Major Role of Microbes in Carbon Fluxes during Austral Winter in the Southern Drake Passage

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Abstract

Carbon cycling in Southern Ocean is a major issue in climate change, hence the need to understand the role of biota in the regulation of carbon fixation and cycling. Southern Ocean is a heterogeneous system, characterized by a strong seasonality, due to long dark winter. Yet, currently little is known about biogeochemical dynamics during this season, particularly in the deeper part of the ocean. We studied bacterial communities and processes in summer and winter cruises in the southern Drake Passage. Here we show that in winter, when the primary production is greatly reduced, Bacteria and Archaea become the major producers of biogenic particles, at the expense of dissolved organic carbon drawdown. Heterotrophic production and chemosynthetic CO2 fixation rates were substantial, also in deep water, and bacterial populations were controlled by protists and viruses. A dynamic food web is also consistent with the observed temporal and spatial variations in archaeal and bacterial communities that might exploit various niches. Thus, Southern Ocean microbial loop may substantially maintain a wintertime food web and system respiration at the expense of summer produced DOC as well as regenerate nutrients and iron. Our findings have important implications for Southern Ocean ecosystem functioning and carbon cycle and its manipulation by iron enrichment to achieve net sequestration of atmospheric CO2.

Introduction

Southern Ocean plays a crucial role in the global carbon cycle, exerting a major control on atmospheric CO2 concentration [1], hence the need to understand the role of biota in the regulation of carbon fixation and cycling. Southern Ocean is a large and heterogeneous biogeochemical system. The coastal and ice edge waters experience intense spring and summer blooms—mainly diatoms and Phaeocystis— that support a food web via zooplankton and krill to penguins and whales. In contrast, the open ocean is the largest high-nutrients low-chlorophyll (HNLC) area, characterized by low primary productivity (PP), mainly iron-limited, as demonstrated by several iron enrichment experiments [2] and observations of blooms induced by natural iron fertilization [3–4]. Iron fertilization to stimulate net phytoplankton growth has been proposed as one method for mitigating rising atmospheric CO2 [5]. Yet, the fate of the carbon accumulating in the productive areas during summertime or in artificially fertilized areas is critical to how biological forces make the Southern Ocean a source or a sink for carbon. There is much interest in whether the southern ocean food web is dominated by microbial loop processes—i.e., in tropical and temperate oligotrophic ocean [6–7]. Previous studies showed weak bacteria-PP coupling and ascribed this to temperature restriction of dissolved organic matter (DOM) utilization [8], limited DOM availability [9] or strong grazing pressure [9–10]. However, others found that bacteria process a significant fraction of primary production during bloom conditions by consuming the phytoplankton derived dissolved organic matter [11–12]. An important feature of the Southern Ocean is the strong seasonality, due to long dark winter with minimal PP. Few studies done in autumn-winter in marginal ice edge zone [13–15] and in the Drake Passage [16], found substantial bacterial activity; however they did not explicitly address microbial loop processes, and were limited to the surface mixing layer [13–15]. Direct evidence of a significant role of the microbial loop over a broad area and in the mesopelagic layer in winter is still lacking.

In summer (February 12-March 24 2004) we sampled the epipelagic layer (~67 m). Our study area had strong gradients of chlorophyll-a (Chl a) (0.05 to 0.74 μg Chl a L-1) from oligotrophic Antarctic Circumpolar Current (ACC) waters to east of Shackleton Transverse Ridge (STR) (Fig. 1a, b). The ACC current is...
steered south by STR and mixes with the iron rich Shelf water, stimulating high PP downstream [4]. Bacterial abundance $(1.9 \times 10^6 \pm 0.9 \times 10^6 \text{ L}^{-1} \text{ s.d.)}$, bacterial carbon production (BCP) $(0.63 \pm 0.57 \mu \text{g C L}^{-1} \text{ d}^{-1} \text{ s.d.)}$ and growth rates ($\mu$) $(0.16 \pm 0.14 \text{ d}^{-1} \text{ s.d.)}$ (Fig. 2) were in the low end of the range for other oligotrophic regions [19]. They were positively correlated with Chl a (Spearman $R$, $p < 0.05$, BCP vs Chl a $R = 0.49$, $\mu$ vs Chl a $R = 0.46$, $n = 21$). Carbon flux into bacteria as fraction of co-local PP is a measure of the strength of bacteria-organic matter (OM) coupling. In summer, BCP was equivalent to $\sim 30\%$ of PP in low chlorophyll ACC waters and $\sim 13\%$ in high PP mixed water. Assuming a conservative growth efficiency (BGE) of 36% [20] the bacterial carbon demand (BCD) over the whole area would be $\sim 40\%$ of PP (Table 1). Thus, bacteria-OM coupling was robust and comparable to other oligotrophic and mesotrophic waters. (Yet, it would spare $\sim 1/2$ of PP, some of which could accumulate and support the wintertime food web).

In winter (July 3-August 15 2006) we occupied 38 stations and sampled depths from surface to 300–750 m (Fig. 1a). In the epipelagic layer, as expected, Chl a and PP were much lower than in summer (Fig. 1b and Table 1) yet, bacterial abundance was similar to summer and these populations were active in terms of $\mu$ and BCP (Fig. 2). Although our summer sampling was limited, the winter and summer ranges for both $\mu$ and BCP were comparable. Indeed, the winter bacterial abundance, BCP and $\mu$ in our study area were comparable to those reported for low latitude oligotrophic systems such as the central North Pacific gyre and Sargasso Sea. Bacteria were also abundant and active in the mesopelagic layer, and could mediate significant carbon flux. The mesopelagic bacterial abundances were within a factor of 2 and

![Figure 1. Sampling area and chlorophyll a distribution.](image1)  
![Figure 2. Depth profile of bacterial parameters in summer 2004 and winter 2006.](image2)
mesopelagic BCP and \( \mu \) were comparable to their ranges in the epipelagic layer (Fig. 2). Notably, the high BCP at depth was due to higher \( \mu \) (0.70 d\(^{-1}\) at 400 m; Fig. 2). Thus, wintertime bacterial populations throughout the epipelagic and mesopelagic layers were abundant, growing and displayed substantial carbon demand. Further, since PP was greatly reduced in winter the epipelagic PP was 3 fold lower than BCP and an order of magnitude lower than BCD (estimating epipelagic BCD at BGE, 39% \[20\]) (Table 1). PP could therefore have supported only 10% of BCD and majority of BCD must have been met by sources other than the contemporaneous PP.

We compared summer and winter data on areal basis to assess possible sources of carbon to support bacteria in winter (Table 1). SeaWiFS composite images of summer 2006 (Jan–Mar) for our study area showed a Chl \( a \) pattern similar to 2004; so we assume that summer 2004 values can be applied to summer 2006. Mesopelagic BCD would usually be met by sinking POC (or DOC mixing from surface \[21\]). In our area sinking particles from contemporaneous PP in winter could have supported only 3% of mesopelagic BCD even if, improbably, all PP sank into the mesopelagial. Since sinking POC could not have supported winter mesopelagic BCD, DOC is a likely source. DOC accumulated in the upper 200 m in the productive summer (Fig. 3a), presumably as slow-to-degrade components of phytoplankton production evade attack by summer bacterial communities. In winter DOC was very low (36.5 ± 2.8 \( \mu \)M C s.d.; Fig. 3a), in low range of constant refractory deep water DOC concentration \[23\]. The summer to winter DOC decline (37.4 g C m\(^{-2}\)) in the epipelagic zone and

### Table 1. Areal data from summer 2004 and winter 2006 cruises.

|                | Number of stations | Depth of integration (m) | PP (mg C m\(^{-2}\) d\(^{-1}\)) | BCP (mg C m\(^{-2}\) d\(^{-1}\)) | Summer excess DOC (g C m\(^{-2}\)) | BCD* (mg C m\(^{-2}\) d\(^{-1}\)) | BCD** (mg C m\(^{-2}\) d\(^{-1}\)) |
|----------------|--------------------|--------------------------|---------------------------------|----------------------------------|----------------------------------|-------------------------------|-------------------------------|
| Summer Epipelagic | 10                 | 65 (17)                  | 354.8 (197.1)                  | 51.9 (30.7)                      | 37.4 (18.8)                      | 143.1 (87.0)                  | 199.5 (118.1)                 |
| Mesopelagic     | 1                  | 685 \(^{\dagger}\)      | N.D.                           | 82.8 \(^{\dagger}\)            | N.D.                            | N.D.                          | N.D.                          |
| Winter Epipelagic | 38                 | 156 (75)                 | 7.6 (5.4)                      | 25.2 (21.3)                     | 65.2 (55.0)                     | 203.4 (171.7)                |
| Mesopelagic     | 38                 | 600 (64)                 | 84.5 (90.5)                    | 227.2 (249.0)                   | 681.2 (729.4)                   |                               |                               |

All parameters for the summer cruise have been integrated over the entire euphotic layer (1% incident PAR at surface; it was similar to the mixed layer depth or deeper). The winter PP has been integrated over the euphotic layer while other parameters have been integrated over the mixed layer depth (epipelagic) and from there down to 750 m (mesopelagic layer). Averages and standard deviation in parenthesis. \( P \) = primary production; \( BCP \) = bacterial carbon production; Summer excess DOC = summer dissolved organic carbon values minus average winter constant value (36.5 ± 2.8 \( \mu \)M C s.d.); BCD = bacterial carbon demand (BCP/bacterial growth efficiency); N.D. = not determined.

\(^{\dagger}\) BCD calculated using a bacterial growth efficiency derived by the curve in ref. \[20\] (~ 36% in summer and 39% in winter).

\(^{\dagger}\) BCD calculated using bacterial growth efficiency of 13% in summer and 6.2% in winter (averaging all data for summer and only HNLC for winter from \[12\]).

\(^{\dagger}\) No s.d. reported because the value is derived from a single depth profile.

DOC data for winter are not reported, since they are considered as constant refractory DOC values, and have been used to determine summer excess DOC.

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Figure 3. Depth distribution of organic matter pools during summer and winter cruises. Summer 2004: blue circles = ACC water; red circles = shelf water; green circles = mixed water; empty square = Winter 2006. (Panel A) DOC = Dissolved organic carbon. The data presented are measurements of total organic carbon, but since POC represents a negligible contribution to total organic carbon (POC represented only between 7% and 2% of the total pool) we can consider the analysis as DOC values. The shaded area covers the range of winter concentration. For a comparison, a range of variations of DOC from the FRUELA cruise study area in summer (Gerlache Strait, Bransfield Strait and Bellinghausen Strait) \[22\] has been reported (black triangles). (Panel B) POC = Particulate organic carbon.

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82.8 g C m⁻² in the mesopelagic zone; Table 1) would suffice to support the observed BCD. In terms of carrying capacity the DOC drawdown could have supported the epipelagic BCD for ~570 d and mesopelagic BCD for ~365 d. Our calculations are based on two assumptions: the conversion factor (CF) from leucine incorporation to carbon production, and the growth efficiency. Regarding CF, we used a well constrained CF of 3.1 kgC mol leu⁻¹ [17], that is the same value that [24] and [25] empirically determined in the Weddell-Scotia Sea and in the Atlantic sector of Southern Ocean. As for BGE, we used the model of [20], that relates temperature to BGE. The summer and winter values (36% and 39%) are close to 40% determined by [24] and in the range of values determined by [26] (9–38%) in the Ross Sea. However, since we did not determine experimentally the BGE for our system, we cannot exclude that using other values found in Southern Ocean, we could have had a different outcome. Applying a lower CF of 1.5 (assuming no dilution factor [12], this value is equivalent to the lower range of CF empirically determined by [27]) and a lower BGE of ~13% in summer and ~6% in winter, empirically determined in the Kerguelen Plateau, Southern Ocean [12], the resulting BCD is even higher (Table 1), and the excess summer DOC would have supported BCD for 184 days in the epipelagic layer and only 122 days in the mesopelagic layer. Integrating over the entire water column, the DOC drawdown would be 120.0 g C m⁻², enough to support BCD for ~136–410 d, depending on the BGE. The excess DOC drawdown may indicate a lower BGE since energy requirement to utilize semi-refractory DOM in winter may be higher. In the central Arctic ocean, in winter, [28] determined a growth efficiency of 0.5%, based on respiration incubation experiments. Alternatively, the excess DOC may have had other fate(s), like horizontal transport [22], vertical export [21], or such as POC → DOM transformation and currently unrecognized biotic sinks. If vertical mixing was the main mechanism of DOC removal from summer surface 200 m rather than biological mineralization [21], we would expect an increase in winter DOC concentration in the layer 200–750 m, which was not the case (integrated winter DOC value in the layer 200–750 m was 238.0 vs 265.5 g C m⁻² in summer). Furthermore, DOC concentration in winter was homogenously very low (see above, Fig. 3a). However, since we sampled only in summer and winter, we cannot exclude the possibility of water masses sinking and being advected northward, earlier in autumn, thus exporting semi-refractory DOC [29].

Interestingly, the winter POC pool, although not sustainable by the meager surface PP, was persistent and vertically homogeneous (Fig. 3b). It may be comprised of refractory non-sinking organic matter. Alternatively, it may be produced dynamically from DOC by polymer cross-linking (above) and provide organic rich microenvironments for bacterial growth in winter. This would be consistent with significant bacterial growth rates and carbon cycling during winter.

In winter bacteria produced biomass at 110 mg C m⁻² d⁻¹ (Table 1), a source of protein rich biomass potentially available to all particle-feeding organisms (depending on the state of bacterial aggregation). Our data on protist and virus abundances from a limited number of stations representative of the whole area and all depths (not shown), support the hypothesis of a dynamic bacterial community channeling DOM-carbon to protists and on to larger animals [30]. A strong predation pressure is consistent with the observed low variability in bacterial biomass despite substantial growth. Protist abundance was 3.2 × 10⁶ ± 0.9 × 10⁶ cell L⁻¹ s.d. (in the range reported for summer in Southern Ocean [31] and in winter in ice-covered Arctic Ocean [32]). Assuming an ingestion rate of 1.3–5.5 bacteria HNF⁻¹ h⁻¹ [32] protists would consume 18% to >200% of our winter BCP. Bacteria/protists ratio of ~400 is within the range of other Antarctic surface waters where significant grazing rates have been measured [31,33]. Viruses were also likely a significant source of bacterial mortality since they persisted at substantial populations (1.4 × 10⁶ ± 0.4 × 10⁶ L⁻¹ s.d.) comparable to a summer study in the Drake Passage [34]. Virus/bacteria ratio was 6–22, found typically in marine waters, including a summer study in Southern ocean that showed significant virus induced bacteria mortality [35]. Thus, BCP could have supported carbon flux to protists and possibly on to larger animals; and an active viral loop that might enhance system respiration [35–36].

We considered that in addition to heterotrophic BCP there might also be chemosynthetic production [37], probably due to Archaea [37–38]. Dark ¹⁴CO₂ assimilation in winter 2006 showed significant but highly variable carbon fixation (Fig. 4), 0.1–40 ng C L⁻¹ d⁻¹ corresponding to 8.8 ± 15.8% s.d. of the total prokaryotic carbon production (bacterial and archael). For the near surface experiments we cannot exclude a potential contribution of dark CO₂ fixation by phytoplankton; however this is probably insignificant, since the incubations lasted from 55 to 100 h and further we did not find a significant correlation between DIC uptake and Chl a (not shown). While our measurements were limited it would be important to determine whether chemosynthetic production in winter is a significant factor in Southern Ocean carbon cycle in a broader geographical context.

We hypothesized that sustained bacterial growth in winter at the expense of DOC semi-refractory to the summer population might have been accompanied by a shift in bacterial community composition as it has been observed in more productive areas [39–40]. Also, bacterial community structure can be influenced by top-down control; grazing and viral lysis can have a different impact on diverse taxa [41]. Whether the summer prokaryotic assemblages persisted into winter and whether there was a spatial variability was addressed by denaturing gradient gel electropho-

![Figure 4. Depth profile of chemotrophic carbon production in winter 2006. DIC = dissolved inorganic carbon. doi:10.1371/journal.pone.0006941.g004](Image)
resis (DGGE) and 16S rRNA sequence analysis of excised bands for both *Bacteria* and *Archaea*.

*Bacteria* communities were different in summer and winter (Fig. 5, Table 2). In summer, in the epipelagic layer (9 stations, 20–120 m, Fig. 5A, Table 3), we detected only seven *Bacteria* phylotypes (and six *Eukarya* plastids)—three *α*-Proteobacteria (associated to *Roseobacter*), three *Cytophaga-Flavobacteria-Bacteroidetes* (CFB) and one *γ*-Proteobacteria. All had 99–100% sequence homology to known uncultured *Bacteria* (Table 2). Winter epipelagic samples (0–150 m, Fig. 5B, Table 3) had much greater community richness—20 phylotypes—possibly reflecting molecular complexity of available substrates. All winter phylotypes had 98–100% sequence similarity to uncultured *Bacteria* from environmental clone libraries, majority (16/20) of them from Antarctic or Arctic waters. The seasonal variation was great. Only one phylotype, a *γ*-Proteobacteria (EF648172, displaying 100% sequence similarity to an uncultured Arctic bacteria) was present both in summer and winter epipelagic samples. [39], analyzing seasonal DGGE profiles in surface coastal waters near Anvers Island, found a striking difference between winter and summer assemblages. Repeating the analysis 6 years later, on samples covering an annual cycle, [47] found a reproducible seasonal trend, with significant differences between winter and summer communities.

In our samples, also the relative contribution of phylogenetic groups changed between seasons: *α*-Proteobacteria and CFB, the dominant groups in summer, decreased to 5 and 15% respectively.
**Table 2.** List of GenBank accession number and closest relative to 16S rDNA bacterial sequences obtained from DGGE.

| Accession # | Band number | % identity | Accession # closest relative | Closest relative | Phylogenetic group | Ref. |
|-------------|-------------|------------|------------------------------|-----------------|-------------------|------|
| **Summer 2004** | | | | | | |
| EF127653 | 1 | 100 | AF355051 | Uncultured Cytophaga/les bacterium Arctic97A-14 | Bacteroidetes | [42] |
| EF127654 | 2 | 100 | DQ184451 | Uncultured γ proteobacterium clone SB04_7 | 16S | γ Proteobacteria | [43] |
| EF127656 | 3 | 97 | AY664327 | Uncultured phototrophic eukaryote, chloroplast | Eukarya | |
| EF127657 | 4 | 99 | EF491388 | Uncultured eukaryote clone S2-72, chloroplast | Eukarya | |
| EF127659 | 5 | 99 | EF414204 | Uncultured phototrophic eukaryote clone MPWIC_C08 | Eukarya | |
| EF127660 | 6 | 99 | AM920863 | Uncultured bacterium clone LV_38 | Bacteroidetes | |
| EF127661 | 7 | 99 | EU005787 | Uncultured marine bacterium clone KG_A3_120m83 | 16S | Bacteroidetes | [44] |
| EF127662 | 8 | 98 | AM747382 | Unc. Marine eukaryote | Eukarya | |
| EF127663 | 9 | 100 | EF395741 | Uncultured phototrophic eukaryote, chloroplast | Eukarya | |
| EF127664 | 10 | 100 | AM920858 | Uncultured bacterium | Eukarya | |
| EF127666 | 11 | 99 | AY794086 | Uncultured Roseobacter sp. clone F1C79 | Proteobacteria | [45] |
| EF127667 | 12 | 99 | EU496898 | Uncultured eukaryote clone S2-72, chloroplast | Eukarya | |
| EF127668 | 13 | 100 | AM921555 | Uncultured bacterium 16S rRNA gene, clone LV_135 | Proteobacteria | |
| **Winter 2006** | | | | | | |
| EF648172 | 1 | 100 | EF127654 | Uncultured proteobacterium isolate DPEU02 | γ Proteobacteria | |
| EF648173 | 2 | 99 | EU005814 | Uncultured marine bacterium clone KG_A3_120m110 | Bacteroidetes | [44] |
| EF648174 | 3 | 99 | EU005908 | Unc mar bacterium clone KG_C11_100m18 16S RNA gene | γ Proteobacteria | [44] |
| EF648175 | 5 | 100 | U70715 | Uncultured prasinophyte clone OMP5 | Plastid | [46] |
| EF648176 | 6 | 100 | EU005720 | Uncultured marine bacterium clone KG_A3_120m16 | Proteobacteria | [44] |
| EF648177 | 7 | 100 | DQ906747 | Unc marine bacterium clone AntCL2E12 16S RNA gene | Bacteroidetes | [47] |
| EF648178 | 8 | 100 | AY628656 | Uncultured prasinophyte clone LS-E12 chloroplast | Plastid | [48] |
| EF648179 | 10 | 100 | AM921438 | Unc bacterium partial 16S rRNA gene, clone S5_116 | α Proteobacteria | |
| EF648180 | 11 | 100 | DQ184424 | Uncultured gamma proteobacterium clone SB04_1 | 16S | γ Proteobacteria | [43] |
| EF648181 | 13 | 100 | EF667989 | bacterium Antarctica-16 | γ Proteobacteria | |
| EF648182 | 14 | 100–99 | AF355039 | Uncultured delta proteobacterium Arctic95C-5 | δ Proteobacteria | [42] |
| EF648183 | 26 | 100 | DQ295238 | Uncultured marine bacterium Ant4E12 | Actinobacteria | [49] |
| EF648184 | 27 | 100 | AJ551107 | Psychrobacter sp. wp30 partial 16S RNA gene, isolate wp30 | Proteobacteria | |
| EF648185 | 29 | 99 | DQ668584 | Uncultured bacterium clone Arctic6-G12 | γ Proteobacteria | [50] |
| EF648186 | 30 | 99 | AF354606 | Uncultured gamma proteobacterium Arctic96BD-19 | γ Proteobacteria | [42] |
| EF648187 | 33 | 100 | AM921265 | Unc bacterium partial 16S rRNA gene, clone PB_53 | α Proteobacteria | |
| EF648188 | 34 | 100 | AM921120 | Unc bacterium partial 16S rRNA gene, clone E1_91 | α Proteobacteria | |
| EF648189 | 39 | 100 | AF469345 | Uncultured alpha proteobacterium CTD44B | Proteobacteria | [51] |
| EF648190 | 43 | 98–100 | DQ255854 | Uncultured marine bacterium clone ANT104A | γ Proteobacteria | [47] |
| EF648191 | 57 | 100 | AF419359 | Uncultured beta proteobacterium MoDE-9 | β Proteobacteria | [52] |
| EF648192 | 66 | 100 | AM921509 | Unc bacterium partial 16S rRNA gene, clone ST_68 | γ Proteobacteria | |
| EF648193 | 71 | 100 | EU005838 | Uncultured marine bacterium clone KG_A3_120m134 | γ Proteobacteria | [44] |
| EF648194 | 76 | 100 | DQ513056 | Uncultured bacterium clone CTD005-48-02 | α Proteobacteria | [53] |
| EF648195 | 79 | 99 | AY704387 | Uncultured bacterium clone CTD005-74B-02 | Bacteroidetes | [53] |
| EF648196 | 82 | 100 | DQ906724 | Uncultured marine bacterium clone AntCL1D8 | Proteobacteria | [47] |
| EF648197 | 87 | 100 | AY664361 | Uncultured Pseudoalteromonas sp. clone JL-BS-K75 | γ Proteobacteria | |
| EF648198 | 91 | 100 | AM921509 | Uncultured bacterium clone 4C230441 | α Proteobacteria | [54] |
| EF648199 | 93 | 98 | AY027805 | Aequorivita lipolytica Y10-2T | Bacteroidetes | [55] |
| EF648200 | 95 | 100 | DQ184430 | Uncultured gamma proteobacterium clone SB04_175 | γ Proteobacteria | |
| EF648201 | 96 | 98 | EU878158 | Unc phototrophic euk isolate DGGE gel band DL27-11 | Plastid | |
| EF648202 | 98 | 100 | DQ513065 | Uncultured clone CTD005-31B-02 | Actinobacteria | [53] |

*phyotypes found in both seasons. Ref. = References of the closest relative.

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in winter, whereas γ-Proteobacteria increased to 50%. In winter, we also found one representative of β-, δ-Proteobacteria and Actinobacteria (and three Eukarya plastids). Roseobacter [56] and the CFB group [57] are commonly associated to phytoplankton bloom; different Roseobacter clusters are well adapted to variable condition of DOM availability [44] and have been shown to account for ~20–25% of total bacterial production in summer [43–44]. CFB is also an important group in Antarctic marine waters and sea-ice microbial community, associated to algal blooms or high primary productivity [58]. So the dominance of α-Proteobacteria and CFB in summer can be related to the availability of freshly/recently produced organic matter. A higher relative abundance of γ-Proteobacteria in winter, and a parallel decrease of α-Proteobacteria, has been observed in the Arctic Ocean [42]. We were successful in culturing one of the γ-Proteobacteria phylotypes in winter (band 13, EF648181) which is associated to Allothermaliales. Also this group has been found only in winter in Arctic waters [42]. Other studies in the Southern Ocean analyzed alternatively the summer [44] or the winter community [47], and found that γ-Proteobacteria and α-Proteobacteria were similarly abundant. The differences can be due to the fact that [44] analyzed bacterial community inside and outside a natural iron fertilized summer bloom, above the Kerguelen plateau, Southern Ocean, whereas we sampled in late summer, at the end of the productive season. [47] sampled from a coastal station, that can be very different from the pelagic environment. Furthermore, the α-Proteobacteria phylotypes were associated mostly to Pelagibacter and SAR11.

In winter we also analyzed mesopelagic samples (200–750 m, Fig. 5b, Table 3). Only 3 out of 14 Bacteria phylotypes were common with the winter epipelagic phylotypes; the bands unique to the mesopelagic communities displayed 99–100% sequence similarity with uncultured environmental bacteria from Arctic and Antarctic Ocean or from deep-sea samples, from seawater surrounding hydrothermal vents (Table 2). In mesopelagic waters α and γ-Proteobacteria phylotypes alone accounted for 79% of community diversity (43 and 36% respectively). Our findings are consistent with the few data available for deep ocean bacterial community structure, that found different phylotypes, related to depth [39–60].

We might expect Archaea, which exploit different niches [39–40], to respond differently than Bacteria. In summer, in the epipelagic layer, we detected 6 archaeal phylotypes compared with 12 in winter (Fig. 6, Table 4). As was the case for Bacteria, archaeal community richness was also greater in winter than in summer. However, unlike Bacteria, all 6 summer archaea phylotypes were common to both seasons (Table 4). Five of 6 were Euryarchaeota and one was Crenarchaeota, and all 6 showed 97–98% sequence similarity to uncultured Arctic or Antarctic Archaea. Six Archaea unique to winter were all Euryarchaeota and showed 97–99% sequence similarity to uncultured Arctic and Antarctic Archaea. Of the 12 epipelagic Archaea in winter 9 were present also in the mesopelagic zones, where we also found 2 phylotypes unique to the mesopelagic zone (98% sequence similarity to uncultured Arctic Archaea).

The abundance and diversity of Archaea in the Southern Ocean, has been widely documented [39–40, 66]. Our results are in agreement with the evidence that both Crenarchaeota and Euryarchaeota are less diverse than marine bacterial phylogenetic group [67]. Previous studies by FISH reported that Crenarchaeota were the dominant group of Archaea in waters off Antarctica Peninsula [40], but a successive study in the Antarctic Circumpolar Current [38], with the same technique and a better protocol of permeabilization of cell wall, showed a higher percentage of Euryarchaeota between 500 and 1000 m, of about 10–20% of total prokaryotes, about twice the previous estimate. Also [63], from a clone library from Arctic samples detected a number of Groups II (Euryarchaeota) clones similar to the Group I (Crenarchaeota). [63] from DGGE analysis never retrieved a sequence of Crennoarchaeota. The authors attributed this to a mismatch in the universal 517r primer, used for DGGE. We detected one Crenarchaeota, and the band is also very intense when present. It is possible that the nested PCR that we performed, increasing selectively the abundance of template DNA, reduce this problem. We are aware of several potential limitations of DGGE, such as chimeras and heteroduplex formation or PCR biases [68], but this technique was very useful for our purposes of relative comparison between different assemblages.

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**Table 3.** List and parameters of stations sampled for bacterial community analysis.

| Station ID | Range of sampling depth (m) | Range of T (°C) | Chl a (µg L⁻¹) | Bacterial abundance (cell L⁻¹ × 10⁶) |
|------------|-----------------------------|-----------------|----------------|-----------------------------------|
| Summer     |                             |                 |                |                                   |
| 13         | 25–100                      | −0.1–3.1        | 0.12–0.20      | 0.8–1.9                           |
| 27         | 25–85                       | −0.7–2.6        | 0.10–0.13      | 0.9–1.2                           |
| 55         | 20–85                       | −0.2–3.0        | 0.08–0.14      | 1.6–2.0                           |
| 42         | 25–90                       | −0.2–3.1        | 0.20–0.67      | 1.5–1.9                           |
| 64         | 20                          | 3.1             | 0.60           | 2.2                               |
| 3          | 20                          | 1.9             | 0.74           | 3.4                               |
| 19         | 30–120                      | −0.1–1.6        | 0.05–0.63      | 1.4–1.8                           |
| 53         | 30                          | 1.5             | 0.59           | 3.7                               |
| 57         | 20                          | 0.7             | 0.32           | 1.8                               |
| Winter     |                             |                 |                |                                   |
| 178        | 5–750                       | −0.7–1.9        | 0.04–0.13      | 0.3–2.1                           |
| 196        | 5–750                       | −0.8–1.7        | 0.01–0.17      | 0.4–2.1                           |
| 197        | 5–750                       | −1–1.7          | 0.01–0.17      | 0.4–2.4                           |
| 220        | 10–150                      | −1.3–1.8        | 0.06–0.07      | 1.7–2.3                           |
| 229        | 10–750                      | −1.8–1.7        | 0.04           | 0.5–1.2                           |

*positive temperatures in summer at surface, in winter at depth.

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Both *Crenarchaeota* and *Euryarchaeota* have been shown to incorporate DIC [38] and this is consistent with our finding of DIC incorporation. The *Crenarchaeota* (EF640727) DGGE band was very intense in Shelf samples where DIC uptake was the highest. It would be interesting to determine if this *Crenarchaeota* is a significant autotrophic producer. Our results support the hypothesis that bacterial phylotypes are more affected by environmental variables associated with summer to winter transition than *Archaea* [40].

This study has shown that active and dynamic *Bacteria* and *Archaea* communities in Southern Ocean play a significant role in Antarctic food web and biogeochemical dynamics through the austral winter. They maintain substantial rates of secondary productivity during protracted periods of diminished primary productivity. They reset the system by organic matter decomposition and nutrient regeneration—setting the stage for the next summer. Our results have important implications for models of the functioning of Antarctic Ocean ecosystem and carbon cycle. The persistence of high microbial loop activity causing strong wintertime system net-heterotrophy is highly relevant to considerations of the efficacy of Southern Ocean iron enrichment to control the level of atmospheric CO₂.

**Materials and Methods**

Chlorophyll-α was determined by high performance liquid chromatography HPLC [69]. Net primary production was estimated by a modification of the Vertically Generalized Productivity Model (VGPM) [70]. The model was parameterized for the Antarctic temperature range considering a large dataset of $^{14}$C PP measurements, including ~30 stations for each of our cruises.

Bacteria, viruses and protists were counted by epifluorescence microscopy. For bacteria and protists, 10 mL formaldehyde fixed samples were DAPI stained (3 μg/mL final concentration) and filtered onto a 0.2 μm polycarbonate black filter (Nuclepore). For viruses, samples were fixed with 0.5% paraformaldehyde, and...
stored at −80°C until analysis. 1 to 3 mL of samples were filtered onto 0.2 μm Anodisc filter and stained with SybrGreen [71].

Heterotrophic production was measured in dark incubations at in situ temperature, in trace metal clean labware, by 3H-leucine incorporation (20 nM concentration; 117 Ci mmol−1) [72] in 4 hours incubations. The incorporated leucine was converted in seawater and exposed overnight to a fume of concentrated HCl. Samples were fixed with 4% final formaldehyde, filtered on 0.22 μm polycarbonate filters, rinsed twice with cold ethanol. Precipitated DNA was resuspended in MilliQ water and stored at −80°C until analysis. 1 to 3 mL of samples were filtered onto 0.2 μm Anodisc filter and stained with SybrGreen [71].

Shifts in bacterial community composition were analyzed by polymerase chain reaction (PCR) amplification of a portion of the bacterial 16S rRNA gene, followed by DGGE and sequencing. Bacterial community DNA was extracted from 500 to 1000 mL of seawater filtered onto 0.2 μm polycarbonate filters, rinsed twice with cold seawater and exposed overnight to a fume of concentrated HCl. Blanks (samples formaldehyde-fixed at T0) were subtracted from samples dpm.

In detail by [68]. After an initial denaturation for 5 min at 94°C, samples were amplified for 30 cycles by touchdown PCR [75]. For Archaea, 16S rRNA gene fragments were added to the tubes and incubated for 12 h at 55°C. The supernatant was transferred in a fresh 15 mL PPCO tube (NUNC). The filter was washed with 20,000 x g, for 20 min, at 4°C to pellet the DNA. The pellet was washed with 70% ice-cold ethanol. Precipitated DNA was resuspended in MilliQ water and quantified fluorometrically (PicoGreen; Molecular Probes).

For Bacteria, a 16S rRNA gene fragment was amplified by PCR using a Eubacteria set, consisting of a universal primer complementary to position 517 to 534 (5'-ATTACCGCGGCTGCT-3') and a bacterial primer complementary to position 341 to 190 bp long for the template was replaced with an equal volume of sterile water, was included in each batch of PCRs. For each cycle, denaturation was at 94°C for 1 min. Annealing was for 1 min at a temperature which decreased 1°C every two cycles from an initial 65°C to a final of 30°C. Primer extension was at 72°C for 3 min in each cycle, with a final 7-min extension following the last cycle. A negative control, in which the template was replaced with an equal volume of sterile water, was included in each batch of PCRs.

For Archaea, 16S rRNA gene fragments ~190 bp long for DGGE analysis were generated by nested PCR. The first PCR

| Accession # | Band number | % identity | Accession # | Closest relative | Phyllogenetic group | Ref. |
|-------------|-------------|------------|-------------|------------------|---------------------|-----|
| Summer 2004 |             |            |             |                  |                     |     |
| EF640719    | 15          | 98%        | DQ146734    | Uncultured archea clone iBS22f.80 | Euryarchaeota II.a | [61]|
| EF640720    | 16          | 97%        | EU486954    | Uncultured crenarchaeote clone FB04aw.90 | Crenarchaeota |     |
| EF640721    | 17          | 98%        | EF486592    | Uncultured euryarchaeote clone M0Sao39.03 | Euryarchaeota II.a | [62]|
| EF640722    | 18          | 97%        | EU199752    | Uncultured euryarchaeote clone SCICEX122424H3 | Euryarchaeota |     |
| EF640723    | 19          | 98%        | EU199730    | Uncultured euryarchaeote clone ANT95156E | Euryarchaeota |     |
| EF640724    | 20          | 98%        | AY288394    | Uncultured euryarchaeote 97D-131-22A | Euryarchaeota II | [63]|
| Winter 2006 |             |            |             |                  |                     |     |
| EF640725    | 100         | 98%        | EU199727    | Uncultured euryarchaeote clone Ant9406E8 | Euryarchaeota |     |
| EF640726    | 104         | 100%       | EF640719    | Uncultured marine archeaclone DPAR02 | Euryarchaeota |     |
| EF640727    | 106         | 100%       | EF640720    | Uncultured marine archeaclone DPAR03 | Crenarchaeota |     |
| EF640728    | 107         | 98%        | EU199759    | Uncultured euryarchaeote clone SCICEX1231220E10 | Euryarchaeota |     |
| EF640729    | 109         | 100%       | EF640723    | Uncultured marine archeaclone DPAR06 | Euryarchaeota |     |
| EF640730    | 114         | 100%       | EF640722    | Uncultured marine archeaclone DPAR05 | Euryarchaeota |     |
| EF640731    | 133         | 99%        | AY856360    | Uncultured archeaclone CTD005-2A | Euryarchaeota III | [64]|
| EF640732    | 139         | 100%       | EF640724    | Uncultured marine archeaclone DPAR07 | Euryarchaeota |     |
| EF640733    | 144         | 97%        | AY288408    | Uncultured euryarchaeote 97E-131-20 | Euryarchaeota | [63]|
| EF640734    | 147         | 99%        | AY288403    | Uncultured euryarchaeote 97D-235-10 | Euryarchaeota | [63]|
| EF640735    | 161         | 97%        | AF257277    | Uncultured marine euryarchaeote DH154-1W1 | Euryarchaeota II | [65]|
| EF640736    | 169         | 100%       | EF640721    | Uncultured marine archeaclone DPAR04 | Euryarchaeota |     |
| EF640737    | 174         | 98%        | AY288381    | Uncultured euryarchaeote 95B-131-15H | Euryarchaeota II | [63]|
| EF640738    | 175         | 98%        | AY288407    | Uncultured euryarchaeote 97E-131-15 | Euryarchaeota II | [63]|

*phylotypes found in both seasons. Ref. = References of the closest relative.
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was performed using specific 21f (TTC CGG TTG ATC CYG CCG GA) and 950r (GCC GGC GTT GAM TGC AAT T) primers at the same PCR conditions described by the author: 30 cycles with a denaturation step at 95°C for 1.5 min, an annealing step at 55°C for 1.5 min, and an extension step at 72°C for 1.5 min [76]. The resulting PCR products were used as templates for the second PCR, which was performed with an Archaea-specific 344f with a 40 bp GC clamp (underlined) (5'-GCCGGCCGCGGCCGC-GCGGGGCTGCCGCGGCGGGCCCACGCGGGCAGCGGGGCGGGA-3') and a universal 518r (5'-ATTACCG-GCGGCTGTGCTGG-3') [63], at the same reaction conditions used for Bacteria.

Between two hundred - five hundred nanograms of PCR product were loaded on 8% polyacrylamide gels (acrylamide-N,N'-methylenebisacrylamide [37:1]) containing denaturant gradients of 30 to 60% from top to bottom for Eubacteria and 20–55% for Archaea top to bottom (where 100% is defined as 7 M urea and 14% [vol/vol] formamide). Electrophoresis was performed with a hot-bath denaturing gradient gel electrophoresis (DGGE) unit (CBS Scientific, Del Mar, Calif.) using 0.5xTAE running buffer (20 mM Tris, 10 mM acetate, 0.5 mM Na2-EDTA, pH 8.2) at 60°C for 6 h at 200 V. Gels were stained for 30 min in SYBR Gold nucleic acid stain (1000x final concentration, Molecular Probes), destained for 10 min in 0.5xTAE, and photographed with UV transillumination. DGGE bands were excised using a sterile razor blade, and DNA was eluted overnight at 37°C in 200 µl of 1x SSC buffer (0.6 M NaCl, 60 mM trisodium citrate, pH 7). The eluant was centrifuged briefly to pellet acrylamide fragments. The supernatant was precipitated with 1/10 volume of LiCl and 3 volumes of ethanol, resuspended in 20 µl of Milli Q water and cloned using the Original TA cloning kit or the TOPO TA cloning l of Milli Q water and DNA was eluted overnight at 37°C for 6 h at 200 V. Gels were stained for 30 min in SYBR Gold nucleic acid stain (1000x final concentration, Molecular Probes), destained for 10 min in 0.5xTAE, and photographed with UV transillumination. DGGE bands were excised using a sterile razor blade, and DNA was eluted overnight at 37°C in 200 µl of 1x SSC buffer (0.6 M NaCl, 60 mM trisodium citrate, pH 7). The eluant was centrifuged briefly to pellet acrylamide fragments. The supernatant was precipitated with 1/10 volume of LiCl and 3 volumes of ethanol, resuspended in 20 µl of Milli Q water and cloned using the Original TA cloning kit or the TOPO TA cloning kit (Invitrogen), following the instructions of the vendor. Insert-containing clones were identified by agarose gel electrophoresis after plasmid EcoRI digestion. DGGE profiles of reamplified, cloned DNA were used to check for heteroduplexes [77] and to confirm the positions of cloned bands relative to the original sample as described previously [60]. Unidirectional sequencing was performed by Agencourt, using M13 primers. Sequences were aligned to known sequences using BLAST (basic local alignment search tool) [78] All sequences were analyzed by the program CHECK_CHIMERA from the Ribosomal Database Project (RDP) [79]. The sequences submitted to GenBank are available under accession numbers listed in Table 2 and Table 4.

POC was measured with a CHN elemental analyzer, from samples collected by filtering 1–2 liters of seawater onto pre-combusted GF/F filters and stored in liquid nitrogen until analysis [4]. TOC samples were acidified (pH 2) with ultrapure HCl and stored in precombusted glass vials at 4°C until analysis with a Shimadzu TOC 5000A.

Statistical differences were tested with non parametric tests, with the software Statistica 6.0 (Statsoft); Kolmogorov-Smirnov test was applied to test differences between means and Spearman R to correlate two variables.

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Author Contributions

Conceived and designed the experiments: MM FM FA. Performed the experiments: MM FM TJ S BGM HW. Analyzed the data: MM FM BGM HW. Contributed reagents/materials/analysis tools: BGM FA. Wrote the paper: MM FM FA.

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