

The role of iron in the bacterial degradation of organic matter derived from *Phaeocystis antarctica*

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Abstract In high-nutrient low-chlorophyll areas, bacterial degradation of organic matter may be iron-limited. The response of heterotrophic bacteria to Fe addition may be directly controlled by Fe availability and/or indirectly controlled through the effect of enhanced phytoplankton productivity and the subsequent supply of organic matter suitable for bacteria. In the present study, the role of Fe on bacterial carbon degradation was investigated through regrowth experiments by monitoring bacterial response to organic substrates derived from *Phaeocystis antarctica* cultures set up in <1 nM Fe (LFe) and in Fe-amended (HFe) Antarctic seawater. Results showed an impact of Fe addition on the morphotype dominance (colonies vs. single cells) of *P. antarctica* and on the quality of *Phaeocystis*-derived organic matter. Fe addition led to a decrease of C/N ratio of *Phaeocystis* material. The bacterial community composition was modified as observed from denaturing gradient gel electrophoresis (DGGE) profiles in LFe as compared to HFe bioassays. The percentage of active bacteria as well as their specific metabolic activities (ectoenzymatic hydrolysis, growth rates and bacterial growth efficiency) were enhanced in HFe bioassays. As a

consequence, the lability of *Phaeocystis*-derived organic matter was altered, i.e., after seven days more than 90% was degraded in HFe and only 9% (dissolved) and 55% (total) organic carbon were degraded in LFe bioassays. By inducing increased bacterial degradation and preventing the accumulation of dissolved organic carbon, the positive effect of Fe supply on the carbon biological pump may partly be counteracted.

Keywords Bacterioplankton · Iron · Organic matter · *Phaeocystis antarctica* · Remineralisation

Introduction

Phaeocystis antarctica recurrently produces large blooms in the Southern Ocean such as in Prydz Bay and the southern Ross Sea (e.g., El-Sayed et al. 1983; Davidson and Marchant 1992; Smith et al. 1996). In the Ross Sea, *P. antarctica* blooms early in the season, in waters characterized by relatively high Fe levels (Sedwick et al. 2000). Among the phytoplankton community, *P. antarctica* showed indeed the strongest response to Fe addition, increasing its relative abundance from <5 up to 20% in 2.5 nM Fe-enriched seawater (Coale et al. 2003).

The question of the fate of the carbon produced by *P. antarctica* in the Southern Ocean is still unresolved. Bacterial degradation has been proposed to be a major pathway in polar waters. However, several

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cases of dissolved organic carbon (DOC) accumulation have been observed in Prydz Bay (e.g., Bölter and Dawson 1982; Davidson and Marchant 1992). This DOC accumulation can either result from the refractory nature of the organic matter and/or from the limitation of its consumption by bacteria (Thingstad et al. 1997).

The biodegradability of organic carbon depends on the physico-chemical properties of the organic matter itself such as molecular size, chemical structure and elemental composition. In high-nutrient low-chlorophyll (HNLC) regions, Fe addition can have a positive effect on primary production and consequently increases the production of organic substrates. In addition, it can have an impact on the quality of the organic matter for example the quality of phytoplankton-derived organic matter is related to nutrient growth conditions with more proteins synthesized under nonlimiting conditions (Lancelot et al. 1986). In the case of *P. antarctica*, it can also control the dominant morphotype, colonies versus flagellated cells (Smith et al. 2003), generally characterized by contrasted elemental ratio (Schoemann et al. 2005).

Bacterial degradation of organic carbon may be limited by temperature (Pomeroy et al. 1991), predation (Thingstad et al. 1997), but also by ambient inorganic nutrients. For example, results reported for northern European *Phaeocystis* blooms suggested that the degradation of *Phaeocystis*-derived organic carbon could be nutrient-limited by phosphorus and nitrogen (Thingstad and Billen 1994). In HNLC waters, inorganic major nutrient limitation of bacterial degradation is unlikely, whereas Fe can be limiting. Dissolved Fe concentrations are extremely low in January (<0.2 nM), at the senescent stage of *P. antarctica* bloom in the Ross Sea (Sedwick et al. 2000). Heterotrophic bacteria, with their high surface-to-volume ratio and ability to produce siderophores (e.g., Granger and Price 1999), are probably very efficient competitors for Fe acquisition even in environments experiencing picomolar concentrations of Fe commonly encountered in HNLC areas. However, the large Fe requirement of the respiratory system requiring 60% more Fe per mol of cytochrome-*c* than the photosynthetic respiratory system (Raven 1988), suggest that these organisms can still be Fe-limited *in situ*.

Bacterial processes such as the ectoenzymatic hydrolysis of polymeric organic matter, the uptake of monomeric substrates and respiration can be directly controlled by Fe availability and/or indirectly controlled through the effect of enhanced phytoplankton productivity and the subsequent supply of organic matter suitable for bacteria. Moreover, Fe availability can affect the bacterial community structure and also the proportion of metabolically active cells playing a role in the degradation of organic matter. During Fe enrichment bottle experiments conducted in the coastal Southern Ocean (Gerlache Strait), Pakulski et al. (1996) found that Fe induces increases of both heterotrophic bacterial abundance and cell-specific growth rates. These experiments, conducted in the dark and in the absence of phytoplankton and bacterivores, suggest a direct stimulation of heterotrophic bacterial growth by Fe enrichment. In other controlled experiments, carbon seemed to be the first growth-limiting factor of the bacteria although Fe quickly became limiting when carbon limitation was alleviated (Church et al. 2000; Kirchman et al. 2000). Fe may also produce an increase in bacterial carbon metabolism efficiency. Indeed, enhanced bacterial growth efficiency (BGE) in Fe-enriched conditions has been previously observed (Tortell et al. 1996; Kirchman et al. 2003). Although increasing Fe availability alone seems to have little effect on bacterial diversity, changes in the organic matter source derived from Fe-enhanced algal production may affect bacterial community structure in favour of species having appropriate enzymes (Hutchins et al. 2001; Arrieta et al. 2004).

In this work we conducted laboratory-controlled experiments to investigate the role of Fe in the bacterial degradation of *P. antarctica*-derived organic matter. Our specific objectives were to determine how Fe affects the production and quality of *Phaeocystis* organic matter and to assess the role of Fe in bacterial processes involved in the degradation of this organic matter. The experimental strategy was to mimic post-phytoplankton bloom events by running bacterial regrowth experiments enriched with *Phaeocystis*-derived organic matter obtained under limiting and nonlimiting Fe conditions. Finally, the effects of Fe availability on the organic matter degradability are discussed in terms of significance for the carbon biological pump.

Materials and methods

Experimental procedure

Regrowth experiments were performed by mixing *P. antarctica*-derived organic matter with an inoculum of Antarctic bacteria. *Phaeocystis*-derived organic matter was prepared from pure cultures of *P. antarctica* grown under Fe-limiting (LFe) and enriched conditions (HFe).

All the experimental procedures were carried out under trace-metal clean conditions as described in Schoemann et al. (2001). Except for the organic matter sampling, flasks and filtration devices used for *Phaeocystis* cultures, the regrowth experiments and seawater sampling were in polycarbonate or high-density polyethylene. For at least 48 h, the polycarbonate labware was soaked in 1 N HCl, whereas the more-resistant polyethylene containers were decontaminated with 7 N HNO₃. Nutrient samples were collected in 1 N HCl acid-cleaned polyethylene vials. The laboratory material was thoroughly rinsed with ultra-high-purity water (Milli-Q Element system, Millipore) before use. All procedures prior to the incubations and during sampling were performed in a class-100 laminar flow bench. The material for organic carbon sampling and measurements, were either in glass or Teflon and carefully cleaned by ashing (4 h at 550°C) or by washing with chromic-sulphuric acid (Merck) or concentrated 6 N HCl.

Phaeocystis cultures

Phaeocystis antarctica strain CCMP1871, originating from the Bellingshausen Sea was obtained from the Provasoli-Guillard National Center for Cultures of Marine Phytoplankton (CCMP, USA). Cultures were performed in natural (low-Fe, high-macronutrient) Antarctic seawater, without the addition of any artificial chelator (e.g., ethylenediaminetetraacetic acid, EDTA) in order to preserve the equilibrium and kinetics of the various natural chemical forms of Fe (Gerringa et al. 2000). The seawater was filtered on a 0.2 µm porosity Sartobran cartridge and sterilized by γ irradiation. *Phaeocystis antarctica* were grown and acclimated for at least five generations to LFe (<1 nM) and HFe (+2 nM) seawater. Cultures were grown at 2°C with a day:night cycle of 16:8 h at

180 µmole quanta m⁻² s⁻¹. An inoculum (15 ml) of *P. antarctica* pre-adapted to LFe and HFe conditions was, respectively, added to 20 l LFe and HFe Antarctic seawater. Surface Antarctic seawater was collected in the southwestern Pacific Antarctic Ocean (135°E–150°E) during CLIVAR SR3 cruise in December 2001. For HFe conditions, Antarctic seawater was enriched with 2 nM FeCl₃ 72 h before the start of the culture. Another spike of 2 nM FeCl₃ was added to the culture after 8 days. In total 4 nM Fe was added in two times to prevent direct precipitation of the added Fe and ensure >2 nM concentrations of Fe in the batch culture. After 15 days, samples were collected during the exponential phase of growth for the regrowth experiments and to measure nutrients, Chl *a*, *P. antarctica* cell number and biomass of both single cells and colonies, particulate organic carbon (POC), particulate nitrogen (PON) and DOC.

Bacteria cultures

The bacteria inoculum was prepared from 0.8 µm prefiltered Antarctic seawater collected four months before the experiments at McMurdo Sound (Antarctica). These bacteria were pre-incubated in LFe (<1 nM) and HFe (+2 nM) Antarctic seawaters for at least five generations. They were grown in the dark at 2°C.

Regrowth experiments

Two pools of organic matter were prepared from exponentially growing LFe and HFe *P. antarctica*: (i) dissolved organic matter (DOM) obtained by gentle filtration (<0.3 atm) on 0.1 µm polycarbonate Nuclepore filters, (ii) total organic matter (TOM), unfiltered, including DOM and POM obtained after phytoplankton lysis by sonication (10 min of sonication at 22 W with pulse of 0.2 s/s) and freeze/thaw (–80°C deep freezer/60°C water bath cycles, repeated three times) methods. The broken cells were observed by epifluorescence microscopy, evidencing the efficiency of this method.

Bacteria were inoculated in a 1:10 volume ratio to each prepared media of organic matter (2 l). Bioassays were then incubated at 2°C in the dark for about 30 days. Sub-samples were collected to monitor the time evolution of bacterial abundance, biomass, genetic diversity, 5-cyano-2,3-ditotyl tetrazolium

chloride (CTC)-positive cells, ectoenzymatic activities, production and organic carbon utilisation.

Analytical procedures

Nutrients

Nitrate and phosphate concentrations were determined according to Koroleff (1983a, b). Fe samples were filtered on 0.1 μm Nuclepore polycarbonate membrane and acidified to $\text{pH} < 2$ with ultraclean quartz distilled concentrated nitric acid (Ultrex, JT Baker). Dissolved Fe concentrations were measured according to a chemiluminescence method adapted from Obata et al. (1993) as applied by Sarthou et al. (2003). The blank is in average equal to 0.06 ± 0.02 nM. The detection limit is equal to 0.03 ± 0.01 nM.

Chlorophyll *a* and *Phaeocystis antarctica* biomass

Chl *a* was measured fluorimetrically following Yentsch and Menzel (1963) after 90% v/v acetone extraction (12 h) in the dark at 4°C of the particulate material retained on 0.8 μm Nuclepore polycarbonate membrane filters. The relative standard deviation of the method was better than 2%.

Samples to determine *P. antarctica* biomass were preserved with glutaraldehyde (0.5% final concentration) for free-living cells and with glutaraldehyde–Lugol's solution (1% final concentration) for colonies. Free-living cells were enumerated by epifluorescence microscopy (400 \times magnification) after 4'-6-diamidino-2-phenylindole (DAPI) staining (Porter and Feig 1980). Colonies were enumerated by inverted light microscopy (100 \times magnification) according to the method of Utermöhl (1958). Colonies and cell biomass were estimated according to Mathot et al. (2000).

Organic carbon

For phytoplankton particulate organic carbon (POC) and particulate nitrogen (PON) determination, some suspended matter was collected on precombusted (450°C) Whatman GF/F filters, dried at 60°C and stored in polystyrene Petri dishes until analysis. POC and PON were analyzed with a Carlo Erba NA 2000 elemental analyzer. The analytical error of the

method is ± 0.8 μM for POC and ± 0.04 μM N for PON.

Samples for dissolved (DOC) and total organic carbon (TOC) were stored in glass tubes, poisoned with azide (final concentration 0.5%) and sealed with Teflon-lined screw caps. They were kept in the dark at 4°C until analysis. Organic carbon was measured by high-temperature catalytic oxidation (HTCO; procedure of Sugimura and Suzuki 1988) with a Dohrmann Apollo 9000 analyzer. Carbon concentration was determined using a five-point calibration curve performed with standards prepared by diluting a stock solution of potassium phthalate in Ultra pure water (Milli-Q Element system, Millipore). Each value corresponds to the average of at least five injections. Samples were measured in duplicate and the relative standard deviation never exceeded 2%. The accuracy of our DOC measurements was tested by measuring reference materials provided by Hansell laboratory (University of Miami). We obtained an average concentration of 45.1 ± 0.7 μM C ($n = 10$) for deep-ocean reference material (Sargasso Sea Deep water, 2,600 m) and 1.4 ± 0.7 μM C ($n = 10$) for low-carbon reference water. Our values are within the nominal values provided by the reference laboratory (44.0 ± 1.5 and 2.0 ± 1.5 μM C, respectively).

Bacterial biomass and activities

Samples for bacterial analyses were preserved with 40% buffered formaldehyde (final concentration 2%). Bacteria were filtered through 0.2- μm black Nuclepore polycarbonate filters of 25-mm diameter and stained by with 4'-6-diamidino-2-phenylindole (DAPI, final concentration 2.5 $\mu\text{g ml}^{-1}$) according to Porter and Feig (1980). A minimum of 1,000 cells were counted with a Leica epifluorescence microscope in at least 10 different fields at 1,000 \times magnification. A relative standard deviation of 15% ($n = 20$) was estimated on bacterial abundance determination. Bacterial biovolumes were determined by image analysis (Lucia 4.6 software) and calculated by treating rods and cocci as cylinders and spheres, respectively (Watson et al. 1977). They were converted to carbon biomass by using the relation established from data measured by Simon and Azam (1989):

$$C = 0.12 V^{0.72},$$

where C is the carbon per cell ($\text{pg C } \mu\text{m}^{-3}$) and V is the biovolume (μm^3).

The number of 5-cyano-2,3-ditotyl tetrazolium chloride (CTC)-reducing bacteria were determined by using a final concentration of 2.5 mM and incubating the sample at 2°C for 2 h (Rodríguez et al. 1992). The reaction was stopped with 40% buffered formaldehyde (final concentration 2%). The samples were further filtered, DAPI stained and observed as described above.

Ectoenzymatic activity was determined fluorimetrically (Kontron SFM 25 fluorimeter) as the maximum hydrolysis rate (H_m) of model substrates for β -glucosidase (4-methylumbelliferyl- β -D-glucoside) and leucine-aminopeptidase (L-leucine-7-amido-4-methyl-coumarin) added at saturating concentration (40 mM solution of L-Leucine-7-amino-4-methyl-coumarin for ectoprotease activity (EPA) and 6 mM solution of 4-methylumbelliferyl- β -D-glucoside). Wavelengths for excitation and emission were 360 and 445 nm for 7-amino-4-methylcoumarine (MCA), and 365 and 455 nm for 4-methylumbelliferone (MUF). Fluorescence in the samples was measured as a function of time over 60 min at 2°C in the dark. Increase of fluorescent units with time was converted into activity from a standard curve prepared with the end product of the reaction, 7-amino-4-methylcoumarin (Sigma) for EPA and 4-methylumbelliferone (Sigma) for EGA. The relative standard deviation was 12% ($n = 20$) on aminopeptidase and 11% ($n = 20$) on glucosidase activities.

Bacterial production was estimated by incorporation of ^3H -thymidine (Fuhrman and Azam 1982). Duplicate 10 ml subsamples were incubated for 1 h in the dark at 2°C in the presence of 20 nM of ^3H -thymidine (Amersham; 40–50 Ci mol^{-1}), filtered on 0.2- μm cellulose acetate membrane filters (Sartorius) and extracted with ice-cold 5% Trichloroacetic acid (TCA) (Becquevort and Smith 2001). Thymidine incorporation was converted into bacterial production using conversion factors of Ducklow et al. (1999) established for the Ross Sea bacterial communities (i.e., 8.6×10^{17} bacteria produced per mole of thymidine incorporated in the cold TCA insoluble material). For bacterial production, the relative standard deviation was 9.3% ($n = 20$). The specific growth rate was estimated from bacterial production and bacterial biomass.

Bacterial growth efficiency (BGE), i.e., the efficiency at which bacterioplankton convert OC into bacterial biomass was calculated from the rate of decline in substrate (OC) and the rate of increase in bacterial biomass (BB) estimated from bacterial production as follow:

$$\text{BGE} = \Delta\text{BB} / -\Delta\text{OC}.$$

Based on this equation, we derived BGE by property–property linear regression of ΔBB vs. $-\Delta\text{OC}$ from the model II regression which could be determined significantly when $P < 0.05$.

The genetic diversity was determined at 0, 6 and 30 days using denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified 16S rRNA genes. The DNA extraction and PCR-DGGE were performed according to Schäfer and Muizer (2001) and using 341F-GC/907R primers. DGGE fingerprints were processed by image analysis (DOC-PRINT, Proxylab). The distance between lanes on the gels was calculated by simply matching distance and the cluster analysis was performed using the SYSTAT Package (V.11, SPSS) [Euclidean distance and unweight pair group method with arithmetic mean (UPGMA)].

Statistical analysis

We used three-way analysis of variations (ANOVA) analysis without replication to test for the separate and interactive effect of Fe enrichment of the *Phaeocystis* culture (LFe and HFe), organic matter fractionation (DOM and TOM), and incubation time on the various parameters measured. Data were initially tested for normal distribution and equality of variances to verify ANOVA assumptions. When these conditions were not met, data were log-transformed. All statistical analyses were conducted using the SYSTAT Package (V.11, SPSS), using a significance level of 0.05. Fitting of least-squares regression was performed using Sigmaplot (V.7, SPSS) to determine whether OC changed with time. A Pearson's correlation was done for ectoenzymatic activities and bacteria growth rates among the various treatments to determine if linear relationships existed among these variables.

Results

Characteristics of LFe and HFe *Phaeocystis* cultures

At the time of subsampling for regrowth experiments, dissolved Fe concentrations were 0.85 nM in the LFe culture and 2.76 nM in the HFe culture. The Chl *a* cell content was lower in the LFe (0.05 pg Chl *a* cell⁻¹ in LFe and 0.24 pg Chl *a* cell⁻¹ in HFe) reflecting Fe stress of *Phaeocystis* cells in the LFe (Table 1). In the LFe culture, 81% of total phytoplanktonic biomass was composed of *Phaeocystis* single cells while in the HFe culture *Phaeocystis* colonies represented 68% of the total biomass. Mucus carbon of *Phaeocystis* colonies represented only a minor fraction of total phytoplanktonic carbon, representing only 2.3% of the C in HFe bioassay. In the LFe conditions, the mucus contribution in total phytoplanktonic carbon was even lower (0.3%). In the latter, the colonies observed were usually composed of two cells embedded in a mucilaginous matrix.

POC and PON concentrations were higher in the HFe culture, but the increases of POC and PON were not proportional (Table 1). This results in contrasted C:N molar ratios with the highest value (8.3) recorded in the LFe culture as compared to the HFe culture (5.0). DOC was higher in the LFe culture than in the HFe culture. DOC initially present in the Antarctic seawater (before *Phaeocystis* inoculation) was 107 μM and thus freshly produced DOC (DOC_{fresh}) during the phytoplanktonic growth, was 73 μM in the LFe culture and 22 μM in the HFe

Table 1 Fe concentrations, Chl *a* per cell, *P. antarctica* cell abundance, particulate organic carbon (POC), particulate organic nitrogen (PON) and dissolved organic carbon (DOC) in the *P. antarctica* cultures under low Fe (<1 nM Fe, LFe) and Fe amended (HFe) conditions used for bacterial regrowth experiments

Parameters	LFe	HFe
Dissolved Fe (nM)	0.85	2.76
Chl <i>a</i> cell ⁻¹ (pg cell ⁻¹)	0.05	0.24
Cell abundance (cells l ⁻¹)	7.44 × 10 ⁶	3.69 × 10 ⁷
POC (μM)	30	89
PON (μM)	3.6	17.9
DOC (μM)	179	128

culture. These DOC_{fresh} values contributed to 71 and 20 % of freshly produced TOC (TOC_{fresh} = (DOC_{fresh} + POC)). TOC_{fresh} amounted to 103 and 111 μM in the LFe and HFe culture, respectively.

Bacterial regrowth experiments

Time evolution of organic carbon

The initial concentrations of organic carbon ranged between 125 and 208 μM (Fig. 1). Organic carbon was similar in the DOM and TOM LFe bioassays (DOM_{LFe} and TOM_{LFe}, Fig. 1a) showing little contribution of POC to total organic carbon (7%). On the contrary, in the HFe bioassays, a larger fraction of the organic matter (41%) was in the particulate form (89 μM, Fig. 1b) due to the high contribution of *Phaeocystis* particulate material in the TOM_{HFe} bioassay. The lowest initial values of organic carbon were found in the DOM_{HFe} bioassay (Fig. 1b).

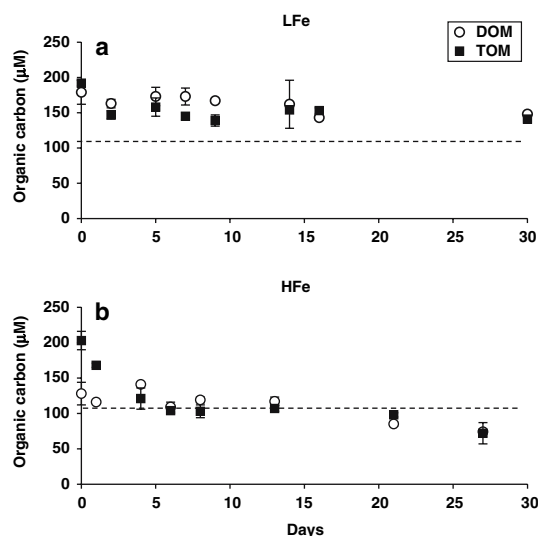


Fig. 1 Time course of organic carbon (μM) during 30 days incubation in low Fe (<1 nM Fe) bioassay (a) and Fe-amended bioassay (b). Dashed line indicates the initial DOC value in the culture seawater. Error bars represent the standard deviation on two replicates. An exponential decay function ($y = a e^{-bx}$) was estimated in each bioassays. DOM_{LFe} bioassay: $a = 174.73$ and $b = 0.0066$, $r^2 = 0.64$, $P < 0.05$; TOM_{LFe} bioassay $a = 163.41$ and $b = 0.0061$, $r^2 = 0.26$, n.s.; DOM_{HFe} bioassay: $a = 131.65$ and $b = 0.0182$, $r^2 = 0.74$, $P < 0.01$; TOM_{HFe} bioassay: $a = 166.77$ and $b = 0.0369$, $r^2 = 0.70$, $P < 0.01$

As reported in Table 2, LFe–HFe conditions as well as time significantly affected ($P < 0.05$) the OC concentrations. In the LFe bioassays, organic carbon decreased very slowly to about 142 μM at day 30 in both DOM_{LFe} and TOM_{LFe} bioassays. After five days of incubation in the HFe bioassays, the organic carbon in the TOM_{HFe} bioassay decreased to similar concentrations as in the DOM_{HFe} bioassay (Fig. 1b), underlining the high lability of fresh *Phaeocystis*-derived particulate organic carbon. Lower concentrations of $\sim 75 \mu\text{M}$ were reached in both HFe bioassays compared to LFe bioassays. These values were less than the initial DOC (107 μM) measured in the culture seawater, suggesting that the addition of fresh organic matter could stimulate the degradation of the organic matter being otherwise semilabile as

previously observed in some other cases (Cherrier et al. 1996).

The percentage of biodegradable organic carbon ($\text{BOC}_{30 \text{ days}}$), estimated by subtracting the OC at day 30 from the OC at day 0 and expressed as a percentage of the OC at day 0, ranged between 18 (DOM_{LFe}) and 64% (TOM_{HFe} ; Table 3). By taking into account only freshly produced OC ($\text{DOC}_{\text{fresh}}$ and POC), these percentages increased and ranged between 44 and >100%. The >100% data were likely related to the stimulation of semilabile organic matter degradation, as suggested above. $\text{BOC}_{30 \text{ days}}$ was enhanced by a factor of 2 in the HFe bioassays, where 100% of the freshly organic matter was degraded after 30 days. After seven days, 100% of $\text{TOC}_{\text{fresh}}$ and 90% of $\text{DOC}_{\text{fresh}}$ were already degraded. In the

Table 2 P -values of a three-way ANOVA without replication performed on organic carbon, bacterial abundance, CTC-positive cell ratio, biovolume, ectoprotease activity (EPA),

specific EPA, ectoglucosidase activity (EGA), specific EGA, EPA/EGA ratio, production, specific growth rate and specific (CTC+) growth rate measured in the four bioassays

Effects parameters	Time	HFe–LFe	DOM–TOM	HFe–LFe \times DOM–TOM
Organic carbon	<0.0001	<0.0001	0.986	<0.0001
Bacterial abundance	0.914	<0.0001	0.052	0.104
CTC-positive cell ratio	<0.0001	<0.0001	<0.0001	<0.0001
Biovolume	0.015	0.158	0.001	0.001
EPA	0.001	0.008	<0.0001	<0.0001
Specific EPA	0.044	0.011	<0.0001	<0.0001
EGA	<0.0001	<0.0001	<0.0001	<0.0001
Specific EGA	0.020	0.045	0.008	0.044
EPA/EGA ratio	0.006	0.043	0.008	0.044
Production	0.000	0.000	0.238	0.040
Specific growth rate	0.001	0.045	0.003	0.035
Specific (CTC+) growth rate	0.001	0.083	0.008	0.392

Grouping factors were time (from 0 to 30 days), HFe–LFe and DOM–TOM conditions

Table 3 Biodegradable organic carbon (BOC) after 30 days ($\text{BOC}_{30 \text{ days}}$, absolute value and percentage of initial organic carbon), biodegradable freshly produced organic carbon after 30 days ($\text{B}_{\text{fr}}\text{OC}_{30 \text{ days}}$, percentage of initial organic carbon) and

after seven days ($\text{B}_{\text{fr}}\text{OC}_{7 \text{ days}}$, percentage of initial organic carbon) in dissolved (DOM) and total organic matter (TOM) under low Fe (<1 nM Fe, LFe) and Fe amended (HFe) conditions used for bacterial regrowth experiments

	LFe		HFe	
	DOM_{LFe}	TOM_{HFe}	DOM_{HFe}	TOM_{HFe}
$\text{BOC}_{30 \text{ days}} (\mu\text{M})$	32	51	53	131
$\text{BOC}_{30 \text{ days}} (\%)$	18	26	42	64
$\text{B}_{\text{fr}}\text{OC}_{30 \text{ days}} (\%)$	44	60	102	105
$\text{B}_{\text{fr}}\text{OC}_{7 \text{ days}} (\%)$	9	55	90	100

The standard deviation for BDOC concentrations was estimated to amount less than 5%

LFe bioassay, TOC (TOM bioassay) was also more biodegradable than DOC (DOM bioassay; Table 3). Some 44 and 60% of freshly produced OC were degraded after 30 days in LFe DOM and TOM bioassays, respectively. The $\text{TOC}_{\text{fresh}}$ was quickly degraded but only 9% of $\text{DOC}_{\text{fresh}}$ was degraded after seven days in LFe bioassays.

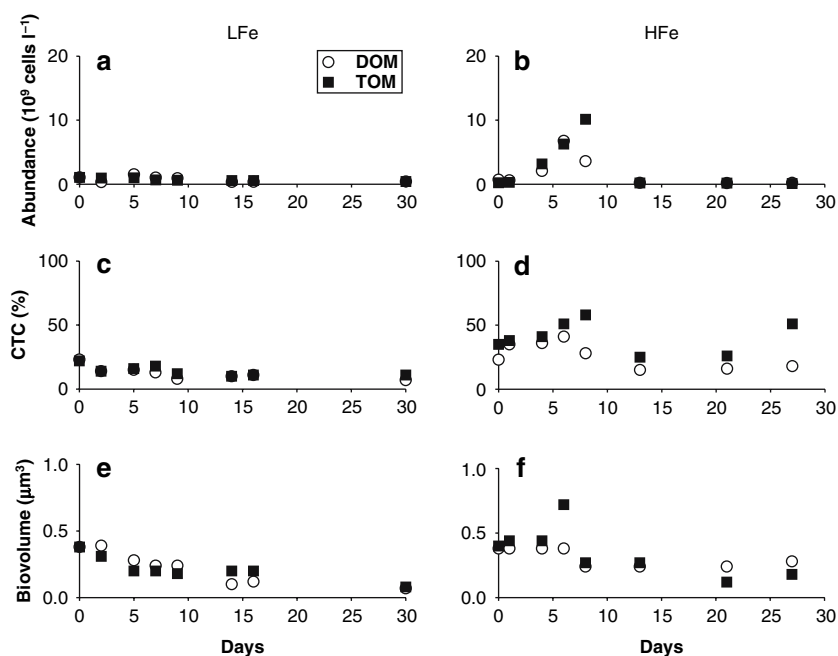
Bacterial communities

Bacterial abundances ranged from 0.3 to 1.5×10^9 cells l^{-1} in the LFe bioassays and from 0.1 to 10.1×10^9 cells l^{-1} in the HFe bioassays (Fig. 2a, b). Indeed, a significant ($P < 0.05$) effect of LFe–HFe conditions on the bacterial abundance was found (Table 2). Bacterial abundance in HFe bioassays increased rapidly during the first 6–8 days of the experiment and then decreased to value close to initial conditions (Fig. 2b). During the first 6 days, the evolution of bacterial abundance was quite similar in the DOM_{HFe} and TOM_{HFe} bioassays, increasing by a factor 10 compared to day 0 (Fig. 2b). The highest abundance in the TOM_{HFe} bioassay was reached at day 8, whereas the abundance decreases rapidly after day 6 in the DOM_{HFe} bioassay (Fig. 2b). No comparable increase in bacterial cells was observed in the LFe bioassays (Fig. 2a). The percentage of

CTC positive cells was always below 23% in the LFe bioassays, while the percentage increased to 41% (day 6) and 61% (day 8) in the DOM_{HFe} and TOM_{HFe} bioassays, respectively (Fig. 2c, d). As reported in Table 2, LFe–HFe as well as DOM–TOM conditions affected significantly ($P < 0.05$) the percentage of CTC positive cells. In the LFe bioassays (Fig. 2e), biovolumes decreased from 0.4 to $0.07 \mu\text{m}^3$. In contrast, the biovolumes in the TOM_{HFe} bioassay (Fig. 2f) reached $0.71 \mu\text{m}^3$ at day 6 and decreased to $0.12 \mu\text{m}^3$. The statistical analysis reported a significant ($P < 0.05$) effect of time and fractionation of organic matter (OM) on the biovolumes (Table 2). LFe–HFe condition did not affect significantly bacterial biovolume (Table 2).

DGGE of PCR-amplified 16S rDNA revealed between 7 and 20 (typically 11) bands in each sample, of which two were detected in all community DNA samples. The maximum number of bands was observed in the HFe bioassays. DGGE fingerprints corresponding to day 0 were similar for DOM and TOM bioassays in the both Fe conditions, whereas five bands were similar in bacterial communities present in HFe compared to those in LFe conditions. As illustrated by the dendrogram in Fig. 3, the assemblage did not change until day 6 in DOM bioassays but was immediately modified in the TOM

Fig. 2 Time course of bacterial abundance (10^9 cells l^{-1}) (a, b), CTC-positive cell ratio (%) (c, d) and bacterial biovolume (μm^3) (e, f) during 30 days incubation in low Fe (< 1 nM Fe) bioassay and Fe amended bioassay



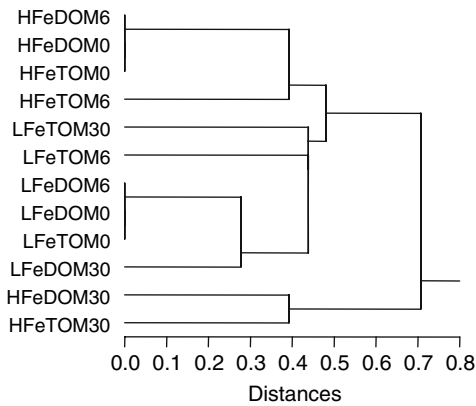


Fig. 3 Dendrogram showing the similarity of DGGE patterns from different sampling times (0, 6 and 30 days) in low Fe (<1 nM Fe) bioassay and Fe-amended bioassay

bioassays. Final detected assemblages, particularly in HFe bioassays, were always significantly different from the initial ones in all bioassays.

Bacterial activities

The ectoenzymatic activities were significantly ($P < 0.05$) affected by LFe–HFe and DOM–TOM conditions (Table 2). The highest ectoenzymatic

activities, leucine-peptidase and β -glucosidase, were reported in the HFe bioassays (Fig. 4d). At day 5, EPA and EGA drastically increased, concomitantly to the bacterial abundance (Fig. 2b). The specific activities (per cell) were not always higher in the HFe bioassay than in the LFe conditions (Table 4). As reported in Table 2, the specific EGA was significantly affected by LFe–HFe conditions. In fact, in the presence of particulate organic matter such as in the TOM bioassay, the specific activities in the LFe condition drastically increased. In this experiment, the EPA as well as the EGA showed the highest values. The EPA/EGA ratios ranged between 104 and 3,449 (Table 4). This ratio was significantly regulated by the HFe–LFe and DOM–TOM conditions (Table 2).

LFe–HFe conditions were a highly significant ($P < 0.0001$) regulating factor for bacterial production (Table 2). Indeed, bacterial production in the HFe bioassays increased after day 4 (Fig. 4f) and rapidly decreased to background levels observed in all bioassays. The growth rates ranged from 0.0004 to 0.0066 h^{-1} in the LFe bioassays and from 0.0001 to 0.0326 h^{-1} in the HFe bioassays (Table 4). LFe–HFe as well as DOM–TOM conditions affected significantly ($P < 0.05$) the specific growth rates (Table 2).

Fig. 4 Time course of ectoprotease activities (EPA, $\mu\text{moles l}^{-1} \text{h}^{-1}$) (a, b), ecto- β -glucosidase activities (EGA, $\text{nmoles l}^{-1} \text{h}^{-1}$) (c, d) and bacterial production ($10^6 \text{ cells l}^{-1} \text{h}^{-1}$) (e, f) during 30 days incubation in low Fe (<1 nM Fe) bioassay and Fe-amended bioassay

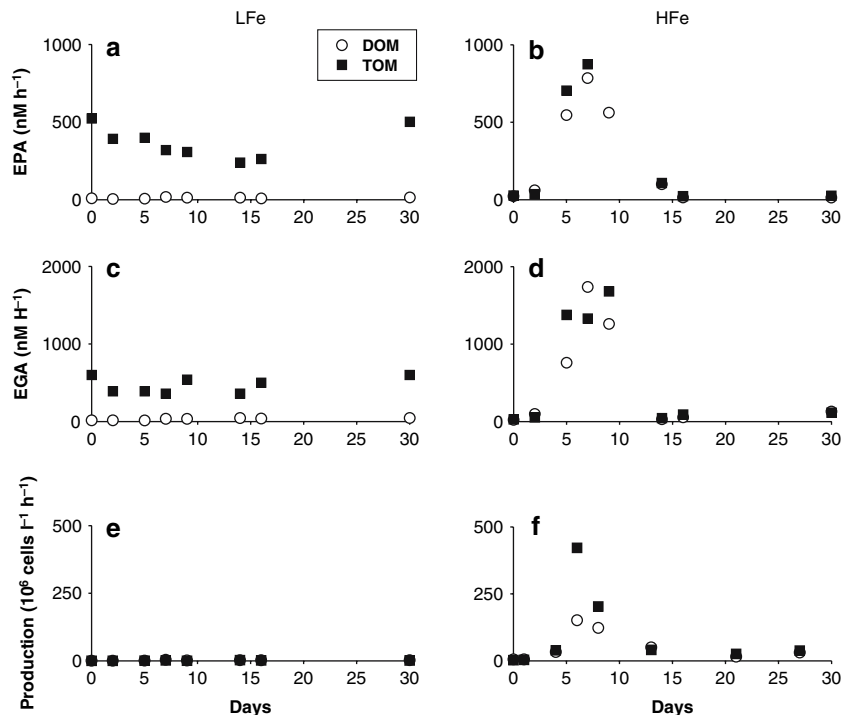


Table 4 Bacterial activities in presence of dissolved (DOM) and total organic matter (TOM) in low-Fe (<1 nM Fe, LFe) and amended-Fe (HFe) conditions; specific ectoenzymatic activities [ectoprotease (EPA) and β -glucosidase (EGA) activities]

and their ratio, specific growth rates for total bacteria and for CTC-positive (CTC+) cells and bacterial growth efficiencies (BGE)

Parameters	LFe	HFe
DOM EPA (10^{-15} moles cell $^{-1}$ h $^{-1}$)	4.4–37.7	31.5–453.7
TOM EPA (10^{-15} moles cell $^{-1}$ h $^{-1}$)	397.8–1194.1	118.5–535.4
DOM EGA (10^{-18} moles cell $^{-1}$ h $^{-1}$)	8.4–125.2	29.5–609.4
TOM EGA (10^{-18} moles cell $^{-1}$ h $^{-1}$)	388.3–4137.1	123.4–984.0
DOM EPA/EGA ratio	220–624	104–3,449
TOM EPA/EGA ratio	289–1,025	224–2,423
DOM growth rate (h $^{-1}$)	0.0004–0.0066	0.0011–0.0326
TOM growth rate (h $^{-1}$)	0.0002–0.0048	0.0001–0.0134
DOM growth rate (CTC+) (h $^{-1}$)	0.0017–0.0771	0.0030–0.0812
TOM growth rate (CTC+) (h $^{-1}$)	0.0007–0.0476	0.0001–0.0892
DOM BGE	0.05 (0.03)	0.29 (0.03)*
TOM BGE	0.15 (0.04)*	0.30 (0.04)*

Bacterial growth efficiency is determined by the slope of the model II regression and values in parentheses represent standard error of the slope

* The values reported were significant at the 0.05 significance level

By taking into account only CTC-positive cells in the estimation of cell specific growth rates, higher values were obtained for all bioassays as expected, and no difference between values in HFe and LFe conditions was observed (Table 2). BGE ranged from 0.05 (n.s. $P > 0.05$) to 0.30 (Table 4). LFe–HFe conditions also affected bacterial growth efficiency, particularly when organic matter was only provided in the dissolved form (Table 4).

Discussion

Fe control on *P. antarctica*-derived organic matter concentration and quality

There are numerous potential sources of organic matter for bacteria which can result from a variety of processes (Nagata 2000). Two major sources are photosynthetic extracellular release (ER) by the algae and products from phytoplankton lysis (Nagata 2000). These two organic pools were represented in our DOM and TOM bioassays, respectively. The relative significance of ER and phytoplankton lysis varies in function of different environmental factors and notably due to inorganic nutrients (Nagata 2000). The biochemical composition of the organic matter

derived from ER and/or phytoplankton lysis is different, consequently they have their specific lability.

In our study, POC concentrations, derived from *P. antarctica* cultures, increased by a factor 3 in the HFe compared to the LFe culture, although $\text{TOC}_{\text{fresh}}$ ($\text{DOC}_{\text{fresh}}$ and POC) was in the same range for both cultures. POC corresponded to 80% of $\text{TOC}_{\text{fresh}}$ in the HFe conditions and only 29% of $\text{TOC}_{\text{fresh}}$ in LFe conditions. The value in the HFe culture is comparable to those measured in the Ross Sea where 70–99% of the accumulated organic matter was partitioned in POM during a *P. antarctica* bloom, with modest net accumulation of DOM stocks (Carlson et al. 1998). In bottle experiments using natural seawater assemblages (Southern Ross Sea), *P. antarctica* did not produce large amounts of DOC during nutrient-replete growth (Smith 1998). In mesoscale Fe fertilization experiments, reports on DOC are very scarce and no consistent trend has been evidenced so far (de Baar et al. 2005). Increase in DOC excretion by phytoplankton was previously observed under phosphate and nitrate limitation and saturating light conditions (Lancelot 1983; Nagata 2000). Phytoplankton excretion of organic matter is interpreted as an overflow mechanism of recently photosynthesized compounds that occurs during

nutrient limitation (Fogg 1983). However, as Fe limits photosynthesis directly, an increase in the excretion under Fe limitation is less probable. Oliver et al. (2004) showed a strong correlation between primary production (PP) and bacterial production (BP) in the Southern Ocean Fe experiment (SOFeX), suggesting a strong dependence of bacterial activity on phytoplankton production. However, the ratio BP:PP stayed low and did not change, indicating that ER did not increase as a result of Fe addition. To our knowledge, there is no available data on excretion under Fe limitation in the literature so far. On the other hand, Fe limitation could enhance lysis of phytoplankton cells and subsequent release of organic compounds (Gobler et al. 1997). Poorvin et al. (2004) showed that lysate-associated Fe can be more available than inorganic Fe. Consequently, lysis could be a mechanism of survival for the whole phytoplankton community allowing Fe recycling and its availability for the surviving cells.

Fe limitation also induces significant changes in phytoplankton growth characteristics, cell metabolism and therefore in the biochemical composition of its organic matter (e.g., Sunda and Huntsman 1995; van Leeuwe and Stefels 1998; Kudo et al. 2000; Lewandowska and Kosakowska 2004). Therefore Fe has an effect on the concentration and quality of organic matter available for bacteria. In our study, Fe controlled the morphotype dominance of *P. antarctica*, i.e., mucilaginous colonies dominated in the HFe culture whereas free-living cells represented most of the biomass in the LFe culture. As a general rule, amendment of Fe was shown to induce a shift in phytoplankton community structure from nanoplankton to microplankton taxa dominating biomass (de Baar et al. 2005). The particularity of *P. antarctica* is that the size range shift is observed within the same species. This change in the morphological form could have resulted in higher C/N ratio for colonies, the mucus exopolymers having higher C/N ratio than the cells (Solomon et al. 2003). However, the mucus carbon contribution to total carbon biomass was only minor, 0.3 and 2.3% in LFe and HFe conditions, respectively. In the Ross Sea, Mathot et al. (2000) also reported relatively low mean mucus carbon contribution of C_{col} of 14% (3–33% of C_{col}). This suggests that the observed difference in C/N could better result from the direct effect of Fe on phytoplankton metabolism rather than from the effect of Fe

on the morphology of *P. antarctica*. Some studies report deviations in the Redfield ratios of marine algae as a function of Fe status. The nature and strength of the deviations vary greatly among species (Tremblay and Price 2002). The most commonly observed response is a decrease of the N/P and C/P ratios under Fe-limited growth, whereas C/N ratio can remain relatively constant over a wide range of Fe concentrations (Geider and La Roche 2002) or increase as a result of Fe limitation under nitrate-replete conditions (Muggli et al. 1996). In our study C/N was below the Redfield value of 6.6 (Redfield et al. 1963) for the organic matter derived from *P. antarctica* in the HFe culture, in agreement with the range of 4–6.4 reported for *P. antarctica* grown under nonlimiting conditions (Schoemann et al. 2005). Accordingly, Smith et al. (2000) estimated C/N values between 5.9 and 6.8 during the exponential phase of *P. antarctica* blooms in the Ross Sea. The C/N increased above Redfield value in the LFe culture. During *P. antarctica* blooms decay in the Ross Sea, increased C/N values of 7.3–8.7 were observed by Smith et al. (2000), likely attributable to Fe limitation (Vaillancourt et al. 2003).

Fe control on the bacterial community composition and activities

The few studies that exist on Fe control of bacterial degradation in HNLC waters agreed that bacterioplankton is first C-limited as a consequence of low primary production due to Fe limitation and not directly limited by Fe (Hutchins et al. 1998; Church et al. 2000; Kirchman et al. 2000). As reported above, the amount of TOC, potential OC available for bacteria, was similar in our HFe and LFe experiments and therefore bacteria could be colimited by two major factors: the availability of Fe and the quality of the organic matter. These factors cannot be discriminated in our experiments. HFe bioassays contained, in addition to increased Fe, a better quality of OM than the LFe bioassays. Moreover, as the distribution in dissolved and particulate organic carbon was affected by Fe availability in the phytoplanktonic cultures, the response of bacteria to different size pool of organic matter (DOM–TOM) was also investigated.

Regrowth experiments show typically a lag phase, an exponential bacterial growth followed by a

stationary phase (Ammerman et al. 1984). In our study, the evolution of bacteria abundance stayed very constant along the time in LFe bioassays. In the presence of OM derived from HFe *Phaeocystis* culture, the bacteria grew and reached a maximal abundance at about 6–8 days, just after the initial decline in DOC and TOC. The exponential phase is nevertheless followed by a sharp decline of bacterial abundance and associated activities such as ectoenzymatic activities and production. Sempéré et al. (1998) attributed the observed bacteria decline in bioassays to bacterial mortality processes.

The degradation of the organic matter depends initially on the presence of bacterial species in the environment able to use it. Indeed, bacterial variability can be comparable to that of phytoplankton, reflecting the ultimate dependence of bacteria on substrates generated by primary production (Giovannoni and Sting 2005). In our experiments, bacterial community structure was modified in HFe bioassays as compared to LFe bioassays. Only six species seemed well adapted to both HFe and LFe conditions. These results seem in contradiction with the first published studies in the field showing that Fe addition had little or no effect on DGGE profiles (Hutchins et al. 2001; Arrieta et al. 2004), suggesting that field bacterial communities are well adapted to episodic Fe inputs. However, in our experiments, the modification of the bacterial community structure could result from the longer time period of bacterial pre-adaptation (>30 days) in the different bioassays. This long pre-adaptation could favour the development of best adapted species for specific Fe/organic ligand complexes (Weaver et al. 2003).

Some <10 to >75% of the bacteria are not active in marine environments (Ducklow 2000). The degradation of organic matter depends on active bacteria. The proportion of active cells in the bacterial community, determined from CTC-positive cells, was also controlled by LFe–HFe condition. The percentages of CTC-positive bacteria in our experiments was in the higher range of the data observed in marine ecosystems (Schumann et al. 2003), particularly in Fe-enriched bioassays where up to 61% of bacterial cells were CTC-positive after one week of incubation. The reduction of CTC to red fluorescing formazan crystals via electron transport activity is used to detect respiring bacterial cells (Rodriguez et al. 1992) and the abundance of the CTC-positive

cells is correlated with in situ respiration rates (Smith 1998). As most of the cellular Fe in heterotrophic bacteria is being dedicated to the respiratory electron chain (Tortell et al. 1999), the percentage of CTC-positive cells could therefore be regulated by Fe availability. However, the highest percentage was measured in the presence of TOM, suggesting that the quality of the organic matter could also have had an impact on the relative abundance of respiring cells.

Little attention has been paid so far to the role of Fe on bacterial organic matter hydrolysis activities. The hydrolysis of organic matter is a necessary step prior to bacterial uptake and remineralization of organic matter. Basically, bacterial growth is directly dependent on the concentration of monomers, the only organic molecules able to be directly transferred inside bacteria. These monomers are supplied either directly by phytoplankton ER or indirectly after bacterial ectoenzymatic hydrolysis of high molecular weight organic matter. In LFe bioassay, a very high activity (protease and β -glucosidasic activities) was measured in the presence of TOM. Under LFe conditions, it could be advantageous for bacteria to increase its enzymatic hydrolytic activity to release and allow the availability of Fe associated to high-molecular-weight organic matter. In the LFe bioassays, significant correlations between specific ectoenzymatic activities and specific growth rates were obtained (Table 5), suggesting that all substrates produced by hydrolytic activities are simultaneously used for growth.

Moreover, our study shows that specific ectoenzymatic activities (per cell) are stimulated in presence of TOM as compared to DOM especially in LFe bioassays. During the in situ Fe fertilization experiment EISENEX, the ectoenzymatic activities were highest inside the Fe patch (Arrieta et al. 2004). The highest specific ectoenzymatic activities in the HFe bioassay could result (i) from a significant input of high-molecular-weight organic matter due to increase in phytoplankton production, and/or (ii) from difference in the percentage of bacteria actively expressing a distinct enzyme. Moreover, a direct role of Fe on the ectoenzymatic hydrolysis cannot be excluded. For example, some aminopeptidases require a metal as cofactor (Lowther and Matthews 2000), suggesting that low metal concentrations in open-ocean seawater could potentially limit protein hydrolysis.

Table 5 Correlation (r) between specific ectoenzymatic activities [ectoprotease (EPA) and ectoglucosidase (EGA) activities] and growth rates in low-Fe (<1 nM, LFe) and Fe-amended (HFe), dissolved (DOM) and total organic matter (TOM) bioassays

	LFe		HFe	
	EPA	EGA	EPA	EGA
DOM	0.81*	0.85*	n.s.	0.66**
TOM	0.91*	0.82*	n.s.	0.61**

* $P < 0.05$, ** $P < 0.1$, n.s., $P > 0.1$

As reported for Antarctic seawaters (e.g., Christian and Karl 1995; Becquevort and Smith 2001), protease activity was 2–3 orders of magnitude higher than that of β -glucosidasic activity in all bioassays. Interestingly, the ratio of protease to β -glucosidasic activities increased in HFe bioassays, mostly due to increase of protease activity. Proteases hydrolyze peptides and proteins, which comprise a part of the utilizable fraction of the marine organic N pool (Kirchman 2000). Consequently, the decrease of elemental organic composition (C/N ratio) observed in HFe conditions could induce protease production.

Our results showed that bacterial abundance and specific growth rate respond to Fe enrichment. In all Fe enrichment experiments, shipboard incubation as well as in situ fertilization, specific growth rates of bacteria increased significantly in response to Fe enrichment (Price et al. 1994; Pakulski et al. 1996; Hutchins et al. 1998; Church et al. 2000; Kirchman et al. 2000; Cochlan 2001; Hall and Safi 2001; Oliver et al. 2004; Arrieta et al. 2004; Suzuki et al. 2005). However, a significant increase in bacterial abundance is not always observed (Cochlan 2001; Hall and Safi 2001) due to a close coupling between bacteria and bacterivorous protozoa. Interestingly, in the present study, the specific growth rates estimated from the CTC-positive cells were on the same range in both Fe conditions, suggesting an actively growing subpopulation of bacteria specifically adapted to low-Fe condition.

Fe enrichment impacts directly and/or indirectly on the bacterial growth efficiency (BGE), i.e., the ratio between growth and carbon uptake. Nevertheless a different limitation was observed in presence of DOM or TOM. Fe conditions affected bacterial growth efficiency, especially when organic matter was provided in the only dissolved form (Table 4). In

LFe conditions, a large fraction of the consumed organic matter was catabolized and the energy produced was primarily used for maintenance rather than for growth, with resulting low growth efficiency (Tortell et al. 1996; Church et al. 2000; Kirchman et al. 2003). As reported above, in LFe bioassays, respiratory electron transport activity seems to be reduced, as suggested by the low relative abundance of CTC-positive cells. The respiratory electron transport system is essential for adenosine triphosphate (ATP) synthesis in aerobic heterotrophic bacteria, and low Fe can result in a significant decrease of BGE with a subsequent effective co-limitation by Fe and C.

The estimation of bacterial growth efficiency from the variation of bacterial biomass and organic carbon consumption could be underestimated due to continual recycling of organic matter by successive bacterial lysis and consumption of this lysed organic matter in our bioassays. In both Fe conditions, the increase of bacterial abundance is controlled by mortality processes. No protozoa were observed by microscopy. Mortality by protozoan grazing can thus be excluded.

On the contrary, lysis and particularly viral lysis could be a major process in these bioassays. In cultures of natural marine bacteria inoculated into 0.2 μm filtered sea water, Wilcox and Furhman (1994) reported that virus abundance increased after few days of bacterial growth. The high abundance and production of bacteria in the HFe bioassay could enhance viral activity and consequently increase specific mortality rates. In agreement with this, during the mesoscale Fe fertilization EISENEX, a higher viral infection of bacterioplankton was estimated in the Fe-enriched patch (Weinbauer et al. 2003). Lysis could be of significance in Fe-limited ecosystems, as Fe released via lysis can be highly bioavailable (Poorvin et al. 2004).

Fe control on organic matter remineralization in a *Phaeocystis*-dominated ecosystem

In nutrient replete bioassays, the organic matter derived from *Phaeocystis* is generally quickly degraded (see review Schoemann et al. 2005). In natural conditions, large accumulation of DOC have been occasionally reported after *Phaeocystis* blooms (Eberlein et al. 1985; Lancelot et al. 1987; Davidson

and Marchant 1992), suggesting a temporal uncoupling between DOM production and bacterial assimilation. Mechanistic explanations are that inorganic nutrients can limit bacterial uptake capacity of otherwise biodegradable DOM and/or alternatively that a part of the produced DOM escapes fast remineralization due to low biochemical biodegradability (Thingstad and Lignell 1997). As reported above and in other studies (Verity et al. 1988; Baumann et al. 1994), the OM derived from nutrient-limited *Phaeocystis* is characterized by high C/N and C/P, giving rise to a substrate for bacteria with unfavourable high carbon content. In temperate ecosystems, bacteria seem first limited by P availability (Thingstad et al. 1997). In HNLC environment, Fe could control bacterial activities and/or organic matter lability and therefore the accumulation of OM in the upper water column.

In HFe condition, *P. antarctica*-derived organic carbon is quickly degraded. As estimated from our experiments, more than 90% of freshly produced organic carbon, for dissolved as well as for total organic carbon, was degraded by bacteria after seven days. These results are the highest percentages of *Phaeocystis*-derived biodegradable organic carbon reported in the literature. For *P. globosa*-derived organic carbon between 57 and 93% could be degraded after seven days, under conditions of nonlimiting nutrients (see Schoemann et al. 2005 and reference herein). Only 7–24% of *P. antarctica*-derived organic carbon was degraded in *in situ* conditions after 7 days (Carlson et al. 1999). In LFe conditions, the degradation is slower; nevertheless up to 54% of TOC could be degraded after 7 days but only 9% of DOC was degraded after 7 days. The high lability of TOM is in agreement with the high microbial hydrolysis of OM measured in LFe TOM bioassay. Nevertheless, a significant part of *P. antarctica*-derived OM was degraded before 30 days in low and high Fe conditions.

Conclusion

Fe has been shown to play a key role in limiting phytoplankton growth and carbon uptake, and in determining the phytoplankton community structure in the Southern Ocean. Therefore, the carbon transfer efficiency to deep water by the biological carbon

pump depends on Fe availability. Heterotrophic bacteria may also influence the biological carbon pump efficiency through the remineralization of POM originating from phytoplankton but also of DOM in the surface and mesopelagic waters. As shown in this study, the degradation of OM is directly and/or indirectly influenced by Fe availability. The quality of dissolved as well as of particulate matter derived from *P. antarctica* improved and was rapidly degraded by bacteria in HFe conditions. A high fraction of organic carbon consumed by bacteria was incorporated in biomass, as suggested by the high bacterial growth efficiency. Very little accumulation of DOM was observed and therefore the potential C export will mainly be due to *Phaeocystis* colonies and aggregates sedimentation. Contrastingly, in LFe conditions, the relative proportion of DOM in the *P. antarctica*-derived organic matter was high. This organic matter was slowly degraded, but even so, after one month, 44% was degraded by bacteria. The particulate organic carbon was as in the HFe conditions, quickly degraded. In this case, the persistence of DOC could participate to carbon exportation by the advection of surface water masses to greater depth. A large fraction of carbon consumed by bacteria was remineralized and respired as suggested by the low bacterial growth efficiency, suggesting a lower relative potential export of C in this case.

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