

Carbon demand, utilization, and metabolic diversity of bacterioplankton in the frontal regimes of the Indian sector of the Southern Ocean

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Abstract Bacterial production, respiration and metabolic diversity were measured up to 120 m depth in the Sub-Antarctic Front (SAF) and Polar Fronts I and II (PFI and PFII) of the Indian Ocean sector of the Southern Ocean during 2010 Austral Summer. Prokaryotic cell count was maximum at PFI and PFII ($\sim 10^9$ cells L^{-1}) and minimum at SAF ($\sim 10^7$ cells L^{-1}). Furthermore, integrated bacterial production was higher at PFI (1.07 mg C m^{-2} h^{-1}) and PFII (0.72 mg C m^{-2} h^{-1}) compared to SAF (0.61 mg C m^{-2} h^{-1}). At PFII, integrated bacterial growth efficiency was higher (8.96) compared to PFI (7.42) and SAF (7.17), signifying that the net contribution of PFII to the microbial loop could be relatively pronounced. Enhanced cell numbers and production at polar fronts indicate that the dissolved organic matter could be converted to secondary biomass through the microbial loop. However, integrated bacterial respiration rate at PFII (0.83 mg C m^{-2} h^{-1}) was lower than that at PFI (1.84 mg C m^{-2} h^{-1}) resulting in higher growth efficiency at PFII. Metabolic flexibility at SAF was clearly brought about by utilization of carboxylic acids like D-malic acid and itaconic acid, and carbohydrates like N-acetyl D-glucosamine, D-cellobiose and D-lactose. Utilization of amino acids like glycyl L-glutamic acid and L-threonine, and an amine, phenylethylamine, was critical in determining the metabolic variability at PFI. PFII hosted microbes that utilized phenolic compounds

(2-hydroxy benzoic acid and 4-hydroxy benzoic acid) and polymers (like Tween 80). Utilization of polyols over carbohydrates in polar waters indicates a niche with lesser influence of the Antarctic melt waters on the bacterioplankton metabolism.

Keywords Bacterial production · Respiration · Carbon demand · EcoPlateTM · Southern Ocean

Introduction

Frontal systems separate water masses of different characteristics. In the Southern Ocean, at the north of the subtropical convergence ($\sim 41^\circ S$), the conditions are warmer and oligotrophic (Lutjeharms 1985; Laubscher et al. 1993) while further south, the Sub-Antarctic and Polar fronts are colder and rich in nitrate and phosphate with low concentrations of dissolved organic matter (DOM) in the latter. The Southern Ocean is among the world's most upwelled oceans, but the strong downwelling counters and returns the bulk of the macronutrients to the ocean interior. In warmer oceans, low concentrations of macronutrients clearly limit biomass accumulation, whereas in strongly upwelled regions, nutrients are supplied from deeper layers which are relatively stripped of trace elements like iron (Smetacek et al. 2004). Low phytoplankton productivity in the Southern Ocean is a result of unfavorable light, trace element deficiency, and high grazing pressure (Chisholm and Morel 1991). The lower trophic levels dominated by autotrophic pico- and nanoplanktons, bacteria, and heterotrophic protists contribute roughly to one-third of the total biomass (Knox 1994). The organic matter produced by pico- and nanoplankton is channeled by protozoa to the top predators while the rate of export of organic carbon influences the nutrient supply to extra-polar ocean and scavenges carbon

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dioxide from surface waters. The contemporary Southern Ocean plays a minor role in the global carbon cycle (Tréguer and Pondaven 2002) because its characteristic microbial community recycles the bulk of organic matter within the winter mixed layer (Smetacek et al. 2004). Several studies from the Southern Ocean report that DOM-enriched sea-ice could regulate the bacterial growth and efficiency (Kahler et al. 1997; Thomas and Dieckmann 2002). Computations of total production indicate that the magnitude of turnover by the microbial network and zooplankton is substantial and that a significant amount of biomass could be available for channeling to higher trophic levels. Many microbes are capable of either taking or regenerating inorganic nutrients in aquatic ecosystems, but heterotrophic bacteria are uniquely involved in both processes (Kirchman 2000). This makes the study of both anabolic and catabolic reactions involving microbes pertinent, more specifically that of the bacteria. The abundance and major bacterial taxa encountered in the Southern Ocean have been widely reported from the other oceans as well (Fenchel 2002; Arrieta et al. 2004). Bacteriophages (Pearce and Wilson 2003) and heterotrophic nanoflagellates (Becquevort et al. 2000) control the abundance of bacteria and maintain near-constant numbers by density-dependent grazing pressure and infections by pathogens (Bonilla-Findji et al. 2008; Evans and Brussaard 2013). Since the abundance is relatively constant, the efficiency of remineralization is critical in holding the nutrients in the mixed layer (Smetacek et al. 2004).

Measurements of bacterial production quantify the amount of dissolved organic carbon transformed into biomass, but do not estimate the amount metabolized. Measurements of bacterial respiration need to be coupled with productivity estimates to compute the flux of dissolved organic carbon through bacteria and to understand the efficiency of the microbial loop in the production of secondary biomass. However, there is a considerable gap in the understanding of the nature of substrates critical in determining the metabolic demand of bacterioplankton. This could play a determinant role in the pathway of carbon cycling and efficiency of the biological pump (Sala et al. 2010). Our main aim in this study was to determine prokaryotic metabolism at the three fronts across a latitudinal gradient in the Indian sector of the Southern Ocean. Regions closer to the Antarctic continent could be influenced by algal exudates associated with melting of sea ice. Nakano (1996) has experimentally demonstrated that bacteria respond to extracellular dissolved organic carbon released from diatoms. Sea-ice melting could release considerable amount of organic aggregates largely composed of polysaccharides and this could result in carbohydrate-dominated bacterial metabolism (Sala et al. 2010). Hence, we hypothesize that the bacterioplankton in the polar fronts could be under significant influence of carbohydrate-based metabolism.

Materials and methods

Study site and water column parameters

Oceanographic Research Vessel (ORV) Sagar Nidhi in her maiden expedition to the Southern Ocean during the Austral summer of 2010 served as a platform to perform several physical and biogeochemical measurements. Figure 1 illustrates some of the major fronts along a meridional transect (57° 30' E) from 45 to 56°S that were identified by the sea surface temperature (SST) according to Ansoorge et al. (2009). A vertical profile was collected at frontal regions up to 120 m using portable CTD (make SBE 9/11 plus, Sea-Bird Electronics, USA). Water samples were collected from standard depths (0, 10, 30, 50, 70, 100, 120 m) at the Sub-Antarctic Front (SAF), Polar Front I (PFI), and Polar Front II (PFII) using INDRONAUT CTD rosette system. Dissolved nitrite, nitrate, phosphate, and silicate were analyzed spectrophotometrically by standard protocols (Grasshoff 1983). Dissolved oxygen was estimated by the Winkler method (Carpenter 1965).

Total cell count

Prokaryotic cells in the water samples (10 mL) collected from all the discrete depths at the three fronts (SAF, PFI, and PFII) were stained with 4', 6-diamidino-2-phenylindole (DAPI) (Hicks et al. 1992) before enumeration. Cells stained with DAPI were first fixed with filter-sterilized, particle-free buffered formaldehyde (final concentration, 3.7 %) to preserve the cell morphology and improve the staining efficiency. Stained cells were filtered onto 0.22 µm black polycarbonate membranes (Nucleopore, Whatman) and counted under an

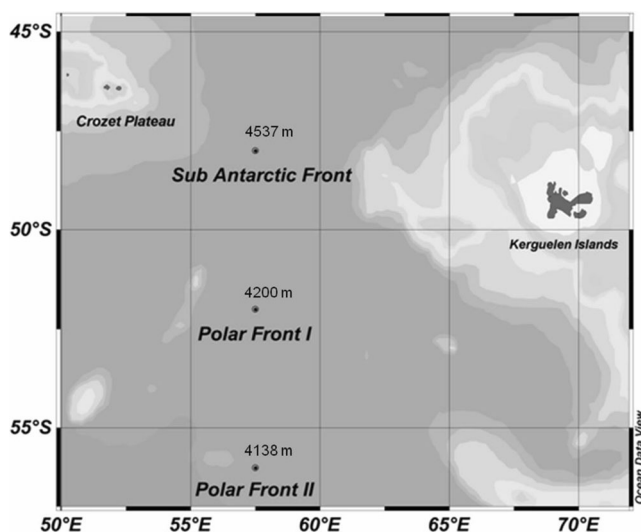


Fig. 1 Location of the stations selected for water sampling during the Indian Expedition to Southern Ocean (2010). Bottom depths (m) are indicated adjacent to the stations

epifluorescence microscope (Olympus BX 51). The filters used for DAPI were Olympus U-MWU2 (Excitation 330–385 nm and Emission 420 nm). Counting was done using a Whipple grid at 100X (Olympus UPLNFLN). Autotrophic pico- and nanoplanktons were discriminated from heterotrophic prokaryotes by the red and orange auto fluorescence observed under blue light excitation (Filter U-MWB2, Excitation 460–490 nm and Emission 520 nm). The total counts presented in this paper pertain to heterotrophic prokaryotes, including Archaea.

Bacterial production, respiration, carbon demand, and growth efficiency

Bacterial production (BP, or more precisely heterotrophic prokaryotic production) was estimated from the rates of protein synthesis (Kirchman 1993) as measured by the incorporation of ^{14}C leucine (specific activity=300 mCi mmol $^{-1}$; BARC, Bombay). Leucine was added at a final concentration of 40 nM to 20 mL of the sample in triplicate and incubated for a period of 1 h at near to ambient temperatures. The activity was stopped by adding 5 % ice-cold trichloro acetic acid (TCA). The samples were then filtered through a 0.22 μm membrane filter (Millipore, GS type) and rinsed with 5 % ice-cold TCA for 5 min, followed by 80 % cold ethanol. One dried filter was then placed in each scintillation vial, filled with 5 mL of dioxan-based liquid scintillation cocktail (Spectrochem), and the radioactivity of each was measured in a scintillation counter (LKB Wallac 1209). A pre-killed sample was used as the blank. Bacterial production was calculated using the conversion factor of 3.1 kg C mol $^{-1}$ of leucine incorporated (Lemée et al. 2002). Production was estimated every 6 h for 24 h. Since it was linear, the time-integrated value is presented here; expressed in mg C m $^{-2}$ h $^{-1}$.

Bacterial respiration (BR) was estimated from the oxygen consumption in filtered (0.8 μm polycarbonate filter, Millipore) water samples (Jensen et al. 1990; Knap et al. 1996) for 72 h at 24-h intervals. The samples were incubated at near to ambient temperatures. Since complete separation from remaining plankton is seldom achieved, a variable fraction of the measured respiration could also be due to other microbial planktonic communities (Robinson 2008). Respiration rate was calculated by measuring dissolved oxygen (DO) in BOD bottles (in triplicate) before and after the incubation of water samples using Winkler's method. For control (0 h), the samples were fixed immediately with Winkler's reagents. Total respiration was calculated as micromolar oxygen consumed per liter per hour by subtracting the amount of dissolved oxygen remaining after the incubation time. The oxygen used up was converted to the carbon respired, assuming a respiratory quotient of 1 (Bonilla-Findji et al. 2008). Bacterial carbon demand (BCD) was calculated

as $\text{BCD} = \text{BP} + \text{BR}$ and bacterial growth efficiency (BGE) as $\text{BGE} = \text{BP}/(\text{BP} + \text{BR})$.

Community-level physiological profiling using EcoPlateTM

Utilization of carbon sources by the bacterial communities were monitored using a Biolog EcoPlateTM (Biolog Inc., USA) containing 31 carbon sources in triplicate and three blanks without any carbon source. The 31 substrates used in the plate can be grouped into six categories: carbohydrates, amino acids, polymers, amines, phenolic compounds, and carboxylic acids. A list of the substrates and categories used in the Biolog EcoPlateTM can be found in Sala et al. (2010). Utilization of any carbon source by the bacteria results in the respiration-dependent reduction of the tetrazolium indicator dye which could be quantified spectrophotometrically. Negative wells remain colorless, as does the reference well having no carbon source. Biolog plates were inoculated with 150 μL of sample in each well, and measurements were carried out for 15 days at near to ambient temperatures. Absorbance was measured at 595 nm in a microplate reader (BMG Fluostar Optima) at 24-h intervals. The plate measurement was considered optimum when the absorbance plateaued for all the wells. While processing this primary data, the average well color development (AWCD) was calculated for each plate to compensate for the influence of inoculum density. $\text{AWCD} = \sum (\text{R}-\text{C})/31$ where R is the absorbance of each well and C is the absorbance of the control well. The primary data was normalized following the equation $(\text{R}-\text{C})/\text{AWCD}$ and averaged for 15 days before subjecting to statistical analysis. Water samples collected from the SAF, PFI, and PFII (depths as given for the water column parameters) were analyzed for obtaining the utilization pattern of the carbon sources.

Statistical analysis

Statistical analyses were carried out using Pearson's Correlation Coefficient and Analysis of Variance in order to explore the possible associations and variances existing between and within different variables. Two-way ANOVA was performed to assess the role of depth as a possible source of variation in the three fronts. This analysis was performed using the statistical program SPSS Statistics 17.0. Bacterial abundance was normalized by log transformation before analysis. Hierarchical Cluster Analysis (HCA) was used to group the discrete sampling points on the basis of similarity in the utilization pattern of carbon substrates. Results of HCA were subjected to factorial analysis using Principal Component Analysis (PCA) to compare the physiological profiles of bacterial communities. PCs were ranked according to the amount of variance of the original samples. The first two PCs accounted for the greatest variance in the samples, hence are discussed. The average of normalized values of carbon

substrate utilization was correlated to these PCs to find the substrates which are characteristically utilized in the three fronts. Bonferroni correction was applied to these correlation coefficients to deduce the most significant correlations. HCA and PCA were done using the statistical software Tanagra 1.4.17. Multivariate analysis of variance (MANOVA) was performed using the statistical software R-2.11. The aim was to evaluate the variability in utilization of various carbon substrates (normalized with AWCD and averaged for the entire period of measurement) among the fronts and depths.

Results

Environmental parameters

Temperature and Salinity: There was significant variation in the temperature profiles among the fronts (7.76×10^{-6} , $df=2$) and depths ($p=4.3 \times 10^{-4}$, $df=6$). At the SAF, PFI, and PFII, the mixed layer was shallow and reached <25 m (Fig. 2a–c). The salinity values (Fig. 2a–c) at SAF (~33.7) were comparable to PFI and PFII (~33.9). Significant variation was observed in the salinity values among the three fronts ($p=1.8 \times 10^{-10}$, $df=2$).

Dissolved Oxygen and Nutrients: There was a progressive increase in the DO content in various frontal regimes south of the SAF and ranged between 6.3 to 7.5 mL L⁻¹ (Fig. 3a–c). Values varied significantly among the frontal systems ($p=1.4 \times 10^{-8}$, $df=2$). There was also a significant variation ($p=5.4 \times 10^{-5}$, $df=2$) in nitrate accumulation within the frontal systems. The concentration ranged from 18.4 to 40 μM with

the maximum at the PFII (Fig. 3a–c). Overall, a significant variation was observed in the vertical distribution of nitrate ($p=0.03$, $df=6$). Phosphate concentration varied with depth at all the fronts ($p=0.03$, $df=6$). Though of low magnitude, a significant variation in the distribution of phosphate was observed ($p=0.03$, $df=2$) among the three fronts (Fig. 3a–c). The ambient phosphate and nitrate showed strong correlation at the PFI ($r=0.69$, $p<0.05$, $n=7$) and PFII ($r=0.72$, $p<0.05$, $n=7$). Vertical profiles of silicate also varied significantly ($p=8.3 \times 10^{-3}$, $df=2$) among the fronts (Fig. 3a–c). Considerable enrichment of silicate (19.1±8.2 μM) was noticed at deeper depths at PFII (Fig. 3d). A significant variation was observed in the vertical distribution of silicate between the PFI and PFII ($p=0.008$, $df=6$).

Total cell count

Figure 4a–c illustrates the vertical profile of prokaryotic cell counts enumerated at the frontal regimes. At the SAF, the vertical distribution of cells varied on several orders (ranged up to 10⁷ cells L⁻¹, Fig. 4a). At PFI and PFII, the distribution was more or less uniform and was on the order of 10⁹ cells L⁻¹ (Fig. 4b and c). In spite of similarity in the vertical distribution pattern, the abundance varied significantly ($p=4.5 \times 10^{-8}$, $df=2$) among the fronts.

Bacterial production, respiration, carbon demand, and growth efficiency

Bacterial production: Integrated bacterial production (Fig. 4a–c) was lower at SAF (0.61 mg C m⁻² h⁻¹) while the highest production rate was recorded at PFI (1.07 mg C m⁻² h⁻¹)

Fig. 2 Temperature (—) and salinity (—) profiles from the **a** Sub-Antarctic Front, **b** Polar Front I, and **c** Polar Front II

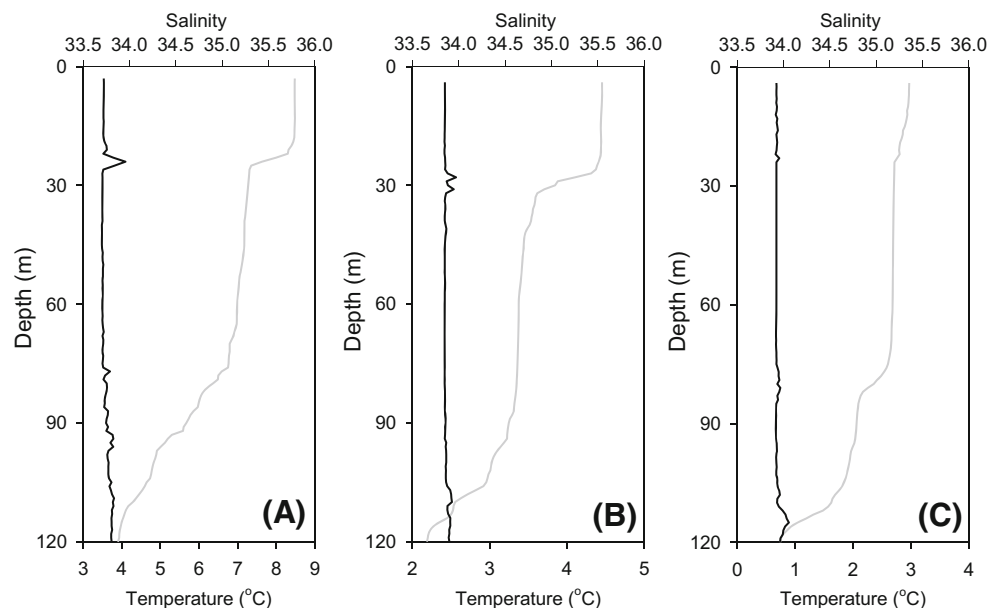
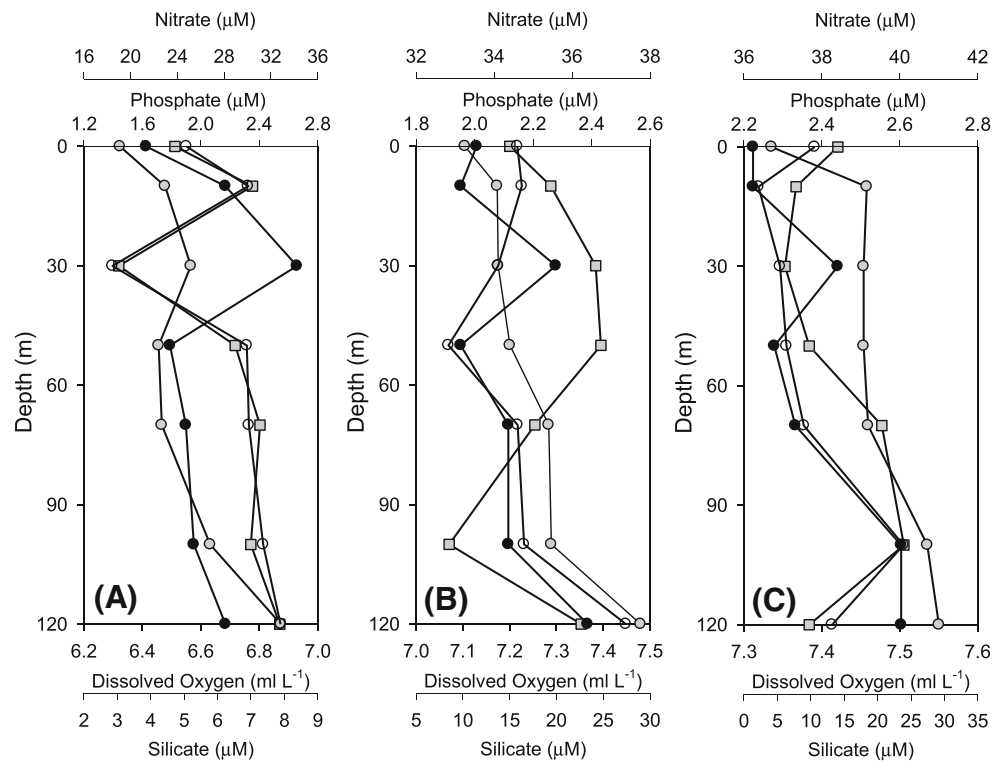


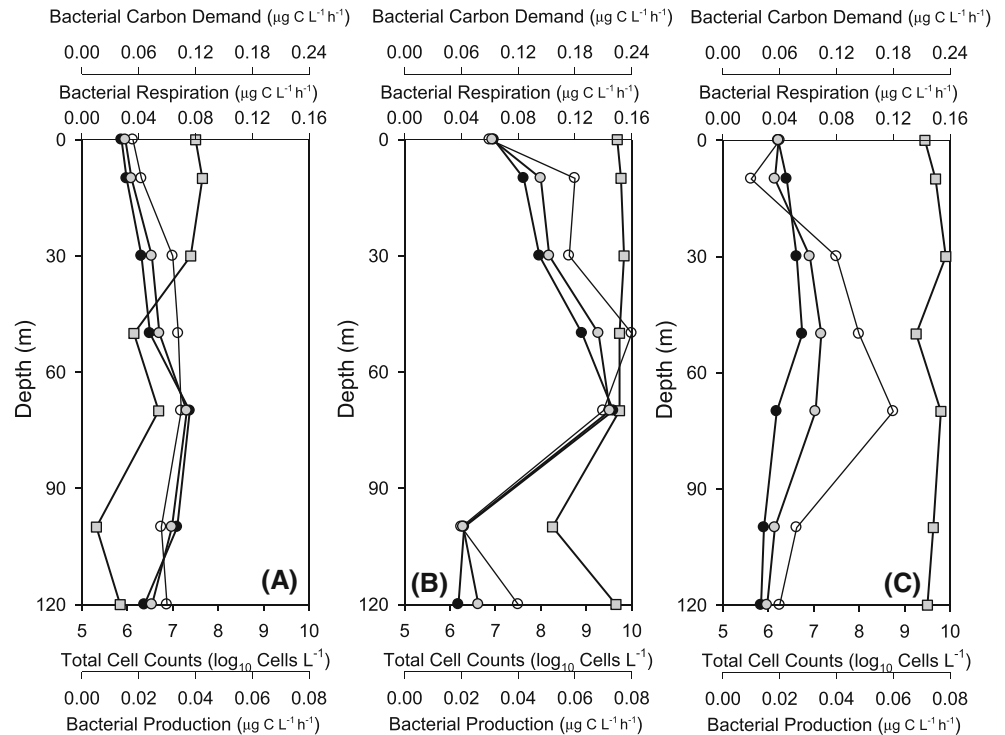
Fig 3 Vertical profiles of nitrate (○-○), phosphate (●-●), silicate (◻-◻) and dissolved oxygen (■-■) in the **a** Sub-Antarctic Front, **b** Polar Front I, and **c** Polar Front II



followed by PFII ($0.72 \text{ mg C m}^{-2} \text{ h}^{-1}$). At SAF, the bacterial production increased from 10 to 70 m and then decreased downwards to 100 m. The peak of bacterial production at PFI and PFII was observed at 50 and 70 m, respectively. It was also noted that bacterial

production at 100 m was comparable for all the fronts. Though of low magnitude, there was significant variation in the bacterial production between the fronts ($p=0.04$, $df=2$). A significant variation was also observed along the depths ($p=0.02$, $df=6$).

Fig 4 Vertical profiles of bacterial production (○-○), bacterial respiration (●-●), bacterial carbon demand (◻-◻), and total cell counts (■-■) in the **a** Sub-Antarctic Front, **b** Polar Front I, and **c** Polar Front II



Bacterial respiration: The highest respiration rate ($1.84 \text{ mg C m}^{-2} \text{ h}^{-1}$) was recorded at PFI (Fig. 4b). Respiration rate was the lowest at PFII ($0.83 \text{ mg C m}^{-2} \text{ h}^{-1}$) while it was $1.14 \text{ mg C m}^{-2} \text{ h}^{-1}$ at SAF (Fig. 4c and a). Unlike the PFII, the SAF showed a gradual increase in rates from surface to deeper depths. Similar to bacterial production, respiration rates were also significantly different between the three fronts ($p=0.01$, $df=2$).

Bacterial carbon demand and growth efficiency: BCD ranged from 0.04 to $0.22 \text{ } \mu\text{m C L}^{-1} \text{ h}^{-1}$ and varied significantly among the fronts ($p=0.01$, $df=2$). The overall range of BGE among the three fronts was 0.18 and 0.61 . The PFI exhibited the highest carbon demand with an integrated value of $2.91 \text{ mg C m}^{-2} \text{ h}^{-1}$ (Fig. 4b) while PFII was found to be more efficient as compared to other fronts and had the lowest carbon demand ($1.55 \text{ mg C m}^{-2} \text{ h}^{-1}$, Fig. 4c). Integrated BCD at SAF was $1.75 \text{ mg C m}^{-2} \text{ h}^{-1}$ (Fig. 4a). Column-integrated values (Table 1) showed that BGE was the highest at the PFII (8.95) while it was comparable at the SAF (7.17) and PFI (7.42). Carbon demand at the PFII (Fig. 4c) gradually increased up to 50 m and then declined at deeper depths. Overall, BCD exhibited significant variation along the depths ($p=0.03$, $df=6$) at all the fronts.

Utilization of sole carbon sources

HCA of the entire data resulted in five clusters together accounting for 9.5 , 23.8 , 38.6 , 14.3 , and 23.8 % of the total variability (Fig. 5). The first and fourth clusters correspond to the samples only from SAF and PFII, respectively. Of the samples from SAF, 28.6 % belonged to the first cluster while 42.9 % of the PFII samples belonged to the fourth cluster. The SAF samples in the first cluster were characterized by utilization of amino acids like L-arginine, L-phenylalanine, and L-serine, and polymers like glycogen, Tween 40, and cyclodextrin. The fourth cluster representing 42.9 % of the samples from PFII, was distinguished by utilization of phenolic compounds like 2-hydroxy benzoic acid and 4-hydroxy benzoic acid followed by Tween 80, a polymer, and i-erythritol, a polyol. The second cluster contained samples from SAF (14.3 %), PFI (28.6 %), and PFII (28.6 %), and it was

Table 1 Estimates of bacterial growth efficiency at various sampling depths at the three fronts

Depth (m)	SAF	PFI	PFII
0	0.39	0.32	0.34
10	0.40	0.42	0.18
30	0.43	0.38	0.43
50	0.41	0.39	0.46
70	0.32	0.32	0.61
100	0.29	0.32	0.47
120	0.40	0.51	0.43
Integrated	7.17	7.42	8.96

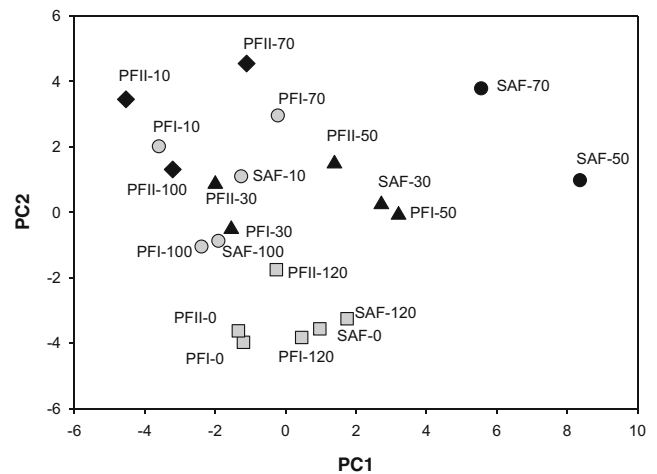


Fig 5 Score plot for PC1 against PC2 of Biolog Ecoplate™ data obtained from Southern Ocean fronts; Cluster 1 (●), Cluster 2 (▲), Cluster 3 (■), Cluster 4 (◆), and Cluster 5 (○). Sampling points can be identified with name of the front and depth (m)

characterized by utilization of D-mannitol, a polyol, and amines like putrescine and phenyl ethylamine. The third cluster comprised samples from SAF, PFI, and PFII, each contributing to 28.6 % of the total samples in the cluster. This cluster was characterized by the utilization of amino acids like L-threonine, glycyl L-glutamic acid, and L-serine. From SAF and PFI, 28.6 and 42.9 % of the samples belonged to the fifth cluster, respectively. This cluster was characterized by utilization of acetyl glucosamine, an amine, and i-erythritol, a polyol, followed by utilization of carbohydrates like cellobiose, xylose, and lactose.

Results of PCA showed that at SAF, PFI, and PFII, the first two principal components (PCs) accounted for 60 % variance in the data. In order to relate the utilization of individual substrates with the sole carbon source utilization patterns, correlation between the substrate variables and PCs were examined. A significant correlation is an indication that the substrate is more important in differentiating the samples (Schultz and Ducklow 2000). There was a considerable change in the metabolic capabilities at SAF with significant correlations with PC1 (Table 2), limited to most of the carboxylic acids (D-malic acid, itaconic acid, etc.) and a few carbohydrates like N-acetyl D-glucosamine, D-cellobiose, and α -D-lactose. This scenario further changed at PFI with significant correlations restricted only to two carbohydrates, i.e. i-erythritol and N-acetyl D-glucosamine (PC1), and a lesser extent to D, L- α -glycerol phosphate and D-galactonic acid- γ -lactone (PC2). The strong correlation shown by the amino acids (glycyl-L-glutamic acid and L-threonine) with PC1 was unique to PFI. At PFII, none of the carbohydrates showed a significant correlation with PC1 while strong correlations were exhibited by polymers, phenolic compounds, and a few carboxylic acids. Results from MANOVA (Table 3) indicated that maximum variability in the microbial metabolism ($p<0.0001$) between the fronts could

Table 2 Pearson's correlation coefficients between various carbon sources and principal components (PC1 and PC2) at discrete sampling points ($n=7$) in the three fronts. Only correlations with $p<0.05$ are shown. Asterisks indicate probabilities that are also below the Bonferroni-corrected probability equivalent to $p=0.05$ (which for this set of correlations is $p=0.007$)

Substrate	PC1 (<i>r</i> value)			PC2 (<i>r</i> value)		
	SAF	PFI	PFII	SAF	PFI	PFII
Carbohydrates						
D,L- α -Glycerol phosphate					-0.73	
D-Galactonic acid- γ -lactone					0.73	
I-Erythritol		-0.73		-0.77		
D-Xylose				-0.76		
N-Acetyl D-glucosamine	-0.87	-0.83				-0.74
D-Cellobiose	-0.85					
α -D-Lactose	-0.78					
Carboxylic acids						
D-Glucosaminic acid	-0.86		0.73		-0.86	
D-Malic acid	0.96*		0.79		-0.87	
Itaconic acid	0.89*		0.73		0.91*	
Pyruvic acid methyl ester	-0.77				0.93*	
D-Galacturonic acid	-0.87				-0.76	
Ketobutyric acid	-0.7					
γ -Hydroxybutyric acid	-0.78					
Amino acids						
Glycyl-L-glutamic acid		0.83		0.78		0.88
L-Threonine		0.73		0.73		0.82
L-Asparagine			-0.84		0.75	
Amines						
Phenylethylamine		0.83				0.73
Putrescine						0.79
Polymers						
Tween 80			-0.79			
Tween 40			-0.82			
Glycogen			-0.78			
Phenolic compounds						
2-Hydroxy benzoic acid			-0.82			
4-Hydroxy benzoic acid			-0.8			

be attributed to the utilization of polymers (like Tween 40, Tween 80, glycogen) and phenolic compounds (like 2-hydroxy benzoic acid and 4-hydroxy benzoic acid), while

utilization of amino acids like L-arginine, L-asparagine, and L-phenylalanine varied considerably ($p<0.0001$) between the frontal depths. Thus, results from PCA provide a clear

Table 3 Significance of the F-statistic derived using MANOVA. Independent variables include: fronts (SAF, PFI, and PFII) and depths (0, 10, 30, 50, 70, 100, and 120 m). The a priori α -level for determining significance was set at 0.05. Significance codes: 0 '***', 0.001 '**' and 0.01 '*'

Substrate	L-Arginine	L-Asparagine	L-Phenylalanine	L-Serine	Glycyl-L-glutamic acid
Front	–	–	–	–	–
Depth	17.7***	6.4**	67.5***	30.1***	35.9***
Substrate	D-Mannitol	2-Hydroxy benzoic acid	4-Hydroxy benzoic acid	Tween 80	Tween 40
Front	–	24.6***	25.7***	9.5***	17.2***
Depth	17.9***	–	–	–	–
Substrate	L-Threonine	Phenylethylamine	Putrescine	α -Cyclodextrin	Glycogen
Front	–	–	–	17.2***	18.6***
Depth	29.3***	10.0***	8.3***	–	–

indication on the metabolic flexibility of microbes at SAF, while MANOVA indicates that the maximum variability in the microbial metabolism between the fronts could be attributed to the utilization of polyols and that between the frontal depths could be due to amino acids (Table 3).

Discussion

Bacterial carbon demand in the Southern Ocean fronts: processes and controls

The three fronts viz. SAF, PFI, and PFII selected for the investigations on bacterial carbon demand were unique and exhibited typical signature traits in terms of in situ temperature and salinity. Phytoplankton biomass is reported to be highly variable in the study region and ranged from 0.9 to 7.5 mg Chl *a* m⁻² (Pavithran et al. 2012). In general, it could be observed that dissolved oxygen, nitrate, phosphate, and silicate in the water column tend to increase southwards. Though bacterial remineralization plays a key role in regulating nutrient availability, the organic material available, their preference, and uptake efficiency are critical in gauging the impact of bacterial contribution to the nutrient pool.

From an ecological perspective, bacterial production is secondary in nature (Fuhrman and Azam 1980) as they convert existing organic molecules into their own biomass and oxidize some of these to final metabolic end products. Bacteria play a pivotal role in the conversion of available dissolved organic carbon into particulate form, thereby promoting production at higher levels. Hence, quantifying this potential in high-nutrient, low-chlorophyll (HNLC) zones and polar waters is vital in examining the factors sustaining biomass based on secondary production. Berggren et al. (2010) have suggested that metabolic responses of aquatic bacterial communities to temperature variations are strongly dependent on the level of growth limiting inorganic nutrients. As observed in the present study and also reported by others (Church et al. 2000), the concentration of major nutrients tends to increase along the southerly gradient and this could have significant impact on the bacterial abundance and carbon demand. The bacterial carbon demand was the highest at PFI followed by SAF and PFII. Enhanced prokaryotic cell numbers (10⁹ cells L⁻¹) and bacterial production at PFI (1.07 mg C m⁻² h⁻¹) and PFII (0.72 mg C m⁻² h⁻¹) indicate that the DOM pool could be efficiently converted to secondary biomass by microbial loop in the polar waters. However, the bacterial respiration rates at PFII (0.83 mg C m⁻² h⁻¹) were lower than PFI (1.84 mg C m⁻² h⁻¹), indicating that the former's net contribution to the microbial loop could be comparatively pronounced. The amount of available organic material and availability of inorganic nutrients are thought to play an

important role in regulating BGE (del Giorgio and Cole 1998). Our estimates of BGE are comparable to other estimates from the Southern Ocean (Bjornsen and Kuparinen 1991; Carlson et al. 1999). BGE was found to increase along the southerly gradient from SAF (7.17) to PFII (8.95). The highest BGE estimated at PFII again demonstrates the efficiency of polar waters in the production of secondary biomass through microbial loop. Recently, Pavithran et al. (2012) had highlighted the significance of microbial food web at PFII based on the elevated number of bacterial cells. However, the role of heterotrophic nanoflagellates (Becquevort et al. 2000) in controlling the abundance of bacterioplankton, non-living particulates, and DOM, and the effect of marine viruses on bacterial production and respiration (Bonilla-Findji et al. 2008) cannot be ruled out. Recent reports indicate that a very low percentage of primary production is utilized by bacteria in polar systems (Kirchman et al. 2009; Ducklow et al. 2012) and this could be an indication of the phytoplankton-bacteria uncoupling, resulting in the incidence of lower rates of bacterial respiration. Kogure et al. (1986) have demonstrated that bacterial production in the Antarctic waters ranged from 0.003–0.03 μg C L⁻¹ h⁻¹ and bacterial abundance and production rate decreased with depth. Our estimates of integrated BP are in agreement with the recent reports on the bacterial carbon metabolism in the Southern Ocean (Teira et al. 2012). In the present study, bacterial production appears to be unlimited by any of the factors examined at SAF and PFI. However, at PFII, bacterial productivity was positively correlated with dissolved oxygen ($r=0.72$, $p<0.05$, $n=7$) and total cell counts ($r=0.66$, $p<0.1$, $n=7$). From an assessment of carbon demand in these fronts, it is explicit that in addition to bacterial abundance, respiration rate is also vital in understanding the efficiency of microbial food web. Contribution by bacteria to the total nutrient pool could be evaluated from the bacterial respiration rate which is a direct indication of remineralization capability; the input could be minimal at PFII and maximum at PFI.

Bacterial catabolic diversity and its implications

Biolog microplates were initially designed for bacterial identification. The increasing requirement to characterize environmental samples has led to the development of Biolog EcoplateTM to assess the functional diversity of microorganisms in the environment (Insam 1997). This assay could be paralleled to an enrichment incubation scaled down to micro level where growth on 31 carbon sources is tested simultaneously in triplicates. Though this technique has limitations (Preston-Mafham et al. 2002), it has been widely used in marine environments (Sala et al. 2006, 2008).

In our study, we found that microbial communities at SAF significantly differed from that of PFI and PFII by exhibiting characteristic utilization of carboxylic acids like D-malic acid

and itaconic acid. PCA indicated that in addition to carboxylic acids, utilization of carbohydrates like N-acetyl D-glucosamine, D-cellobiose, and α -D-lactose were critical in determining the metabolic demands at SAF. D-cellobiose and N-acetyl D-glucosamine are common in marine environments and are present in phytoplankton cell walls and bacterial peptidoglycan, respectively. Davis and Benner (2005) have experimentally demonstrated that chitin, a source of N-acetyl D-glucosamine could be degraded at very high rates. The metabolic profile at PFI was much different from that of other fronts with utilization of amino acids along with i-erythritol and N-acetyl D-glucosamine being critical in determining the metabolic demands. In the present study, it was observed that glycyl-L-glutamic acid was significantly utilized over the others, and this amino acid is reported to be selectively preserved in particulate organic matter in polar waters (Hubberten et al. 1995). Owing to the continued melting of first-year sea ice, a considerable amount of DOM may become available to heterotrophic bacterioplankton in Austral summer (Arrigo and Thomas 2004; Riedel et al. 2006). Very few studies have been conducted to date to characterize the nature of these DOM from and within the sea ice. Studies have also demonstrated that it can influence the bacterial abundance and production through certain allelopathic interactions (Monfort et al. 2000; Weissbach et al. 2011). As discussed by Brierley and Thomas (2002); and Thomas and Papadimitriou (2003), the production of DOM is closely linked to the production of extracellular polysaccharides by algae (Kremsb et al. 2002). Hence, oceanic realms under the influence of sea ice melting could host microbes that prefer carbohydrates (Sala et al. 2005, 2010). Contrary to this assumption, at PFII where the influence of melt water could be the highest, phenolic compounds and polymers were found to be critical in determining the variability in microbial metabolism. As evident from MANOVA, utilization of phenolic compounds and polymers also contributed significantly to the metabolic variability between the three fronts which could be attributed to its characteristic utilization at the PFII. Polyols could be hydrolyzed by polar bacterial strains (Tan and R uger 1999) and have a profound role in survival at low temperatures (Wynn-Williams 1990). This gives an indication that the degree of melting may not be profound enough to have a significant impact on the nutritional aspects of microbiota.

Conclusion

Among the three fronts studied, the bacterial carbon demand was maximum at PFI ($2.91 \text{ mg C m}^{-2} \text{ h}^{-1}$) and minimum at PFII ($1.55 \text{ mg C m}^{-2} \text{ h}^{-1}$). Contribution of microbial loop to the secondary biomass at PFII was enhanced due to comparatively low bacterial respiration rate ($0.83 \text{ mg C m}^{-2} \text{ h}^{-1}$).

Significant utilization of polyols over carbohydrates in polar waters indicates lesser influence of the Antarctic melt waters on the metabolic capacities of bacterioplankton.

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