**Mixing of water masses caused by a drifting iceberg affects bacterial activity,
community composition and substrate utilization capability in the Southern Ocean**

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Originality-Significance Statement:

The extensive regional warming of Antarctica has already caused rapid retreat of ice-shelves and elevated production of free-drifting icebergs. These consequences of climate change may affect local and even global biogeochemical cycles. Here we studied the influence of an iceberg on bacterial activity and community composition, and on the ability of the bacterial community to exploit carbon substrates during the austral summer in the coastal Southern Ocean. Through in situ measurements and short/long term incubations of bacterioplankton with specific carbon substrates we show that a drifting iceberg dramatically influences local hydrography, and bacterial biomass, activity, community composition and carbon substrate processing. These findings indicate that the future increasing number of free-drifting icebergs will significantly affect carbon sequestration in the highly productive Southern Ocean, and possibly in polar regions in general.

Summary

The number of icebergs produced from ice-shelf disintegration has increased over the past decade in Antarctica. These drifting icebergs mix the water column, influence stratification and nutrient condition, and can affect local productivity and food web composition. Data on whether icebergs affect bacterioplankton function and composition are scarce, however. We assessed the influence of iceberg drift on bacterial community composition and on their ability to exploit carbon substrates during summer in the coastal Southern Ocean. An elevated bacterial production and a different community composition were observed in iceberg-influenced waters relative to the undisturbed water column nearby. These major differences were confirmed in short-term incubations with bromodeoxyuridine followed by CARD-FISH. Furthermore, one-week bottle incubations amended with inorganic nutrients and carbon substrates (a mix of substrates, glutamine, N-acetylglucosamine, or pyruvate) revealed contrasting capacity of bacterioplankton to utilize specific carbon substrates in the iceberg-influenced waters compared to the undisturbed site. Our study demonstrates that the hydrographical perturbations introduced by a drifting iceberg can affect activity, composition, and substrate utilization capability of marine bacterioplankton. Consequently, in a context of global warming, increased frequency of drifting icebergs in polar regions holds the potential to affect carbon and nutrient biogeochemistry at local and possibly regional scales.

Introduction

Polar oceans play a significant role in the global carbon cycle and account for 4 to 11% of the global carbon export to the ocean interior (Laws *et al.*, 2000). Extensive regional warming of surface air temperatures in Antarctica (Thompson and Solomon, 2002; Steig *et al.*, 2009), combined with increased ocean warming (Martinson, 2012; Jacobs *et al.*, 2012), has caused rapid retreat of ice-shelves and glaciers (Thomas *et al.*, 2004; Cook *et al.*, 2005) resulting in an increased number of free-drifting icebergs (Scambos *et al.*, 2000). Wind-driven motion of these floating masses of ice that often reach several hundred meters deep, causes vertical water mixing in their vicinity and wake (Helly *et al.*, 2011) leading to upwelling of nutrient-rich deep water. Combined with the release of micronutrients from the melting ice (Raiswell *et al.*, 2008; Lin *et al.*, 2011), this enrichment can cause enhanced local primary production and elevated zooplankton biomass (Smith *et al.*, 2007; Schwarz and Schodlok, 2009; Kaufmann *et al.*, 2011), which may double the carbon export compared to regions less influenced by icebergs (Smith *et al.*, 2011). Hence, drifting icebergs have the potential to significantly alter carbon sequestration in the polar regions.

In the coastal Ross Sea, Antarctica, heterotrophic bacteria process 20-50% of the seasonal primary production in the surface waters (Ducklow, 2003). Since bacterial abundance and activity are controlled mainly by substrate availability, grazing and virus induced mortality, enhanced primary production and food-web activities associated with an increased number of icebergs would presumably affect bacterial processing of dissolved organic carbon (DOC) and thereby also the fate of carbon and nutrients in the region.

However, earlier studies found no significant effects of icebergs on bacterioplankton during winter (Murray *et al.*, 2011); nevertheless, it is plausible that a bacterial response would be more pronounced during the much more productive summer season. Indeed, certain areas of the Southern Ocean, such as polynyas, undergo temporally restricted but intense pulses of

primary production in summer and bacterioplankton respond rapidly to this environmental change (Ducklow and Yager, 2007).

The marine DOC pool is a complex mix of substrates that is partly available for bacteria. Cold-adapted heterotrophic bacteria are known to respond to natural and experimental substrate input in high-latitude seas (e.g. Yager and Deming, 1999; Abell and Bowman, 2005; Simon *et al.*, 2012). Uptake and utilization of DOC can be carried out by bacterial taxa specializing in using certain substrates (Cottrell and Kirchman, 2000; Riemann and Azam, 2002; Gomez-Consarnau *et al.*, 2012), by more generalist taxa with the capacity to exploit a wide range of carbon compounds (Button *et al.*, 2004; Mou *et al.*, 2008), or both. Either way, changes in the composition of the bacterioplankton community will likely affect carbon processing and nutrient recycling. In the context of future increased frequencies of icebergs in the Southern Ocean (Scambos *et al.*, 2000) it is therefore of interest to examine not only bacterioplankton abundance and productivity, but also community composition and patterns of substrate utilization in the wake of icebergs. As part of a range of studies reporting physical and biological effects of a drifting iceberg in the Amundsen Sea Polynya (ASP) within the coastal Southern Ocean (Alderkamp *et al.*, 2015; Randall-Goodwin *et al.*, 2015; Sherrell *et al.*, 2015; Wilson *et al.*, 2015; Williams *et al.*, 2016), we studied effects of the iceberg on bacterioplankton. The goal of this research was to study if hydrographic perturbations caused by a drifting iceberg could locally enhance bacterioplankton production and change bacterial community composition while also altering the bacterial capacity to use specific dissolved organic compounds for growth.

Results

The effects of a drifting iceberg on the hydrography were assessed by measurements from several stations in its wake (Randall-Goodwin *et al.*, 2015). The consequences for

bacterioplankton abundance, production and community composition were then compared for an “Iceberg station” close to the iceberg, and a “Control station” representing a station not affected. Moreover, at these two stations one-week bottle incubations examined the ability of the bacterioplankton to utilize carbon substrates, and short term incubations examined actively growing cells within key bacterial groups.

In situ conditions in the vicinity and wake of the iceberg

The Iceberg station (s57.26) and other iceberg-influenced stations (s57.02 and s57.11) were located in an area downstream and closest to the studied iceberg (see Randall-Goodwin *et al.*, 2015). The control station and non-influenced stations were located away from the studied iceberg (Control stations s13 and s35) or upstream (s57.35). Pronounced hydrological effects were observed at the Iceberg station and the other iceberg-influenced stations where entrainment of mCDW (modified Circumpolar Deep Water) and glacial meltwater into the winter mixed layer was evident (Fig. 1B,C). Further details on these hydrodynamics are available in Randall-Goodwin *et al.* (2015). They report that it was not possible to determine whether the melt water contributions to the mixed layer originated from the iceberg itself or from the nearby ice-shelf. Moreover, elevated iron concentrations around the wake of the iceberg from 100 m to the bottom (Sherrell *et al.*, 2015) and DIN (Dissolved Inorganic Nitrogen) drawdown (Yager *et al.*, 2016; Williams *et al.*, 2016) reflect mixing of the mCDW and WW (Winter Water), consistent with the water mass mixing induced by the iceberg keel (Randall-Goodwin *et al.*, 2015). The Control station, ~20.7 km away from the iceberg, showed a stratified water column structure typical for the ASP (Yager *et al.*, 2012). While similar levels of Chl. *a* were observed at the DCMs (Deep Chlorophyll Maximum) of the Iceberg and Control stations (Table 1), the depth-integrated Chl. *a* at the Iceberg station was slightly higher (Alderkamp *et al.*, 2015). DOC concentrations were higher ($+9 \pm 2.8 \mu\text{mol L}^{-1}$

¹) at both depths of the Iceberg station (Table 1). Integrated over 100 m, bacterial production was highest at the Control station (William *et al.*, 2016). Nevertheless, the bacterial production per cell at the Iceberg station was 2-fold higher at the DCM and 50-fold higher for the deeper waters (Table 1).

Substrate utilization experiments: bacterial abundance and production

Bacterial abundance and production increased in all treatments after an initial lag phase of 96 h (Fig. 2). For the DCM waters, minor differences were observed among substrate treatments and control treatments for both Control and Iceberg stations, respectively. In the Iceberg DCM water, although bacterial production and abundance did not show significant differences between treatments, bacterial abundances in the treatments with pyruvate and the mix of substrates were 1.5-fold lower compared to the control treatments. The abundance in the treatment with NAG (N-acetylglucosamine) was 1.6-fold higher than the control treatments. All initial time points with DCM water from both stations showed 5-10-fold higher bacterial abundance and production than those of deep water (Fig. 2). After 168 h, the bacterial abundance and production in treatments with water from the Iceberg station were about 5-fold and 10-100-fold higher, respectively, than those from the Control station.

Significant differences in the growth responses to substrate amendments were observed in Deep water incubations (Fig. 2C). Inorganic nutrients stimulated a growth response in the deep waters at the Control station (Fig. 2A); bacterial abundance was significantly higher in incubations with inorganic nutrient additions (ANOVA, $p = 0.012$). Treatments amended with glutamine or the mix of substrates showed a significantly lower growth compared to the controls (ANOVA, $p = 0.008$; $p = 0.009$, respectively). Pyruvate and NAG stimulated growth in the Iceberg Deep water; bacterial abundance and production were about twice as high as the control in treatments with pyruvate (ANOVA, $p = 0.004$ and 0.010 , respectively) or NAG

(ANOVA, $p = 0.015$ and 0.005 , respectively). Very low production response (5-10 fold lower than the average of the other treatments) was detected in treatments with mixed substrates or with glutamine; presumably due to use of the same cellular transporters for leucine and glutamine uptake.

● *Bacterial community composition*

A total of 264,278 bacterial 16S rRNA gene sequences remained after quality control, yielding on average 6,191 reads per sample and a total of 1,468 OTUs in the whole dataset (at 99 % similarity). Rarefaction curves normalized to 2,371 reads per sample leveled off at 70 to 180 OTUs for most samples.

In situ: bacterial community composition at iceberg and control stations

Community richness (Chao1) *in situ* was higher at the Control station, especially in the deep water, compared to the Iceberg station (Deep water richness: 520 and 303 for the Control and Iceberg station, respectively; Table S1). However, the Shannon diversity index was highest in the deep water from the Iceberg station (Deep water diversity: 4.4 and 5.14 for the Control and Iceberg station, respectively).

Beta diversity analyses (based on weighted UniFrac distances) showed that *in situ* communities (inocula) from the Control DCM and deep waters and Iceberg DCM water clustered together in the dendrograms (Fig. 3). These bacterioplankton communities were dominated by phylum Bacteroidetes (45 - 56% of the reads), and classes γ - Proteobacteria (35 - 41%) and α -Proteobacteria (6 – 11%; Fig. 4A). The three dominant phyla (and proteobacteria classes, hereafter referred as phyla) in the entire dataset were mainly represented by OTUs related to *Polaribacter* (Bacteroidetes), Oceanospirillales (mainly *Oleispira*) and the OMG SAR92 (γ - Proteobacteria), and SAR11 (α -Proteobacteria; data not shown, Fig. 4B).

The deep community at the Iceberg station was different from the deep community at the Control station (Fig. 4A) and was dominated by OTUs related to δ -Proteobacteria (33%), γ -Proteobacteria (22%), α -Proteobacteria (15%), and Bacteroidetes (11%; Fig. 4A). Less abundant groups included e.g. SAR 406 and Actinobacteria. δ -Proteobacteria were mainly represented by SAR324, γ -Proteobacteria by SUP05, and α -Proteobacteria by SAR11 and some taxa affiliated to Rhodospirales. Bacteroidetes were represented by Cryomorphaceae and *Polaribacter*.

Response of community composition to substrate amendments

The bacterial communities that grew up after 168 h incubation were less diverse than in the inocula (average Shannon diversity was 4.5 in the inocula and 2.1 in the incubation after 168h, Table S1). Yet, after the experimental incubations, bacterial community composition clustered significantly according to station/depth (ADONIS $r^2=0.87$ $p=0.001$, Fig. 3), indicating that each sample type supported a different actively growing community. While no major treatment effect was observed for the Control DCM water (all treatments supported a similar community shift relative to the inoculum, including the no-amendment control), treatments of the Control Deep water with pyruvate (PERMANOVA $p=0.08$) and especially with glutamine clustered together and differed (PERMANOVA $p=0.028$) from the no-amendment controls. In the Iceberg DCM treatments, glutamine samples (PERMANOVA $p=0.017$) and NAG (PERMANOVA $p=0.007$) were significantly different compared to the no-amendment control samples from the no-amendment control. In the Iceberg Deep treatments, a strong community response was observed for pyruvate (PERMANOVA $p=0.007$), NAG (PERMANOVA $p=0.002$) and mixed substrates (PERMANOVA $p=0.15$), respectively, showing a significantly different community when compared to the no-amendment control.

As with the in situ communities described above, the phylum composition after incubation clustered according to the initial station/depths, but large differences in response to treatment were observed between stations/depths (Fig. 4A). While all treatments from the Control DCM, Control Deep, and Iceberg DCM waters continued to be dominated by the initially high percentages ($77 \pm 17\%$) of Bacteroidetes (mainly *Polaribacter*), treatments from the Iceberg Deep waters shifted from their initial composition to be dominated by γ -Proteobacteria ($91 \pm 7\%$, mainly Colwelliaceae; Fig. 4A, B).

Ternary plots (Fig. 5) were used to visualize OTUs responding to specific treatments of interest (highlighted in Fig. 3). In such plots, orientation towards the corners indicates preference for a particular substrate. In incubations of the Control Deep water (Fig. 5A), 22 OTUs showed a strong positive response to glutamine. For instance, OTUs related to SUP05, SAR11, and Flavobacteriaceae appeared stimulated by glutamine but not by any other treatments. In contrast, OTUs related to Oceanospirillaceae, SAR92 and *Polaribacter* responded to all substrate-treatments, but *Colwellia* seemed to be more responsive to substrates other than glutamine and pyruvate. No OTUs appeared to be stimulated by pyruvate. In incubations of the Iceberg Deep water (Fig. 5B), OTUs related to *Polaribacter* and *Pseudoalteromonas* appeared to respond particularly to NAG. *Colwellia* responded to all the substrates (although less to the mix), while Oceanospirillaceae and SAR11 proliferated in treatments amended with NAG and the mix of substrates, but not in the pyruvate treatment. Similar to the Control Deep water incubations, none of the abundant OTUs responded solely to the addition of pyruvate.

For the incubations of Iceberg DCM water, OTUs related to *Polaribacter*, Oceanospirillaceae and *Colwellia* responded to all substrates enrichments (Fig. S1). Many less abundant taxa (not included in the ternary plots) appeared unique to each treatment (Fig. S2). Most of these taxa also appeared specific to each of the environments (station/depth).

Short term incubations

Short term incubations were performed in order to study the proportion of actively growing cells (i.e. uptake of BrdU, which is an indication of genome replication) within key bacterial groups, and compare this to responses in the long-term incubations. Therefore, the proportions of cells incorporating BrdU (hereafter called BrdU⁺) were determined for both stations and depths (Table 2). Target probes were chosen according to dominant taxa observed in the long term incubation experiments. Unfortunately, in some samples the signal of some probes was too weak to enumerate positive cells (described as not determined in Table 2).

Only $9 \pm 7\%$ of DAPI-stained cells incorporated BrdU (Table 2). The fraction of BrdU⁺ cells were generally higher in the Iceberg station ($11 \pm 9\%$) than in the Control station ($5 \pm 3\%$); likewise, for DCM ($10 \pm 8\%$) waters relative to deep ($7 \pm 7\%$) waters. A higher number of Archaea ($+ 77 \pm 23\%$) was observed in the Iceberg Deep waters compared to the Control and Iceberg DCM waters but the deep Archaea did not seem to incorporate BrdU. Importantly, the fraction of BrdU⁺ Archaea cells was highest ($+ 30\%$) in the Iceberg DCM (Table 2), suggesting that they were activated by conditions there. At both stations, the abundance of Bacteria assessed by CARD-FISH was higher ($+ 76 \pm 6\%$) in DCM waters than in deep waters. Only $5 \pm 1\%$ of these cells incorporated measurable amounts of BrdU, with no detectable incorporation in the deep waters of the Control station. The abundance of hybridized *Polaribacter* and SAR11 clade cells was highest ($+ 93 \pm 8\%$ and $+ 76 \pm 22\%$ for *Polaribacter* and SAR11, respectively) in the Iceberg DCM water. Nevertheless, the fractions of BrdU⁺ cells for both groups were highest in the Iceberg deep water highest ($+ 66 \pm 48\%$ and $+ 94 \pm 7\%$ for *Polaribacter* and SAR11, respectively). *Roseobacter* were present at the DCM of both stations and in Iceberg deep waters, but the fraction of BrdU⁺ cells was highest of all ($+ 78 \pm 1\%$) in the Control DCM waters. SUP05 clade hybridized cells were most

abundant in the Control DCM waters (+ 96%) but the fraction of BrdU+ cells was highest at the Iceberg DCM (+ 76%). Hence, there was no strong coupling between abundance and growth, at least not for the most abundant populations.

Discussion

Climate change impact on high-latitude marine ecosystems may result in increased massive ice-shelf and glacial calving events and iceberg production. Our results indicate that the water mass perturbations caused by a drifting iceberg, leading to upward mixing of nutrient-rich deep waters (Randall-Goodwin *et al.*, 2015), enhances bacterial production, changes bacterial community composition, and affects the ability of the bacterial assemblage to utilize dissolved organic carbon. This implies that the increased frequency of icebergs in the Southern Ocean caused by progressive global warming may have large consequences for bacterial carbon and nutrient cycling and thereby for the overall carbon sequestration in the region.

Icebergs: hot-spots for bacterial productivity

The drifting iceberg we studied generated deep mixing of the water column. The warm and nutrient-rich modified circumpolar deep water (mCDW) in the Amundsen Sea Polynya (ASP), which included the iron-rich and melt laden outflow from the ice-shelf, was mixed into the winter water (WW) in the mid water column (Randall-Goodwin *et al.*, 2015). We hypothesized that the iceberg-driven mixing (and its resulting impact on primary production and export) would affect the bacterioplankton during summer in the highly productive ASP. Bacterial production estimated at the Control station and at the Iceberg DCM was similar to rates measured across the ASP (Williams *et al.*, 2016) and in the Ross Sea Polynya during the Austral Summer (Ducklow *et al.*, 1999); however, bacterial production was elevated more than 50-fold in the deep Iceberg water relative to the deep Control waters. Similarly, the

fraction of BrdU incorporating cells indicated that within the examined bacterial groups, more active cells were present in the Iceberg Deep water compared to the Control Deep water. Moreover, Archaea, presumably brought up with the deep water, were stimulated by the mixing in the Iceberg DCM.

In the Atlantic Ocean, semi-labile dissolved organic matter (DOM) accumulating in surface waters was readily remineralized by the deep bacteria when exported to greater depth by convective overturn (Carlson *et al.*, 2004). Similarly, in the ASP, the aphotic bacterioplankton was shown to be stimulated by surface water and sea-ice DOM (Sipler and Connelly, 2015). Sherrell *et al.*, (2015) and Alderkamp *et al.*, (2015) suggest that the high primary production at this Iceberg station may be due to the vertical mixing of iron and nutrient rich deep waters into shallower depths. This enhanced primary production could at least partly explain the elevated bacterial production observed at this station. Furthermore, introduction of specific bacterial taxa to upper waters from depth could be an alternative and non-exclusive explanation for these observations. This process may be comparable to the upwelling associated with the Antarctic ice shelf. Indeed, in parallel studies based on samples from the same cruise, typical deep-water bacterial taxa were observed in surface waters near the ice shelf (Delmont *et al.*, 2014; Richert *et al.*, 2015).

Iceberg affects bacterial community composition

Bacterial community compositions at both depths of the Control station and in the Iceberg DCM water were similar to previous observations from the Southern Ocean in early summer with a predominance of Bacteroidetes, γ - and α -Proteobacteria (Murray and Grzymalski, 2007; Ghiglione and Murray, 2011; Grzymalski *et al.*, 2012). In contrast, in the Iceberg Deep water, we observed a distinct community composition with lower relative abundance of Bacteroidetes, a higher relative abundance of α -Proteobacteria (mainly SAR11 and Rhodospirillales) and other Proteobacteria (mainly δ -, related to SAR324 and

Methylophylales), and the presence of the environmental clade SAR406 and Actinobacteria.

These taxa are usually present in deeper mCDW, where the Bacteroidetes phylum is less abundant while the proportion of SAR406 and δ -Proteobacteria is elevated relative to surface waters (Ghiglione *et al.*, 2012). The increased prevalence of Colwelliaceae - related OTUs observed at the iceberg station could also reflect the high particle export rate at this station (Ducklow *et al.*, 2015; Williams *et al.*, 2016; Yager *et al.*, 2016) as *Colwellia* have been shown to be associated with sinking *Phaeocystis* in the ASP (Delmont *et al.*, 2014, 2015). Moreover, some of the taxa that were predominant at depth in the vicinity of the iceberg keel, e.g. Rhodospirillales, SUP05, SAR406, SAR11, and especially SAR324, are all known to be capable of demethylation or sulfur oxidation processes (Gordon and Giovannoni, 1996; Howard *et al.*, 2008; Tripp *et al.*, 2008) and could respond to the high export (Ducklow *et al.*, 2015) of the locally abundant (Alderkamp *et al.*, 2015) and conceivably DMSP producing (Baumann *et al.*, 1993) *Phaeocystis antarctica*. Indeed, cells within SAR11, *Polaribacter*, *Roseobacter* and SUP05 were actively growing (incorporating BrdU) at the iceberg-influenced station.

To summarize, some taxa present in the Iceberg Deep water have the capacity to utilize compounds derived from phytoplankton, such as DMSP (Tripp *et al.*, 2008; McCarren *et al.*, 2010), which are exported to depth in association with sinking particles (Delmont *et al.*, 2015). This finding may be particularly important in the vicinity of icebergs due to the locally enhanced vertical mixing (Randall-Goodwin *et al.*, 2015), enhanced biological activity (Alderkamp *et al.*, 2015), and export of particulate matter from the surface (Smith *et al.*, 2011).

Iceberg disturbance of the water column affected the capacity of the bacterial assemblages to utilize carbon substrates

We performed enrichment experiments with carbon substrates to examine whether the presence of the iceberg affected the bacterial carbon utilization potential. Such long-term (one week) incubations should be interpreted with caution since bottle effects may cause community shifts away from the original composition (Eilers *et al.*, 2000). Nevertheless, like used here, they can be forcefully used to demonstrate the metabolic potential of particular microbial clades in the water column. After one week of incubation, the most striking responses to the amendments were observed in the Iceberg Deep treatments; especially strong compositional responses were observed to NAG, but also to pyruvate and the mix of substrates (Fig. 3). Moreover, the bacterial production in the treatments with NAG and pyruvate was more than twice as high as in the control treatments, and more than 100-fold higher than in the corresponding Control Deep treatments. Yet, whereas no consistent community response to glutamine was observed for the Iceberg Deep water, a pronounced response was observed for the Control Deep water. These differential responses to model substrates suggest that the hydrodynamics associated with the iceberg influenced the ability of the indigenous bacterial assemblages to utilize various carbon substrates.

In all treatments, Alteromonadales and Colwelliaceae (γ -Proteobacteria), and *Polaribacter* (Flavobacteriales, Bacteroidetes) dominated after 7 d. These cold-adapted taxa are known opportunists (Massana *et al.*, 2001; Töpper *et al.*, 2012) and may have increased their relative population sizes because of the experimental confinement (Eilers *et al.*, 2000). Nevertheless, the high proportions of BrdU⁺ *Polaribacter* cells also in the short term incubations suggests that at least *Polaribacter* was an active component of the bacterial community at the iceberg station, especially at depth.

OTUs related to *Polaribacter* showed station-specific responses and were, in the Iceberg Deep water, stimulated exclusively by NAG. This may reflect a presence of different ecotypes of *Polaribacter in situ*; possibly due to genetic adaptation in response to

environmental selection, as suggested for other bacteria (Shi *et al.*, 2012). Consequently, the stimulation of *Polaribacter* and *Pseudoaltermonas* phylotypes by NAG, specifically in incubations from the Iceberg Deep water, may be seen as an *in situ* capacity to utilize NAG. Indeed, this could represent an advantageous growth strategy at depth upon mixing with surface waters, which may contain accumulated NAG (Aluwihare *et al.*, 2005) originating from locally elevated phytoplankton and zooplankton biomass (Kaufmann *et al.*, 2011). This suggested strategy would be consistent with the slightly higher ratio of diatoms to *Phaeocystis* at this station (Alderkamp *et al.*, 2015) and the distinct zooplankton composition observed at the iceberg station relative to other stations of the ASP (Wilson *et al.*, 2015). In summary, the presence of the iceberg affected the vertical distribution of specific bacterial taxa and the ability of bacterial communities to exploit selected carbon sources.

While icebergs did not affect bacterioplankton during the Austral winter (Murray *et al.*, 2011), our study indicates that water column disturbance generated by a drifting iceberg, and presumably the locally enhanced primary production (Smith *et al.*, 2007) in the vicinity of the iceberg, did affect bacterial activity, community composition and substrate utilization capacity at depth during summer in the Amundsen Sea Polynya. Due to the ample logistic challenges associated with sampling in this extreme environment, our study was constrained to a single iceberg. While this limits direct extrapolation and generalization of our results on bacterioplankton, the extensive hydrographic disturbance (Randall-Goodwin *et al.*, 2015) and stimulation of higher trophic levels (Alderkamp *et al.*, 2015; Wilson *et al.*, 2015) are well-documented for the examined iceberg, as well as for several other icebergs (Smith *et al.*, 2007; Schwarz and Schodlok, 2009; Kaufmann *et al.*, 2011). Consequently, the vertical water mixing in the vicinity and wake of drifting icebergs (Helly *et al.*, 2011, Randall-Goodwin *et al.*, 2015) stimulates plankton productivity (Smith *et al.*, 2007; Alderkamp *et al.*, 2015; Wilson *et al.*, 2015), which conceivably includes the bacterioplankton (this study). Taken

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together, these observations suggest that a future increased frequency of icebergs, or other physical mixing of the water column, could potentially have large implications for carbon sequestration and nutrient fluxes in polar regions. More data on hydrographical disturbance, and its consequence for the activity of indigenous microbes, are, however, needed for more and differently sized icebergs in different localities and seasons, before firm predictions on consequences for microbial carbon and nutrient cycling can be established.

Experimental Procedures

In-situ sampling

Sampling was conducted during the ASPIRE (Amundsen Sea Polynya Research Expedition) cruise from November 2010 to January 2011 on board R/V *N. B. Palmer* in the Amundsen Sea Polynya (ASP; Fig. 1A). The studied iceberg (113.7°W; 73.7°S) was a free-floating and quasi-tabular iceberg (~3 x 1 km) with an estimated keel depth of 340 – 450 m. The height (37-50 m) was measured by sextant and used to calculate the iceberg depth using isostatic principles. The iceberg was drifting southeast to northwest with an estimated drift speed of 0.3 knots under light southerly winds (see Figure 5; Randall-Goodwin *et al.*, 2015). The iceberg likely originated from the Dotson Ice Shelf (DIS) located 30 km southeast of the sampled station. The hydrographical perturbations generated by the iceberg were studied through multiple CTD (conductivity, temperature, depth) profiles all around the iceberg over a period of ~2.5 d both downstream and upstream of the drift trajectory (Randall-Goodwin *et al.*, 2015). For the study of bacterioplankton responses to substrate enrichment, waters from a station free of any iceberg influence (hereafter called “Control station” 112.667°W; 73.571°S, s13) and a station under influence of a free-drifting iceberg (hereafter called “Iceberg station”; 113.223°W; 73.648°S, s57.26) were sampled on 19 December 2010 and 03 January 2011, respectively (Fig. 1; Table 1). For each station, we sampled the deep chlorophyll maximum (DCM) at 40 and 10 m, respectively, and a deeper layer, corresponding to the interface between modified circumpolar deep water (mCDW) and winter water (WW) for the Control station (440 m) or the base of the mixed water layer at the iceberg station (300 m) shaped by the iceberg disturbance of the water column (Randall-Goodwin *et al.*, 2015). Data on selected parameters are shown in Table 1. Water was collected with 12 L Niskin bottles mounted on a CTD rosette. To assess the iceberg disturbance of the water column, comparisons were made to measurements made at other stations near or far from the iceberg (Fig. 1). Bacterioplankton

parameters at these other stations are reported in Williams *et al.*, (2016) and Richert *et al.*, (2015).

Experimental set-up

Enrichment experiments with carbon substrates were performed to examine whether the presence of the iceberg affected the bacterial carbon utilization potential. Substrates representing different classes of compounds were selected based on their documented relevance for marine bacteria (Suttle *et al.*, 1991; Obernosterer *et al.*, 1999; Riemann and Azam, 2002). Moreover, some of these substrates are known to stimulate Antarctic bacteria (Church *et al.*, 2000; Simon and Rosenstock, 2007; Ducklow *et al.*, 2011). For each depth, >20 L water was filtered through a rinsed 0.2 μm capsule filter (Acropak 500, Pall) and collected in large containers. This 0.2- μm filtered water was then inoculated with bacteria (20% v/v, 0.65- μm gravity-filtered; Durapore filters, Millipore) from the respective depth and station. Aliquots were then distributed in 1-L bottles, and divided into 6 treatments in triplicate: (1) control with no added inorganic nutrients or substrates; (2) control with inorganic nutrients (10 μM NaNO_3 ; 10 μM NH_4Cl ; 3 μM NaH_2PO_4 ; 3 μM Fe(III)Cl); (3) equimolar mix of carbon substrates (bovine serum albumine, chitobiase, leucine, thymine, N-acetylglucosamine (NAG), glucose, pyruvate, oxalate; 50 μM total concentration) and inorganic nutrients; and three treatments with inorganic nutrients as in (2) and a single carbon substrate: (4) glutamine (50 μM); (5) NAG (50 μM); or (6) Pyruvate (50 μM). The bottles were incubated for 7 d at *in situ* temperature (ca. -1°C). In order to measure only the response of heterotrophic bacteria and avoid production of organic matter by small phytoplankton, incubations were performed in the dark. All materials in contact with the samples were acid washed in 10% hydrochloric acid and repeatedly rinsed with Q-grade water (Millipore) prior to use. For bacterial abundance, samples were taken at the start, after 48 h, 96 h and at the end

of the incubation (168 h). For bacterial production, samples were taken at the start, after 48 h, at the end of the incubation (also at 96 h for the iceberg station), and assays were performed onboard. For bacterial community composition, samples were taken at the start and end of the incubation. The filters for DNA extractions, fixed samples for bacterial abundance and BrdU filters were transported to the R/V Oden in McMurdo at the end of the cruise, and kept at -80°C until analysis in Sweden.

Bacterial abundance and production

Samples (1.5 ml) for bacterial enumeration were fixed with EM grade glutaraldehyde (Sigma; 1 % final conc.), flash frozen in liquid N₂ and stored at -80 °C. Samples were stained with Sybr Green I (Invitrogen) and counted on a FASCanto II flow cytometer (Becton Dickinson) as previously described (Gasol and del Giorgio, 2000). Fluorescent beads (Truecount®, Becton Dickinson) were used to calibrate the flow rate. Bacterial production was measured by [H³]-Leucine incorporation (Kirchman *et al.*, 1985) modified for microcentrifugation (Smith and Azam 1992). Triplicate 1.5 ml aliquots were incubated with [H³]-Leucine (25 nM final conc.) in sterile 2.0 ml polypropylene tubes for 4 h at -1.5°C.

Samples with 5% trichloroacetic acid added prior to isotope served as blanks. Leucine incorporation was converted to carbon production using a conservative conversion factor of 1.5 kg C mol leucine⁻¹ (Simon and Azam 1989; Kirchman 2001; Ducklow *et al.*, 2002) according to Williams *et al.* (2016). The data were analyzed using the factorial analysis of variance (ANOVA) with SYSTAT 13. Post hoc comparisons were done using Tukey's honestly significant difference test.

Bacterial community composition

For each of the 72 bottle incubations (six treatments in triplicates for both depths and stations), cells were collected from 1 L of water by vacuum filtration onto 0.2 µm pore size,

47 mm diameter Supor membrane filters (Pall). Filters were subsequently frozen at -80 °C in 1 ml sucrose lysis buffer (20 % sucrose, 50 mM EDTA, 50 mM TrisHCl, pH = 8). DNA was extracted using an enzyme/phenol-chloroform protocol (Riemann *et al.*, 2000) but with a 30-min lysozyme digestion at 37 °C and an overnight proteinase K digestion (20 mg ml⁻¹ final conc.) at 55 °C (Boström *et al.*, 2004). Bacterial 16S rDNA (V3-V4 region) was PCR amplified using Phusion Hot start high fidelity DNA polymerase (Thermo Scientific), 0.06 ng DNA µl⁻¹, and the primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) (Herlemann *et al.*, 2011). Primer 805R carried a 7 bp sample-specific barcode and both primers had fused 454-Titanium Lib-L adapters. For each sample, products from triplicate PCR reactions were pooled, purified (Agencourt AMPure XP kit, Beckman Coulter), and quantified using PicoGreen double stranded DNA staining dye (Invitrogen) as recommended by the manufacturers. The samples were mixed in equimolar amounts and sequenced by 454 pyrosequencing using Titanium chemistry at the SNP/SEQ SciLife platform hosted by Uppsala University, Sweden.

Sequence analyses

All 454 reads with mismatches in the primer or lengths below 200 bp and quality scores < 25 were discarded. Reads that passed the quality check were denoised using AmpliconNoise v.1.24, and subsequently checked for chimeras using Perseus (Quince *et al.*, 2011). High quality reads were subsequently processed using the Quantitative Insights Into Microbial Ecology pipeline (QIIME v1.3; Caporaso *et al.*, 2010b). Reads were clustered into operational taxonomic units (OTUs) at 99% pairwise identity using UCLUST. One representative sequence per OTU was extracted and the entire set of representative sequences was aligned using PyNAST (Caporaso *et al.*, 2010a) based on the Greengenes reference alignment (<http://greengenes.lbl.gov>; DeSantis *et al.*, 2006) as template. A maximum likelihood phylogenetic tree was constructed based on this alignment using RAxMLHPC-MPI (v7.2.8;

Stamatakis, 2006) with the GTR+CAT/G+I (GTRCATI) evolutionary model under rapid hill climbing mode. A total of 100 inferences were run for topology and the inference with the best likelihood was used for downstream analyses. Tree construction was run at the Supercomputer MareNostrum (<http://www.bsc.es/>) with 100 processors. Taxonomy assignments were made using the Ribosomal Database Project (RDP) classifier (Wang *et al.*, 2007). Singletons and OTUs present in only one sample were removed. Out of 76 samples, six samples with less than 600 reads were removed from the analysis as this small amount of data is likely not sufficient to properly represent the underlying community composition. All sequences have been deposited in the European Nucleotide Archive - Short Read Archive under accession number PRJEB4866.

Alpha and beta diversity indices were estimated for all samples after randomized subsampling to 2,371 reads per sample for equal sequencing depth in all samples. For alpha diversity, we calculated Chao1 (richness) and Shannon (richness and evenness) indices. Rarefaction analyses were performed to determine alpha diversity saturation. Beta diversity was investigated using weighted UniFrac distances, which take into account the phylogenetic differences between communities (Lozupone and Knight, 2005). The UniFrac distance matrix was used for weighted normalized UPGMA clustering analyses. The support for the UPGMA branching pattern was assessed using jackknifing with 100 permutations. Nodes with $\geq 80\%$ jackknife support were considered robust (statistically significant similarities). Communities with robust support were analyzed in more detail. Differences between communities were tested with PERMANOVA (Permutational Multivariate Analysis of Variance) with pairwise test and 9999 permutations in Primer E (Clarke *et al.*, 2014). All alpha and beta diversity analyses were run in QIIME.

Short term incubations

To explore the growth of key bacterial groups, 50 ml samples from both stations and depths were incubated at *in situ* temperature and in the dark with bromodeoxyuridine (BrdU, 20 μ M final conc.) and thymidine (33 nM final conc.) for 7 h. Subsequently, samples were fixed with paraformaldehyde (PFA, 2% final conc.) at 4 °C for 10 h. Samples pre-fixed with PFA served as negative controls. Samples were then filtered onto 0.2 μ m, 45 mm white polycarbonate membrane filters (Millipore). Preparations were stored at -20°C until further processing.

Cell permeabilization and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) were performed as described by Tischer *et al.*, (2012). All hybridizations were carried out under the conditions previously described for each of the probes (Table S1) targeting Archaea (Stahl and Amann, 1991), Bacteria (Amann *et al.*, 1990), *Oceanospirillum* (Eilers *et al.*, 2000), *Polaribacter* (Malmstrom *et al.*, 2007), *Roseobacter* (Brinkmeyer *et al.*, 2000), SAR11 clade (Morris *et al.*, 2002) and SUP05 clade (Sunamura *et al.*, 2004). Hybridized filters were placed in amplification solution (1x PBS (pH 7.6), 2 M NaCl, 10% dextran sulphate, 0.1% blocking reagent, 0.0015% H₂O₂) containing 2.5 μ g ml⁻¹ tyramides with custom labeled green fluorescent dye Alexa₄₈₈ (Molecular probes). BrdU staining was performed according to Pernthaler and Pernthaler (2005). The filter sections were covered with a mix of 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, 1 μ g mL⁻¹) and citifluor antifading agent (Citifluor AF2, Citifluor Ltd.) for estimating total cell numbers and to prevent fading of the fluorescent signal. All filters were kept dark at -20°C until analysis.

Image acquisition for each filter section was done using an Axioplan II epifluorescence microscope (Carl Zeiss). Tools for automatic image acquisition and image quality were implemented by Zeder and Pernthaler (2009), which resulted in 20-35 high quality images per filter section. The software Cell Profiler (Lamprecht *et al.*, 2007) was applied for image analysis by detecting and enumerating probe-, BrdU- and DAPI- positive cells.

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Dinasquet *et al.*, Table 1

Table 1. Environmental variables for the sampled stations and depths. Dissolved organic carbon (DOC) and chlorophyll *a* (Chl. *a*) were measured by standard methods. DCM: deep chlorophyll maximum; AASW: Antarctic surface water; WW: winter water; mCDW: modified circumpolar deep water.

	Control station		Iceberg station	
	DCM	deep	DCM	deep
Depth (m)	40	440	10	300
Water mass	AASW	Interface WW/mCDW	AASW	Mixed water WW/mCDW
Temperature (°C)	-1.25	-0.19	-0.41	-1.67
Salinity	33.94	34.31	33.93	34.14
Chl. <i>a</i> (µg L ⁻¹)	7.5	0.03	7.8	0.09
Phosphate (µmol L ⁻¹)	1.54	2.16	1.57	2.13
Nitrate/Nitrite (µmol L ⁻¹)	23.7	32.7	23.4	31.4
Ammonium(µmol L ⁻¹)	0.27	-	0.12	-
Silica (µmol L ⁻¹)	83.5	101	89	96.6
DOC (µmol L ⁻¹)	70.6	58.8	77.6	69.7
Bacterial abundance (x 10 ⁵ cells ml ⁻¹)	6.5	1.2	3.0	1.2
Bacterial production (µg C cell ⁻¹ d ⁻¹)	4.8 x 10 ⁻⁹	1.1 x 10 ⁻¹⁰	1.04 x 10 ⁻⁸	5 x 10 ⁻⁹

Dinasquet *et al.*, Table 2

Table 2. BrdU incorporation after 7 h of incubation. Data show the cells incorporating BrdU (BrdU⁺) as a % of hybridized cells and the number of hybridized cells for the different probes (cells ml⁻¹). n.d. not determined.

	Control station		Iceberg station	
	DCM	deep	DCM	deep
DAPI	7.2 x 10 ⁴	4.1 x 10 ⁴	6.6 x 10 ⁴	5.9 x 10 ⁴
Archaea	n.d.	0% 5.6 x 10 ²	30% 3.6 x 10 ³	0% 9.2 x 10 ³
Eubacteria	6% 1.13 x 10 ⁵	0% 3.2 x 10 ³	4% 7.4 x 10 ⁴	6% 1.4 x 10 ⁴
<i>Polaribacter</i>	n.d.	0% 4.0 x 10 ²	42% 3.2 x 10 ⁴	62% 4.3 x 10 ³
SAR11 clade	0% 8.0 x 10 ³	n.d.	1% 2.0 x 10 ⁴	9% 1.6 x 10 ³
<i>Roseobacter</i>	76% 2.6 x 10 ³	n.d.	18% 4.1 x 10 ³	16% 7.8 x 10 ²
SUP05 clade	17% 2.9 x 10 ⁴	n.d.	70% 1.1 x 10 ³	n.d.

Figure legends

Figure 1. Site description. Location of the ASPIRE stations (blue are iceberg-influenced, green are non influenced/control stations) in the Amundsen Sea Polynya, Antarctica (a); Temperature/Salinity plot of sampled stations (blue solid lines are iceberg-influenced, green lines are control stations, diamonds and circles represent the iceberg and control stations, respectively, presented in this study) with the key water masses identified, adapted from Randall-Goodwin *et al.*, 2015. Ice shelves stations: DIS (Dotson Ice Shelf station) and GIS (Getz Ice Shelf station) are presented to compare disturbed and undisturbed stations to stations closed to ice shelves outflow. (b); Temperature section of a transect going through the two studied stations and four other stations sampled during the cruise (c). Water mass abbreviations: AASW: Antarctic surface water; WW: winter water; mCDW: modified circumpolar deep water.

Figure 2. Bacterial abundance (A, C) and production (B, D) in the different treatments during the incubation; at the control station (A, B) and at the iceberg station (C, D). Note the break in the scales for the control station (A, B). White symbol represent deep treatments while black symbols represent DCM treatments. Error bars represent the SD for the triplicate bottles.

DCM: deep Chlorophyll maximum

Figure 3. UPGMA dendrogram based on weighted normalized Unifrac distances between inocula and after 168 h of incubation. Dots represent nodes with jackknife support $\geq 82\%$. Gray ellipses highlight clusters of interest with robust jackknife supports (except for the NAG group in Iceberg DCM with only 64% support). Replicate treatments are displayed. nts: nutrients; DCM: deep Chlorophyll maximum.

Figure 4. Relative abundance of bacterial phyla and proteobacterial sub-classes (a) and of γ -Proteobacteria orders (b) in the inocula and after 168 h of incubation. Others: unclassified and rare phyla accounting for less than 0.002% of total relative abundance. nuts: nutrients; DCM: deep Chlorophyll maximum

Figure 5. Ternary plots of OTU distribution across three different treatments after 168 h of incubation of water from the Control Deep station samples (a) and the Iceberg Deep station (b). Only OTUs representing ≥ 10 reads across the three treatments are represented in the plots. Circle size represents the abundance of the OTU reads across the three treatments (average of triplicate samples). To account for the variation in bacterial abundance between treatments, OTU abundance was calculated by multiplying the relative OTU frequencies with total bacterial abundance. Circles with no colors belong to taxa that were represented only by one OTU across the three treatments. “others” is an average of all treatments with no significant differences between sample communities of Control Deep station samples.

Figure 1. Site description. Location of the ASPIRE stations (blue are iceberg-influenced, green are non influenced/control stations) in the Amundsen Sea Polynya, Antarctica (a); Temperature/Salinity plot of sampled stations (blue solid lines are iceberg-influenced, green lines are control stations, diamonds and circles represent the iceberg and control stations, respectively, presented in this study) with the key water masses identified, adapted from Randall-Goodwin et al., 2015. Ice shelves stations: DIS (Dotson Ice Shelf station) and GIS (Getz Ice Shelf station) are presented to compare disturbed and undisturbed stations to stations closed to ice shelves outflow. (b); Temperature section of a transect going through the two studied stations and four other stations sampled during the cruise (c). Water mass abbreviations: AASW: Antarctic surface water; WW: winter water; mCDW: modified circumpolar deep water.

238x209mm (300 x 300 DPI)

Figure 2. Bacterial abundance (A, C) and production (B, D) in the different treatments during the incubation; at the control station (A, B) and at the iceberg station (C, D). Note the break in the scales for the control station (A, B). White symbol represent deep treatments while black symbols represent DCM treatments. Error bars represent the SD for the triplicate bottles. DCM: deep Chlorophyll maximum

303x209mm (72 x 72 DPI)

Figure 3. UPGMA dendrogram based on weighted normalized Unifrac distances between inocula and after 168 h of incubation. Dots represent nodes with jackknife support $\geq 82\%$. Gray ellipses highlight clusters of interest with robust jackknife supports (except for the NAG group in Iceberg DCM with only 64% support). Replicate treatments are displayed. nuts: nutrients; DCM: deep Chlorophyll maximum.

166x149mm (300 x 300 DPI)

Figure 4. Relative abundance of bacterial phyla and proteobacterial sub-classes (a) and of γ -Proteobacteria orders (b) in the inocula and after 168 h of incubation. Others: unclassified and rare phyla accounting for less than 0.002% of total relative abundance. nuts: nutrients; DCM: deep Chlorophyll maximum

199x245mm (300 x 300 DPI)

Figure 5. Ternary plots of OTU distribution across three different treatments after 168 h of incubation of water from the Control Deep station samples (a) and the Iceberg Deep station (b). Only OTUs representing ≥ 10 reads across the three treatments are represented in the plots. Circle size represents the abundance of the OTU reads across the three treatments (average of triplicate samples). To account for the variation in bacterial abundance between treatments, OTU abundance was calculated by multiplying the relative OTU frequencies with total bacterial abundance. Circles with no colors belong to taxa that were represented only by one OTU across the three treatments. "others" is an average of all treatments with no significant differences between sample communities of Control Deep station samples.

131x186mm (300 x 300 DPI)