosms 3, 7 and 2; the intermediate group consists of mesocosms 4, 8 and 1; and the high pCO$_2$ addition group is made up of mesocosms 6, 5 and 9.

Collective sampling was performed daily in the morning using an integrated water sample (0–12 m). From this water subsamples were obtained for counting phytoplankton, heterotrophic prokaryotes (HP) and viruses. Besides this, we sampled 2–3 times per week for the microzooplankton grazing incubations using a gentle vacuum-driven pump system. Samples were protected against daylight by black plastic bags. In the laboratory the samples were maintained and processed (for grazing assay and counting) at in situ temperature and dimmed light. The abundances of phytoplankton were determined on fresh samples. HP and viruses were fixed for 30 min at 7°C with glutaraldehyde (25 %, EM-grade) at a final concentration of 0.5 % before snap freezing in liquid nitrogen and storage at −80°C until analysis.

2.2 Microbial abundances

The microbes were enumerated using a bench-top Becton Dickinson FACSCalibur flow cytometer (FCM) equipped with a 488 nm argon laser. The photautotrophic cells (<20 µm) were counted fresh and discriminated by their pigment autofluorescence (Marie et al., 1999). Only eukaryotic photosynthetic organisms were detected. Based on their autofluorescence and side scatter signal, the phytoplankton community could be divided into 6 clusters: picophytoplankton I and II, nanophytoplankton I and IV. Nanophytoplankton III and IV displayed higher side scatter signals than other clusters similar in size. Average cell size of the different clusters was determined by serial gravity filtration of a very small volume of sample (<10 mL) through different
Results

3.1 Phytoplankton abundances and net growth

The abundance of each phytoplankton cluster is presented in Fig. 1. Total phytoplankton abundance was strongly dominated by picophytoplankton I (Fig. 1a), generally making up more than 90 % of the total count, except for r1 to r11 when their share declined to 60 % due to a bloom of nanophytoplankton (Fig. 1c). Picophytoplankton I showed a steady increase until t18, after which the bloom declined sharply (Fig. 1a). The net growth rates during phase 1, derived from the standing stock and calculated using the natural logarithm, were on average 0.14 ± 0.03, 0.15 ± 0.03 and 0.17 ± 0.02 for the low, intermediate and high pCO2 mesocosms, respectively. During phase 2, after inorganic nutrient addition, the net growth rates of the picophytoplankton I were slightly higher, ranging from 0.14 to 0.24 d⁻¹ (no significant correlation with pCO2, however). Highest net peak abundances, i.e. 1.1–1.5 × 10⁵ cells mL⁻¹, were recorded for the high pCO2 mesocosms 5, 6, and 9 (ANOVA, p < 0.000). Picophytoplankton I increased until t7 at a net growth rate between 0.30 and 0.38 d⁻¹ (Fig. 1b). The highest abundances of 3.5–4.9 × 10⁵ cells mL⁻¹ during phase 1 were observed for the intermediate and low pCO2 mesocosms (ANOVA, p < 0.000).

Nanophytoplankton I increased steeply from the start of the experiment, growing at net growth rates between 0.51 and 0.55 d⁻¹ (with the exception of mesocosm 4 showing a growth rate of 0.42 d⁻¹), and peaked at t4–6 with maximum abundances between 1.6 and 1.8 × 10⁶ cells mL⁻¹ (Fig. 1c). A second increase was found during the last week of the experiment with highest abundances typically in the higher pCO2 mesocosms. Nanophytoplankton II were present in
low numbers (generally below 150 cells mL$^{-1}$) and could no longer be detected in either the mesocosms or the natural fjord water from \(t10\) onwards (Fig. 1d). Around the same time (\(t6\)), a new phytoplankton population, nanophytoplankton III, appeared that was undetectable until then (Fig. 1e). It remained present throughout the duration of the experiment at a rather stable concentration (<150 cells mL$^{-1}$), with significantly higher algal numbers for the high \(p\)CO$_2$ mesocosms during phase 2 and 3 (ANOVA, \(p < 0.000\)). Nanophytoplankton IV showed similar algal concentrations, with significantly higher abundances during phase 3 in the high \(p\)CO$_2$ addition mesocosms (Fig. 1f; ANOVA, \(p < 0.000\)).

Figure 2 shows the phytoplankton (<20 µm) cellular carbon (algal POC). The first maximum at \(t5\) is due to the peak in nanophytoplankton around that time (Fig. 2a). Despite their relatively low numbers, their larger size results in algal POC concentrations similar to or higher than that of the more abundant (up to 70-fold higher cell abundances) but very small picoeukaryotic algae that are responsible for the second maximum of algal POC (Fig. 2b).

### 3.2 \(p\)CO$_2$-related phytoplankton trends

As expected, all mesocosms resembled each other for microbial abundances during the initial phase 0 (\(t7\) to \(t3\)). Shortly after completing CO$_2$ additions (at \(t7\); phase 1), the standing stock of picophytoplankton II in the three high \(p\)CO$_2$ mesocosms, 5, 6 and 9, showed a negative response (Fig. 3a). This response was, however, not maintained and even reversed after nutrients were added at \(t13\). It has to be noted that picophytoplankton II was made up by 3 subpopulations that were hard to separate. A weak positive correlation with \(p\)CO$_2$ during phase 1, thus prior to nutrient addition, was only observed for picophytoplankton I (\(y = 16.6x + 20963, r^2 = 0.516\)). Upon the addition of inorganic nutrients (phase 2),
and $-0.19 \pm 0.07 \text{d}^{-1}$ for $p\text{CO}_2 \leq 600 \mu\text{atm}$; Fig. 5c). At the end of the experiment ($t_{27}$), the picophytoplankton I show a negative correlation with $p\text{CO}_2$, however, we argue below that at this stage into the experiment such a correlation is the indirect effect of viral lysis and grazing dynamics.

### 3.3 Phytoplankton viruses and microzooplankton grazing

The viral community could be discriminated into 5 distinct clusters, of which two (V4 and V5) are considered most likely algal viruses (Fig. 6; Brussaard and Martínez Martínez, 2008). The abundance of viruses V4 started to increase upon the development of the nanophytoplankton I bloom and showed its strongest increase concomitantly with the decline of this phytoplankton group (Figs. 1c and 6a). Moreover, V5 increased sharply the moment the bloom of picophytoplankton I declined ($t_{18}$; Figs. 1a and 6b), indicating a regulating role of viruses on the dynamics of also this phytoplankton population.

Microzooplankton grazing rates (Table 1) showed high grazing on picophytoplankton I during the first days of the experiment (phase 0; around $0.45 \pm 0.14 \text{d}^{-1}$). Grazing rates dropped after phase 0 down to $0.1–0.2 \text{d}^{-1}$. No correlation with $p\text{CO}_2$ was observed. For unknown reasons, the dilution assay during the first half of the experiment (phase 0 and 1) did not provide good grazing data for the picophytoplankton II. During the second half of the experiment (phase 2 and 3), grazing on picophytoplankton II was substantial (on average $0.39 \pm 0.30 \text{d}^{-1}$), and although grazing rates seem somewhat higher at high $p\text{CO}_2$, the low number of grazing experiments does not validate a statistically significant conclusion. Microzooplankton grazing on nanophytoplankton I was on average $0.44 \pm 0.36 \text{d}^{-1}$ throughout the experiment, with high rates during phase 2 ($0.81 \pm 0.26 \text{d}^{-1}$ vs. $0.28 \pm 0.40 \text{d}^{-1}$ for other phases).

### 3.4 HP and viral dynamics

The abundance of HP in all mesocosms dropped abruptly during the first week (by $1.5 \times 10^6 \text{mL}^{-1}$; Fig. 7a), which was fully due to the decline in the high nucleic acid (HDNA) HP population. The % HDNA HP dropped from around 80 to 40–55 % at $t_7$ (Fig. 7b). Viruses increased in abundance around $1 \times 10^7 \text{mL}^{-1}$ (Fig. 8a). The concomitant decline of HP and increase in viruses resulted in a steeply increasing total virus to prokaryote ratio (VPR), from 25 up to 150 (Fig. 8b). After $t_7$ the total HP community increased steadily again (Fig. 7a). The % HDNA HP also increased again up to a maximum of 70 % at $t_{14}$ (Fig. 7b), after which it decreased steadily, resulting in an equal contribution of HDNA and LDNA HP towards the end of the experiment (phase 3). HP net growth rates, derived from $t_5$ to the end of the experiment, correlated negatively to $p\text{CO}_2$, which consequently resulted in a negative correlation between HP abundance at $t_{28}$...
Table 1. Microzooplankton grazing rates on phytoplankton ($d^{-1}$) for 3 mesocosms representing low, intermediate (mid) and high $p$CO$_2$ levels. Mesocosm M3 (low, i.e. present time $p$CO$_2$) was sampled as reference to mesocosm M5 and M8. Failed assays for specific phytoplankton clusters are indicated by –. Standard error between parentheses. Statistical significance indicated by * $p = 0.1–0.05$; ** $p = 0.05–0.01$; *** $p = 0.01–0.001$; and **** $p = 0.001–0$.

<table>
<thead>
<tr>
<th>Mesocosms ($p$CO$_2$)</th>
<th>Picophytoplankton I</th>
<th>Picophytoplankton II</th>
<th>Nanophytoplankton I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Low 3</td>
<td>Mid 8</td>
<td>High 5</td>
</tr>
<tr>
<td>1</td>
<td>0.62 (0.15)*</td>
<td>0.37 (0.15)</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>0.50 (0.09)**</td>
<td>0.31 (0.07)**</td>
<td>0.07</td>
</tr>
<tr>
<td>7</td>
<td>0.09 (0.14)</td>
<td>0.10 (0.05)*</td>
<td>0.07</td>
</tr>
<tr>
<td>12</td>
<td>–</td>
<td>0.02 (0.03)</td>
<td>0.05 (0.05)</td>
</tr>
<tr>
<td>20</td>
<td>0.04 (0.03)</td>
<td>0.15 (0.05)**</td>
<td>0.39 (0.12)**</td>
</tr>
<tr>
<td>22</td>
<td>0 (0.05)</td>
<td>0.12 (0.04)**</td>
<td>0.04 (0.24)</td>
</tr>
<tr>
<td>24</td>
<td>0.19 (0.06)*</td>
<td>0.09 (0.03)***</td>
<td>0.36 (0.06)*****</td>
</tr>
<tr>
<td>28</td>
<td>0.01 (0.05)</td>
<td>0.18 (0.04)****</td>
<td>0.31 (0.07)***</td>
</tr>
</tbody>
</table>

Fig. 3. Phytoplankton cell abundance versus actual $p$CO$_2$ for picophytoplankton II on t7 (a), picophytoplankton I on t18 (b), picophytoplankton II on t18 (c), and nanophytoplankton I on t18 (d). For (b–d) the CO$_2$ concentrations plotted were the averages of t8 to t18. For color and symbol coding, see Fig. 1. Linear regression statistics provided in plots.

and $p$CO$_2$ levels (Fig. 7c). No such trend was found for virus abundances. However, the slopes of the linear regression of viruses versus HP per mesocosm plotted against $p$CO$_2$ levels resulted in a linear correlation (Fig. 8c), which suggests a stronger impact of viruses on HP dynamics at higher $p$CO$_2$.

4 Discussion

The Arctic Ocean ecosystem is particularly vulnerable to OA related alterations due to the relatively high CO$_2$ solubility and low carbonate saturation states of its cold surface waters. Thus far, however, there is only little known about the consequences of OA on the base of the food web. In a mesocosm CO$_2$-enrichment experiment (overall CO$_2$ levels ranged from $\sim$ 180 to 1100µatm) in Kongsfjorden off Svalbard, we studied the consequences of OA on a natural pelagic microbial
community. Overall, our results show that elevated CO₂ stimulated the growth of the smallest size range of primary producers, thereby substantially affecting the structure and functioning of the pelagic food web in the future.

4.1 Phase 0

The initial phase of the experiment showed an increase in standing stock of the phytoplankton clusters picophytoplankton II (2–3 µm diameter) and nanophytoplankton I (3–8 µm diameter). For nanophytoplankton I the gross growth rate based on the net growth rates and grazing rate (dominant loss factor) compares well with the 0.87 d⁻¹ gross growth rate obtained by ¹³C-uptake for a phytoplankton group that included nanophytoplankton (De Kluijver et al., 2013). Inorganic nutrients were depleted due to the spring bloom preceding our experiment, and thus, the smaller-sized phytoplankton had a competitive advantage by growing on remineralised ammonia and inorganic phosphate (Schulz et al., 2013). Furthermore, utilization of organic nutrients and mixotrophy may also have allowed growth by these phytoplankton groups (Schulz et al., 2013). Light microscopy data showed that nano-sized (around 5 µm cell diameter, thus potentially belonging to nanophytoplankton I) *Pseudopedinella* sp., a known mixotroph, peaked around t4 (A. Stuhr, personal communication, 2012). Another mixotroph chrysophyte, i.e. *Ochromonas* sp., also developed during phase 0 and into phase I (A. Stuhr, personal communication, 2012). However, expressed in organic carbon, these chrysophytes were not the dominant nanoeukayotes. Phytoplankton pigment analysis showed dominance of Prasinophyceae and Haptophyceae (Schulz et al., 2013), which are known to consist largely of pico- and nanophytoplankton, respectively. Members of Prasinophyceae have been found to be the most abundant picophytoplankton (Zhu et al., 2005), although recently Cuvelier et al. (2010) found that picoprymnesiophytes (Haptophyceae) may also contribute to global picophytoplankton

![Fig. 4. Total algal POC versus pCO₂. The CO₂ concentrations plotted were the averages of t8 to t18. For color and symbol coding, see Fig. 1. Linear regression statistics provided in plots.](image)

![Fig. 5. Specific growth rates determined from the net phytoplankton abundances versus pCO₂. Nanophytoplankton I from t6–t13 (a), picophytoplankton II from t7 to t22 (b), and picophytoplankton I from t18–t26 (c). A negative growth rate indicates cell loss. The CO₂ concentrations plotted were the averages for the specific time periods indicated. For color and symbol coding, see Fig. 1. Linear regression statistics provided in plots.](image)
found to depend on host metabolism (e.g. HP host generation times; see review by Weinbauer, 2004), and HDNA HP have been reported to represent the metabolically active members of the microbial community (Lebaron et al., 2001). However, some contrasting studies did show that LDNA HP can also be an active part of the microbial population (Zubkov et al., 2001; Brussaard et al., 2005; Wang et al., 2009). Preferential grazing on HDNA HP has been implied (Gasol et al., 1999; Vaqué, 2001), although cell size instead of metabolic activity might have been the underlying reason. Still, the selective loss of HDNA HP due to viral lysis has not been, to our knowledge, yet reported.

### 4.2 Phase 1

This phase is the only period in which the sole effect of the CO₂ treatment could be determined as nutrients were not added yet (t13). Phase 1 was dominated in algal POC by nanophytoplankton, peaking around t5. The study by Schulz et al. (2013) showed that the Haptophyceae was the principal algal class. Although no obvious correlation between the peak abundances and pCO₂ was recorded, we did find a significant negative correlation of the net loss rates (obtained from the decline in net abundances) with pCO₂. The relatively high net loss rates for nanophytoplankton I in the low CO₂ mesocosms (0.30 d⁻¹ as compared to 0.20 d⁻¹ for the high CO₂ mesocosms) corresponded well with the higher microzooplankton grazing rates found at the low CO₂ level (0.35 d⁻¹ vs. 0.20 d⁻¹ at high pCO₂). OA has been reported not to affect microzooplankton abundance or grazing activity (Suffrian et al., 2008), however in contrast to our study, neither phytoplankton composition nor succession differed between their CO₂ treatments (Riebesell et al., 2007). Still, OA during the present study did not significantly affect the diversity of the protozooplankton (ciliates and heterotrophic dinoflagellates), indicating to a high tolerance of this Arctic protozooplankton community to changes in pCO₂/pH (Aberle et al., 2013). Although grazing on nanophytoplankton I matched the net loss rates, viral lysis also accounted for substantial loss (based on increase of virus V4 and concurrent decline of nanophytoplankton I), which implies that the nanophytoplankton still grew but that the higher loss rates resulted in the decline of the algal bloom. De Kluijver et al. (2013) indeed still recorded ¹³C utilization by the nanophytoplankton during t5 to t10.

Assuming half the net decline in nanophytoplankton I abundance to be due to viral lysis, we found viral burst sizes (i.e. the number of newly produced viruses released per lysing host cell; derived from the net increase in V4 abundance and half of the net decline in algal host abundance) of 540–764 for the different mesocosms. The literature shows comparable burst sizes for nanophytoplankton host-virus model systems from the Haptophyceae class, e.g. 350–600 for *Phaeocystis globosa* and *P. pouchetii* and

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**Fig. 6.** Temporal dynamics of virus cluster V4, indicative of larger algal viruses infecting nanophytoplankton I (a), and V5, most likely infecting picophytoplankton I (b). For color and symbol coding, see Fig. 1. Note the different y-axis scales.
The utilization of the organic matter released by viral lysis of the HP (phase 0) and the nanophytoplankton (phase 1) resulted in a steady increase in HP abundance after t5. Based on half of the nanophytoplankton lost due to viral lysis, a HP carbon conversion of 12 fg C cell$^{-1}$ (Fukuda et al., 1998), a HP growth efficiency of 30% (Motegi et al., 2013) and taking 50% mortality of the heterotrophic HP into account (Motegi et al., 2013), we estimated that the cellular carbon released by viral lysis (about 30 µg C L$^{-1}$) sustained about a third of the gross HP carbon demand during phase 2. Extracellular enzyme activities and bacterial protein production indeed increased already during phase 0 and peaked during t5 to t10 (Piontek et al., 2013), concomitantly with the virally induced decline in nanphytoplankton I.

The abundance of picophytoplankton II peaked at t7 but was significantly (ANOVA p < 0.000) lower for the high CO$_2$ mesocosms (∼650–1100 µatm). It is unclear whether these algae became negatively affected in their growth at the higher future CO$_2$ levels or the loss rates for the high pCO$_2$ mesocosms were enhanced (grazing estimates on picophytoplankton II during this phase failed for the low pCO$_2$ mesocosm). Hopkins et al. (2010) reported a similar finding for a mesocosm experiment in a fjord in Norway, showing lower
abundances of large picophytoplankton under high $p$CO$_2$ (750 µatm) than in the present day CO$_2$ enclosure. Still, picophytoplankton II formed a complex cluster with 2–3 subpopulations that could not be separated from each other. A shift to one subpopulation in response to elevated $p$CO$_2$ may also have been responsible for the obtained results.

During phase 1 the abundances of the small-sized picophytoplankton I (average 1.2 µm cell diameter) increased steadily at 0.15 ± 0.03 d$^{-1}$ (not significantly different from each other). Correcting for grazing loss, the gross growth rates were overall 0.1 d$^{-1}$ higher. Already prior to nutrient addition, a weak positive correlation between abundance and $p$CO$_2$ emerged, suggesting that elevated CO$_2$ stimulated the growth of these very small-sized phytoplankton (0.8–2 µm). The trend developed strongly into phase 2 (see Sect. 4.3), emphasizing that elevated CO$_2$ stimulates growth and nutrient utilization (Schulz et al., 2013) leading to enhanced production of the smallest size range of primary producers.

4.3 Phase 2

Phase 2 represents the initial period upon the addition of inorganic nutrients, allowing for potential growth of larger-sized phytoplankton. Elevated CO$_2$ in combination with nutrient addition stimulated the growth of the smaller-sized phytoplankton, as shown by the positive correlation of algal POC at t18 with $p$CO$_2$. These results were supported by the higher uptake rates of the added inorganic nutrients for the highest $p$CO$_2$ mesocosms (Schulz et al., 2013). Interestingly, the prevailing group of algae during this period was still the picophytoplankton (particularly cluster I) and to a lesser extent the nanophytoplankton. Algal pigment analysis and light microscopic screening indicated that Prasinophyceae was the dominant algal class, and larger-sized phytoplankton like diatoms were largely absent still (Schulz et al., 2013).

Our results clearly show that the very small-sized picophytoplankton became dominant under increased CO$_2$ levels, indicating that this group does not or still inefficiently applies carbon concentrating mechanisms (CCMs). Diffusion of CO$_2$ is typically not expected to limit photosynthesis in the very small-sized phytoplankton (Giordano et al., 2005), although CCMs were identified for the picophytoplankter *Micromonas pusilla* (Worden et al., 2009). Yet, despite their small size (and thus large surface to volume ratio) their diffusive CO$_2$ uptake seems insufficient and their CO$_2$ sensitive photosynthesis benefitted from elevated $p$CO$_2$. Only a small response to OA (e.g. 10%; The Royal Society, 2005 and references therein) can still result in dominance over time under the assumptions that the loss factors (grazing, viral lysis) do not show direct responses to elevated $p$CO$_2$ and other growth variables are not limiting the growth. We found no evidence that grazing or viral lysis rates were directly affected by elevated CO$_2$ levels, and nutrients and irradiance were non-limiting at the time. The higher abundances of picophytoplankton I at high $p$CO$_2$ resulted thus from somewhat enhanced growth rates. Paulino et al. (2008) reported an increased abundance of picophytoplankton in a high CO$_2$ mesocosm; however, this was towards the end of the experiment when all nutrients were depleted. During another Norwegian fjord mesocosm experiment, still nutrient-replete, the total number of small-sized picophytoplankton did not change under increased $p$CO$_2$ (750 µatm; Hopkins et al., 2010; Newbold et al., 2012). The abundances based on quantitative PCR of specifically the picophytoplankter *Micromonas pusilla*, nevertheless, enhanced significantly (Meakin and Wyman, 2011). A similar trend was observed a few years earlier at the same location, time of the year and experimental set-up, this time based on a *M. pusilla*-like flow cytometric signature (Engel et al., 2008).

High grazing pressure kept the abundances of nanophytoplankton I under control during phase 2 (on average 0.81 ± 0.26 d$^{-1}$). Our data suggest that grazing was not affected by $p$CO$_2$ (a relationship with $p$CO$_2$ could not be tested for shortage of grazing rates in the high CO$_2$ mesocosms) and viral lysis was not a substantial loss factor at the time, implying that the higher standing stock of the nanophytoplankton I under elevated CO$_2$ was also due to higher growth rates (as for the picophytoplankton I).

For the picocoeukayotes I viral lysis was the dominant loss factor (0.2–0.3 d$^{-1}$ while grazing <0.1 d$^{-1}$), with the steepest increase of virus population V observed for the highest $p$CO$_2$ mesocosms (in agreement with the strongest decline of picophytoplankton I at these CO$_2$ levels). Estimated burst sizes ranged between 119 and 245 (derived from the net increase in V5 abundance and net decline in algal host abundance, matching the published values for prasinophyte picophytoplankton host-virus model systems (e.g. 72–360 for *Micromonas pusilla*; Waters and Chan, 1982; Baudoux et al., 2008). The resultant release of cellular organic carbon due to viral lysis of the picophytoplankton I increased with CO$_2$ level, i.e. 7, 10 and 20 µg C L$^{-1}$ for the low, intermediate and high $p$CO$_2$ mesocosms. Based on the HP net growth rate of 0.2 × 105 cells mL$^{-1}$ d$^{-1}$, a growth efficiency of 30% and low mortality (Motegi et al., 2013), viral lysis accounted for 15 to 42% of the gross HP C demand.

4.4 Phase 3

Despite the enhanced release of the lysing algal cells in the highest $p$CO$_2$ mesocosms that continued into phase 3, the HP standing stock was significantly lower at elevated CO$_2$ (ANOVA, $p < 0.000$). We speculate that this may have been
due to higher viral lysis rates as a result of higher VPR and therefore encounter rate.

During this last phase of the experiment, picophytoplankton were still most abundant but the biomass share of the nanophytoplankton became more important, equaling picophytoplankton. Schulz et al. (2013) showed that the Haptophyceae, containing many nanoeukaryotic phytoplankton species, increased in abundance during this phase. The same authors showed, furthermore, that larger-sized photoautotrophs such as the Dinophyceae became an important group and were positively correlated to elevated CO$_2$. In contrast, the growth of diatoms and Chlorophyceae was reduced at elevated CO$_2$, most likely due to diminished nutrient availability (inorganic nutrients became depleted during the second half of Phase 3). Species in these phytoplankton classes often have efficient CCMs that allow for optimal photosynthesis and growth at ambient (and past) CO$_2$ concentrations and, therefore, are not (or only minimally) affected by increased CO$_2$ levels (Giordano et al., 2005; Engel et al., 2008; Rost et al., 2008).

5 Conclusions

The most prominent finding of our study is the evident impact of OA on the composition and growth of the Arctic phytoplankton community, i.e. the picoeukaryotic photoautotrophs and to a lesser extent the nanophytoplankton prospered. This mesocosm experiment took into account at least 4 of the 7 future research recommendations stated by Rost et al. (2008), namely it mimicked the environmental conditions as closely as possible, was community based (microbial food web), involved the interactive effects of multiple environmental variables in combination with CO$_2$, and tried to unravel the underlying mechanisms for the observed responses by taking into account loss factors such as grazing and viral lysis. These aspects in combination with our focus on the smaller-sized phytoplankton contributed largely to a more detailed and inclusive understanding of ecological responses to changes in CO$_2$.

A shift towards the smallest phytoplankton as a result of OA will have direct consequences for the structure and functioning of the pelagic food web and thus for the biogeochemical cycles. Due to the very small cell size, one or two additional trophic levels of smaller grazers are evident, which will, as compared to the more traditional food chain (large phytoplankton, suspension-feeding zooplankton and fish), result in reduced transfer of matter and energy to higher predators and increased importance of the microbial food web. Furthermore, the dominant pico- and nanophytoplankton groups were found prone to viral lysis, thereby shunting the carbon that enters the biological pool via photosynthesis from carbon accumulation in living organisms into the dissolved pools of organic carbon (Suttle, 2007). Microbial communities incorporate these pools, thereby converting much of it to CO$_2$ again by respiration and subsequently influencing the efficiency of the biological pump (Brussaard et al., 2008).

The here discussed ecological changes by OA do not stand alone as global climate change impacts the polar waters also through temperature rise, shift in wind, precipitation and circulation (Schofield et al., 2010). Phytoplankton growth-relevant factors such as light, $p$CO$_2$, temperature, nutrient availability and salinity are all under change due to global warming. The increment in surface water temperature will reduce the solubility of CO$_2$ in the Arctic seawater, and the mean cell size of the phytoplankton community (Daudefresne et al., 2009). Moreover, increased surface water temperature and glacier and sea-ice melt will strengthen vertical stratification, which in the Canadian Arctic waters has been reported to stimulate picophytoplankton and reduce nanophytoplankton abundance (Li et al., 2009). Thus, in the global climate-driven changing Arctic Ocean, the smallest phytoplankton are expected to flourish as a general result of OA that is further reinforced in summer by increased temperature-imposed vertical stratification.

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