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Probing natural iron fertilization near the Kerguelen (Southern Ocean) using natural phytoplankton assemblages and diatom cultures

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Abstract

Natural phytoplankton assemblages collected in surface waters above the Kerguelen Plateau or in the open-ocean and single-species cultures of Southern Ocean diatoms were used to address the existence and effects of natural iron fertilization near the Kerguelen Islands (Southern Ocean). The phytoplankton was transferred during so-called translocation experiments into water collected at the surface over the Plateau, open-ocean surface water or water collected close to the sediment of the Plateau. These watertypes differed in iron (iron-rich deep water and iron-poor surface water) and silicic acid concentration (silicic acid-rich Plateau deep and open-ocean surface water, silicic acid-poor Plateau surface water). As a general trend in the natural phytoplankton assemblages, cell numbers, chlorophyll autofluorescence, photosynthetic efficiency of photosystem II, chlorophyll *a* and phytoplankton carbon concentrations increased especially after translocation into Plateau deep water. This response was most pronounced in terms of increase in carbon assimilation in the larger-sized phytoplankton (>8 μm in cell diameter), mainly diatoms. Effects of translocation on bacteria and viruses followed those of the phytoplankton. Experiments with single-species cultures of large diatoms (*Fragilariopsis kerguelensis*, *Thalassiosira* sp., *Chaetoceros dichaeta*), which have high iron requirements, confirmed the observations made for the natural phytoplankton assemblages.

Assuming a continuous flux of deep water to the surface over the Kerguelen Plateau, the translocation experiments provide evidence that this water contains the growth-stimulating factor, most likely iron, responsible for the formation of a phytoplankton bloom as is observed over the Kerguelen Plateau.

Keywords: Iron; Phytoplankton; Kerguelen; Diatoms; Microbial foodweb; Southern Ocean

1. Introduction

During in situ iron (Fe) enrichment and laboratory experiments, the role of Fe in limiting phytoplankton growth in high-nutrient low-chlorophyll (HNLC) regions has been well studied (reviewed in de Baar and Boyd, 2000; Boyd, 2002; Coale et al., 2004; de Baar et al., 1995), leaving the important role of Fe in carbon cycling beyond doubt. Still, full insight in Fe biogeochemistry (recycling, fluxes), sources of Fe (from sediment or from aeolian input) and, most importantly, the bioavailability of Fe (ionic or organically complexed) is lacking.

Iron can enter the world oceans via dust originating from deserts (Jickells et al., 2005), causing increased Fe bioavailability for phytoplankton (Visser et al., 2003). Alternatively, Fe from sediments or riverine input also can be an important source of this micronutrient, but most of this is supposed to be restricted to coastal areas (Poulton and Raiswell, 2002). However, in case of a shallow plateau with water depth of only several hundreds of meters in open-ocean waters, Fe from the deep may become available in surface waters (Blain et al., 2001; Bucciarelli et al., 2001). A natural laboratory to study Fe fertilization from the sediments can be found around the Kerguelen Islands and the adjacent Plateau to the southeast. In the otherwise true HNLC conditions of the Indian sector of the Southern Ocean, yearly a dense phytoplankton bloom develops over the Kerguelen Plateau. This results in a

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massive CO_2 sink (Blain et al., 2001, 2007, 2008a; Bucciarelli et al., 2001). During the *KE*rguelen *O*cean and *P*lateau *C*ompared *S*tudy, (January–February 2005, KEOPS) expedition the possible source of Fe from Plateau sediment or dust from the Kerguelen Islands, and its potential implications on microbial foodweb and biogeochemical cycles were investigated. The strategy comparing the natural Fe-fertilized Plateau and (as a reference) the open-ocean HNLC sites was chosen because the same processes could be studied in two contrasting natural environments in a single field survey (Blain et al., 2008a). Ample chemical, physical and biological background information for the experimental site was available thanks to years of research in the area (Blain et al., 2001; Bucciarelli et al., 2001).

The source(s) of Fe were not easily identified during the fieldwork, and a potential Fe–silicic acid co-limitation further complicated the analyses of the numerical and/or the functional responses of the phytoplankton. The dissolved Fe (DFe) concentrations were quite similar and low in surface water over the Plateau and in the open ocean. Only close to the sediment were elevated DFe concentrations measured (Blain et al., 2008b). Nitrate concentrations were high (and therefore considered to be non-limiting) both in Plateau and HNLC surface waters. Silicic acid concentrations, on the contrary, were low over the Plateau at about 2 μM and high (>25 μM) in Plateau deep water and in HNLC surface (and deep) ocean waters.

By definition, only living organisms can determine the bioavailability of any nutrient. Especially in the light of the recent debate on the Fe'model, the Fe(II)s model and the FeL model of (reductive) Fe uptake in phytoplankton (Shaked et al., 2005; Salmon et al., 2006), the use of living phytoplankton to determine bioavailability was deemed very appropriate. In this study, natural phytoplankton assemblages and single-species cultures of Southern Ocean diatoms were used to test the hypothesis that Fe originating from the Kerguelen Plateau sediment provided a (continuous) source of Fe, releasing this growth-limiting factor. In order to mimic natural conditions as much as possible, the choice was made only to work with freshly taken natural filtered seawater, collected at three different contrasting locations in so-called translocation experiments. Phytoplankton-free water collected at the Plateau (surface and deep water) and at the open ocean (HNLC surface water) served as growth medium for natural phytoplankton assemblages collected at the Plateau or at the open HNLC ocean. Moreover, the same watertypes were used as growth medium for single-species cultures of Southern Ocean diatoms, thus excluding differences in the phytoplankton community structure. This experimental set-up was used to determine whether upwelled deep water from the sediments of the Kerguelen Plateau could provide enough iron to the surface waters to produce a bloom similar to those seen in field studies and satellite images. In the experiments, the responses of the plankton community were measured as changes in the numerical abundance of the phytoplankton, bacteria and viruses, the photochemical efficiency of photosystem II (F_v/F_m) and changes in flow cytometrically derived bio-optical properties, like scatter (= size) and cellular chlorophyll *a* and carbon concentration (Schreiber et al., 1993; Gorbunov et al., 1999; Sosik and Olson, 2002; Veldhuis and Timmermans, 2007).

2. Methods

2.1. The experimental area

The Kerguelen Plateau can be regarded as an unique natural laboratory in the Southern Ocean as it is located in the core of a key oceanic area in the context of global climate change (Sarmiento et al., 1998), allowing comparison of a Fe-enriched Plateau water with the surrounding HNLC open ocean. Field campaigns and experiments have given insights into the concentration of DFe and the phytoplankton community in the water column above the Kerguelen Plateau (Blain et al., 2001; Bucciarelli et al., 2001). In addition, MODIS satellite data provide a good overview of the spatial and temporal variability of phytoplankton bloom events helping to choose the appropriate time of the year and location for field studies. Yearly, a dense phytoplankton bloom develops southeast of the Kerguelen. During the KEOPS cruise (19 January–13 February 2005) with R.V. *Marion Dufresne*, the mechanisms and effects of natural fertilization of the oceanic water by the Kerguelen Plateau on the biological pump and on the cycles of other chemical compounds relevant for climate were studied in detail, and compared to the adjacent HNLC conditions of the open Southern Ocean.

2.2. Sampling

Sampling of water was done by use of a 6-pack of 10-L Go-Flo bottles mounted on poly-urethane coated frame (NIOZ-made). The frame was lowered to the desired sampling depth using a winch with 8-mm Kevlar wire. Bottles were closed simultaneously using an all Teflon messenger. Depth for closing the bottles was selected based on data from a prior CTD cast. Upon retrieval the Go-Flo bottles were carried to an over-pressurized class 100 clean air container van, the water filtered over 0.2-μm Sartobran filters (using a low N_2 overpressure), gently mixed in large 20-L polycarbonate (PC) carboys and finally dispensed in 2-L or 250-mL PC bottles. Clean techniques were applied throughout. Check on Fe contamination-free work was done by DFe analyses (Table 1). Three different filtered watertypes were used: Plateau surface water and Plateau deep water collected at the main sampling station A3 (position 50°38′S, 72°05′E) situated over the Kerguelen Plateau, and open-ocean surface water (HNLC surface water) collected at the main sampling station C11 (position 51°39′S, 78°00′E). The chemical composition of these watertypes is described in Table 1.

Table 1
Overview of main characteristics of watertypes used in the translocation experiments with natural phytoplankton assemblages and single species of Southern Ocean diatoms, and overview of the resulting nutrient and DFe concentrations in the translocation experiments

Station	Watertype	Depth (m)	Nitrate (μM)	Phosphate (μM)	Silicic acid (μM)	DFe (nM)
A3	Plateau surface	40	23.7	1.55	1.76	0.09
A3	Plateau deep	400	38.6	2.87	61.86	0.35
C11	HNLC surface	40	30.8	2.09	25.2	0.09

Translocation experiment	Nitrate (μM)	Phosphate (μM)	Silicic acid (μM)	DFe (nM)
Plateau surface + Plateau surface	23.7	1.55	1.76	0.09
Plateau surface + Plateau deep	31.2	2.2	31.8	0.22
Plateau surface + HNLC surface	27.2	1.8	13.5	0.09
HNLC surface + Plateau surface	27.2	1.8	13.5	0.09
HNLC surface + Plateau deep	34.7	2.5	43.5	0.22
HNLC surface + HNLC surface	30.8	2.09	25.2	0.09

A full description of the hydrographic conditions during the KEOPS campaign can be found in detail in Park et al. (2008) and Blain et al. (2007, 2008a). Briefly, the macronutrient concentration over the first 100 m of the water column varied little and was on average 26.7 ± 3.1 μM for nitrate plus nitrite, 1.8 ± 0.3 μM for phosphate (all stations pooled). Average silicic acid concentrations in surface waters were 2.1 ± 1.0 and 25.1 ± 0.7 μM in the bloom over the Plateau and in the HNLC area, respectively (Mosseri et al., 2008). In surface water, DFe concentrations were 0.1 nM at all stations, but increased with depth in the Plateau area (Blain et al., 2007, 2008a). In the filtered water used for the translocation and single-species experiments DFe concentrations for Plateau surface, Plateau deep and HNLC surface water were determined to be 0.08 ± 0.01, 0.36 ± 0.02 and 0.09 ± 0.01 nM, respectively, values closely reflecting the ambient Fe concentrations measured (Blain et al., 2008b). A full overview of the parameters investigated during the KEOPS cruise can be found at: http://www.obs-vlfr.fr.proof/vt/op/ec/keops/keo.htm.

2.2.1. Translocation experiments

Natural phytoplankton was collected from unfiltered water immediately drawn from Go-Flo bottles filled in dedicated cast (40 m depth). Samples taken in the surface mixed layer at station A3 (Plateau phytoplankton) and C11 (HNLC phytoplankton), served as inoculum for the three watertypes specified above. In practice, a 1:1 dilution was made, resulting in six different experimental conditions: Plateau and HNLC phytoplankton in Plateau surface, Plateau deep and HNLC surface water. The resulting macronutrient and DFe concentrations after mixing of the different watertypes are given in Table 1. All incubations were done in duplicate, in acid-cleaned, sterile 2-L PC bottles, without any further additions. The bottles were incubated on deck under ambient light (neutrally shielded at 50–60% of the surface photosynthetic active radiation level) and temperature was kept constant using running seawater. The bottles were opened under clean air conditions only. During the 16-day experimental period, 10-mL subsamples were taken for flow cytometric (FCM) enumeration of phytoplankton, bacteria and viruses and for pulse amplitude modulated (PAM)-fluorometry (see below).

2.2.2. Single-species experiments

Filtered water from the three locations specified above was used as growth medium for single-species incubation using the Southern Ocean diatoms *Chaetoceros brevis* (prismatic on a elliptic base, single cells, 4–6 μm in larger cell dimension), *Fragilariopis kerguelensis* (chain-forming elongated cells 20 μm wide, 80 μm long), *Thalassiosira* sp. (chain-forming cylindrical cells, 80 μm in larger cell dimension), and *Chaetoceros dichaeta* (prismatic on a elliptic base, chain-forming cells, 80 μm in larger cell dimension). These diatom species were brought from the home laboratory to R.V. *Marion Dufresne*, and cultured in Fe-poor medium before the start of the experiments. The species were used based on existing knowledge regarding their response to Fe availability (Timmermans et al., 2001, 2004). Incubations were done in a 2 °C climate room, with a 16 h light: 8 h dark regime and a light intensity 80 μmol photons $m^{-2} s^{-1}$. The 250 mL acid-cleaned PC bottles were fixed on a rotating wheel to ensure proper, but gentle mixing. On board, during an 11-day experimental period, 10-mL subsamples were taken for flow cytometric enumeration of *C. brevis*, microscopical analyses of the other three species and PAM-fluorometry. In order to shed light on the exact role of Fe, and the possible interaction with silicic acid, additional single-species experiments with *F. kerguelensis* and *C. dichaeta* were performed in deep water containing 1 nM desferroxiamine B (DFB), a fungal siderophore very selective in binding Fe. On the samples drawn from the single-species experiments the same analyses (FCM, microscopy and PAM fluorometry) were performed as on board (see below).

2.3. Analyses

Under clean condition collected samples were routinely analyzed for their phytoplankton community composition and numerical abundance (also *C. brevis* in the single-species experiments) and chlorophyll autofluorescence (FluorFCM) with a benchtop flow cytometer (COULTER XL-MCL) within 1 h after sampling. Prior to analysis the samples were stored on melting ice and kept in the dark. Chl *a* autofluorescence (excitation 488 and emission >630 nm) and phycoerythrin autofluorescence (emission 575 ± 20 nm) were measured next to the forward light scatter (FLS, as indicator of cell size). The machine drift was tested using calibration beads with known size (3 and 10 μm) and fluorescence properties on a day-to-day basis (Veldhuis and Kraay, 2000). The flow cytometer remained very stable, despite occasionally bad weather, and signals of the calibration beads varied no more than 5%. A cell size of approximately 30 μm was the operational upper size limit of the phytoplankton community that could be quantified in reliable numbers.

The flow cytometrically derived parameter FLS showed a linear relationship with the equivalent spherical diameter (esd) derived from fractionated filtrations (no data shown). A mathematical relationship (Veldhuis and Timmermans, 2007) was used to estimate the esd of the different algal groups in each sample.

The phytoplankton community showed a continuous range of cell size versus cellular Chl *a* autofluorescence but in general six major groups could be distinguished. The three dominant groups were: small eukaryotes (esd 2–4 μm, indicated as EUK I), eukaryotes (esd 4–8 μm, most likely including *Phaeocystis*, indicated as EUK II), and large eukaryotes (esd ranging from 8 to 30 μm, dominated by diatoms, indicated as EUK III). Furthermore, based on their pigment composition and size distribution *Synechococcus* cells, pico eukaryotes (esd <2 μm), and a "high density" group (consisting of resting spores of diatoms) were identified as separate phytoplankton groups; but these individual groups were all less than 3% of the total cell numbers. For all groups, cell biovolume was calculated assuming a spherical cell (cf. Gall et al., 2001). The biovolumes were then used to calculate Chl *a* and carbon content per group for the whole phytoplankton assemblage (cf. Veldhuis and Timmermans, 2007). The empirical relationship as determined by Verity and co-workers (Verity et al., 1992) was used for conversion of biovolume to cell carbon:

$$0.433 \times (\mathrm{BV})^{0.863}\ \mathrm{pg\,C\,cell^{-1}}.$$

A conversion factor was established of the flow cytometric derived Chl *a* autofluorescence signal into a cellular Chl *a* content for the different phytoplankton size categories. The integrated Chl *a* contents of the different size categories (by FCM) matched well with the chemically determined Chl *a* values measured using the whole phytoplankton community, using a subset of Chl *a* data excluding samples dominated by very large cells (>30 μm).

A Zeiss Axiovert 25 inverted microscope was used for counting phytoplankton >30 μm in the natural assemblages (translocation experiments) as well as *F. kerguelensis*, *Thalassiosira* sp. and *C. dichaeta* (single-species experiments) using settling chambers after gravimetric preconcentration at the start and end of the experimental period. As routine only 100 cells >30 μm were counted in the translocation experiments. In the single-species cultures also the number of cells per chain and the size of individual cells were measured.

Samples for counting bacteria and viruses were fixed with glutaraldehyde (25%, EM-grade) to a final concentration of 0.5%, after which they were flash frozen in liquid nitrogen and stored at $-80\,^{\circ}\mathrm{C}$ until analysis. Enumeration of the samples was done by flow cytometry according to Brussaard (2004a) using a Beckton-Dickinson FACSCalibur FCM with a 15-mW 488 nm air-cooled argon-ion laser. Thawed samples were diluted (dilution factor $\geqslant5$ for bacteria and $\geqslant50$ for viruses) in 0.2-μm filtered sterile TE-buffer (pH 8) and stained with the nucleic acid-specific dye SYBR Green I (Invitrogen) at a final concentration of 1×10^{-4} and 0.5×10^{-4} of the commercial stock for bacteria and viruses, respectively. Bacteria and viruses were discriminated on the basis of their green fluorescence and side scatter signature.

The photochemical efficiency of photosystem II (F_v/F_m) of the whole phytoplankton community and single-species cultures was measured using a PAM fluorometer (PHYTOPAM, Walz, Germany). Freshly collected samples were stored on ice in the dark for at least 30 min prior to measurement. Measurements of F_0 and F_m, minimum and maximum fluorescence levels, respectively, were used to calculate F_v (variable fluorescence; $F_v = F_m - F_0$) and F_v/F_m (potential photochemical efficiency of photosystem II; Schreiber et al., 1993). F_v/F_m has been shown to be a powerful tool to indicate the nutritional status of marine phytoplankton (Geider and LaRoche, 1994; Behrenfeld and Kolber, 1999). Especially nitrogen and Fe stress are clearly reflected in low F_v/F_m values (typically 0.2–0.3 range), whereas nutrient replete phytoplankton possessed F_v/F_m values of >0.5.

Concentrations of nitrate and silicic acid in 0.2-μm filtered samples were determined spectrophotometrically, using standard techniques (Grasshoff, 1983). DFe was measured on board and back in the home laboratory using a chemiluminescence method (de Jong et al., 1998) in 0.2-μm filtered samples (Sartobran).

3. Results

3.1. Translocation experiment

The chlorophyll autofluorescence (F_0 as measured with the PAM fluorometer, or FluorFCM measured with the flow cytometer), as indicator of phytoplankton biomass,

was for Plateau and HNLC phytoplankton at the start of the experiments similar to those found in the field (Timmermans, unpublished data), indicative of absence of treatment effects. The F_0 of the Plateau phytoplankton increased after an initial lag phase of 6 days, upon the addition of Plateau deep or HNLC surface water (Fig. 1A, Table 2). The increase at the end of the experiment was the largest in the HNLC surface water treatment, but on Day 12 the Plateau deep-water treatment reached the maximum (Fig. 1A). In contrast, the FluorFCM showed a far more gradual response (Fig. 1B). When transferred into Plateau surface water the F_0 response of the Plateau phytoplankton was only minimal. The values of F_0 for the HNLC phytoplankton were generally lower than those from the Plateau phytoplankton (Fig. 1A). The F_0 response of the HNLC phytoplankton became evident at the end of the 16-day experimental period, but differences were small between the different treatments (Fig. 1A, Table 2), whereas the FluorFCM signal already increased after 6 days (Fig. 1B) with the largest effect in the Plateau deep-water treatment (Table 2). Measurements of F_v/F_m (photosynthetic efficiency) indicated that Plateau phytoplankton performed moderately when transferred to Plateau deep water ($F_v/F_m = 0.38$), after transfer into Plateau surface or HNLC surface water F_v/F_m of 0.26 was measured (Table 2). For the HNLC phytoplankton translocations the F_v/F_m were always higher than for the Plateau phytoplankton translocations, and they were apparently not influenced by the treatments (F_v/F_m range between 0.45 and 0.48).

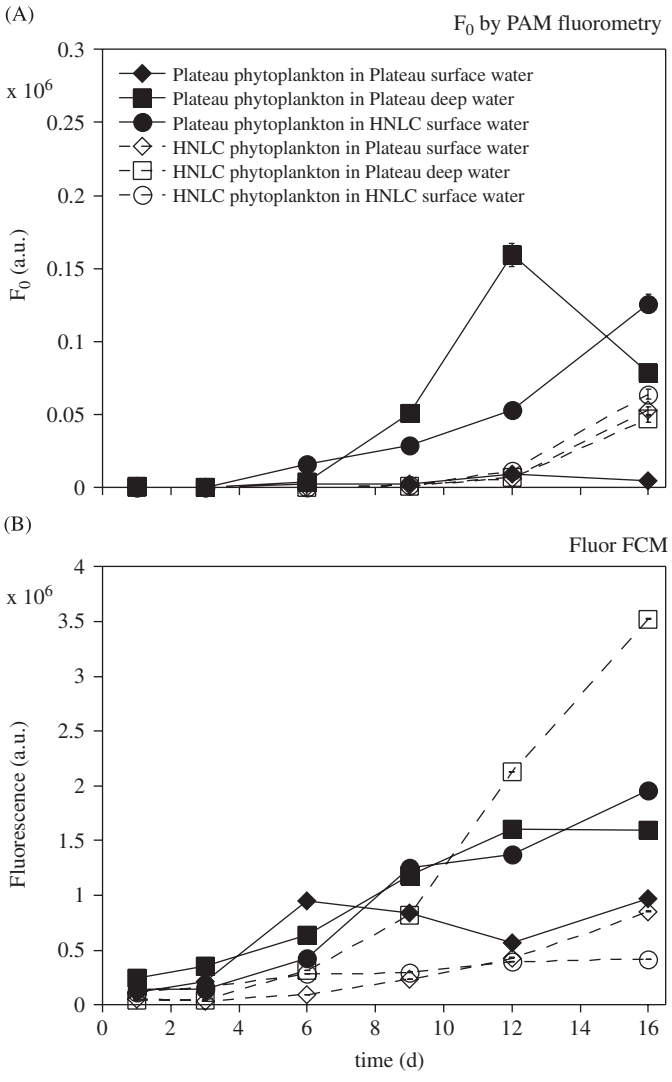

Fig. 1. Average Chl *a* autofluorescence ($n = 2$) measured as (A) F_0 by PAM Fluorometer (a.u.) and (B) FluorFCM by FCM (a.u.) versus time (d) using Plateau and HNLC phytoplankton in Plateau surface (black and open diamonds), Plateau deep (black and open squares) or HNLC surface (black and open circles) water. When no error bars (S.D.) are visible, they are smaller than the symbol (a.u. = arbitrary units).

Total cell numbers of the smaller-sized Plateau phytoplankton (<30 μm diameter, as measured flow cytometrically) showed relative modest increases (Fig. 2A). Starting concentrations ranged between 2.5 and 3.0×10^3 cells mL^{-1}, which was about 50% of the original cell numbers in the field (Timmermans, unpublished data) and increased to about 17×10^3 cells mL^{-1}. There were only little differences between the treatments for the Plateau phytoplankton (Fig. 2A), with increases ranging between 10 and 15×10^3 cells mL^{-1} (Table 2). The numbers of the three phytoplankton groups EUK I–III of Plateau phytoplankton leveled gradually off towards the end of the experimental period in treatments with Plateau surface and HNLC surface water, and a similar trend was seen for EUK I in the Plateau deep water (no data shown). With HNLC phytoplankton, initial cell abundances were between 1.0 and 1.3×10^3 cells mL^{-1}, but in all treatments a substantial increase was observed. The highest cell numbers were reached when incubated in Plateau deep water (41×10^3 cells mL^{-1}, Fig. 2A, Table 2). This increase was mainly caused by the smaller-sized phytoplankton EUK I (Fig. 2B). Translocation into Plateau surface or HNLC surface water also resulted in increased cell numbers with little differences between the treatments (Fig. 2A, Table 2).

At the start of the Plateau phytoplankton experiments, *Eucampia antarctica* dominated the diatoms >30 μm (but only 10% of the total cell numbers), followed by *F. kerguelensis* (50% and 8% respectively of total diatom numbers, with 42% unidentified species). This was in fair agreement with the field observations at Plateau station A3 (Armand et al., 2008). After the 16-day experimental period, considerable shifts in numerical and taxonomical abundance had occurred: *Phaeocystis antarctica* (in colonies, with a diameter of 1–2 mm) fully dominated in cells numbers (respectively 86% and 82% in Plateau surface and Plateau deep water) with some *E. antarctica* and *F. kerguelensis* cells. In the Plateau phytoplankton translocations in HNLC surface water, no colonies of *P. antarctica* were found at the end, *E. antarctica* (38%) and *F. kerguelensis* (33%) were the dominant species present in about equal numbers. In the HNLC phytoplankton translocations less pronounced numerical and taxonomical shifts were observed for the large size phytoplankton cells

Table 2
Overview of the main results from the Plateau and HNLC phytoplankton translocation experiments

Parameter	Plateau phytoplankton			HNLC phytoplankton		
	Watertype			Watertype		
	Plateau		HNLC	Plateau		HNLC
	Surface	Deep	Surface	Surface	Deep	Oceanic
F_0 Δ (a.u.)	0.4×10^4	7.8×10^4	**12.5×10^4**	5.3×10^4	4.7×10^4	6.3×10^4
FluorFCM Δ (αv)	8.5×10^6	14.8×10^6	**18.1×10^6**	8.1×10^6	**35.1×10^6**	3.8×10^6
F_v/F_m (a.u.)	0.26 ± 0.05	**0.38 ± 0.03**	0.26 ± 0.03	**0.48 ± 0.01**	**0.48 ± 0.02**	0.45 ± 0.09
Abundance cells $<30\,\mu m$ Δ (cells mL^{-1})	**1.5×10^4**	1.4×10^4	1.0×10^4	1.9×10^4	4.1×10^4	2.1×10^4
μ (F_0) (d^{-1})	0.09 ± 0.01	0.31 ± 0.05	**0.44 ± 0.09**	0.51 ± 0.06	**0.64 ± 0.14**	0.54 ± 0.16
μ (cells) (d^{-1})	**0.13 ± 0.02**	0.12 ± 0.02	0.09 ± 0.02	0.19 ± 0.01	**0.25 ± 0.01**	0.18 ± 0.01
Chl *a* Δ ($\mu g\,L^{-1}$)	2.0	**12.2**	8.5	12.5	**21.2**	12.3
C Δ ($\mu g\,L^{-1}$)	381	**3770**	1516	456	**1257**	412
Bacteria Δ (cells mL^{-1})	7.5×10^5	**20.0×10^5**	10.0×10^5	**4.9×10^5**	3.5×10^5	1.9×10^5
Viruses Δ (cells mL^{-1})	2.8×10^7	**5.8×10^7**	3.2×10^7	1.0×10^7	**1.1×10^7**	**1.1×10^7**
Putative algal virus Δ (%)	−0.5	**1.4**	−0.8	8.4	**10.5**	5.6

For all parameters, the absolute changes from start to end of the experiments (Δ) are indicated. Per phytoplankton assemblage used, the highest value per watertype is in bold.

($>30\,\mu m$). Initially, *F. kerguelensis* dominated the large diatoms in numbers (70–80%, cf. Armand et al., 2008), and translocation in Plateau surface, Plateau deep or HNLC surface water resulted in only minor changes. In comparison with the total phytoplankton cell numbers, the number of large diatoms in the HNLC phytoplankton remained low (<5%).

Based on the autofluorescence biomass measurements (F_0 by PAM fluorometer) and total cell numbers (cells $<30\,\mu m$, by FCM) growth rates were calculated (Table 2). Using F_0, the Plateau phytoplankton grew the fastest (community average of $0.44\,d^{-1}$) in HNLC surface water, and somewhat slower in Plateau deep water (community average of $0.31\,d^{-1}$). If we take into account the maximum F_0 at Day 12, growth rates of Plateau phytoplankton incubated in Plateau deep water would be $0.48\,d^{-1}$. With a community growth rate of $0.09\,d^{-1}$ the Plateau phytoplankton grew minimally in Plateau surface water (Table 2). Calculation of F_0 based community growth rates for HNLC phytoplankton resulted in values varying between $0.51\,d^{-1}$ (Plateau surface water) and $0.64\,d^{-1}$ (Plateau deep water). These values were for all treatments higher than the growth rates calculated based on the cell numbers (Table 2). Based on the cell numbers Plateau phytoplankton grew slowly and with few differences between the treatments ($0.09–0.13\,d^{-1}$). The HNLC phytoplankton grew slightly best after transfer to Plateau deep water ($0.25\,d^{-1}$) and slightly less in Plateau surface and HNLC surface water (Table 2). The differences in community growth rates based on F_0 and cell numbers were the largest for the Plateau phytoplankton experiments. This is in agreement with the observations that in these experiments the contribution of cells $>30\,\mu m$ increased substantially, this fraction of phytoplankton was not included in the FCM measurements. In the translocation experiments with HNLC phytoplankton the differences in growth rates were smaller (still F_0 based larger than FCM based), an observation in line with the smaller increase in cells $>30\,\mu m$ during these experiments.

Based on the FCM measurement, total Chl *a* concentrations (Fig. 3), as well as Chl *a* per phytoplankton group were calculated. The initial total Chl *a* concentration of Plateau and HNLC phytoplankton were 1.52 and $0.33\,\mu g\,Chl\,a\,L^{-1}$, respectively. These values matched well with the $1.3\,\mu g\,Chl\,a\,L^{-1}$ over the Kerguelen Plateau station A3 (19 January 2005) and $0.25\,\mu g\,Chl\,a\,L^{-1}$ in the open-ocean HNLC station C11 (26 January 2005) in the field. In experiments with Plateau phytoplankton, the largest increase in Chl *a* concentrations was measured in Plateau deep water ($12.2\,\mu g\,L^{-1}$), against only a $2.0\,\mu g\,L^{-1}$ increase in Plateau surface water (Fig. 3A, Table 2). For HNLC phytoplankton the Chl *a* increase was also the largest when transferred into Plateau deep water ($21.2\,\mu g\,L^{-1}$), even exceeding the Chl *a* concentrations at the end of the experiment with the Plateau phytoplankton, which started with five times higher concentration as compared to the HNLC phytoplankton. This increase of HNLC phytoplankton supplemented with Plateau deep water resulted in a numerical increase in all main phytoplankton EUK I–III, irrespective of their size (Fig. 3B).

Total C ($\mu g\,L^{-1}$) as well as the C content of the different size classes of the phytoplankton were calculated based on the FCM measurement of cell numbers and cell size (Fig. 4). Initial carbon concentrations in the Plateau and HNLC phytoplankton experiments were 86 ± 6 and $12 \pm 2\,\mu g\,C\,L^{-1}$, which were in good agreement with the total carbon biomass reported for station A3 and C11 (Armand et al., 2008). Total C showed only a moderate increase of $381\,\mu g\,L^{-1}$ in the experiment in which Plateau phytoplankton was transferred into Plateau surface water, when transferred into Plateau deep water a strong increase

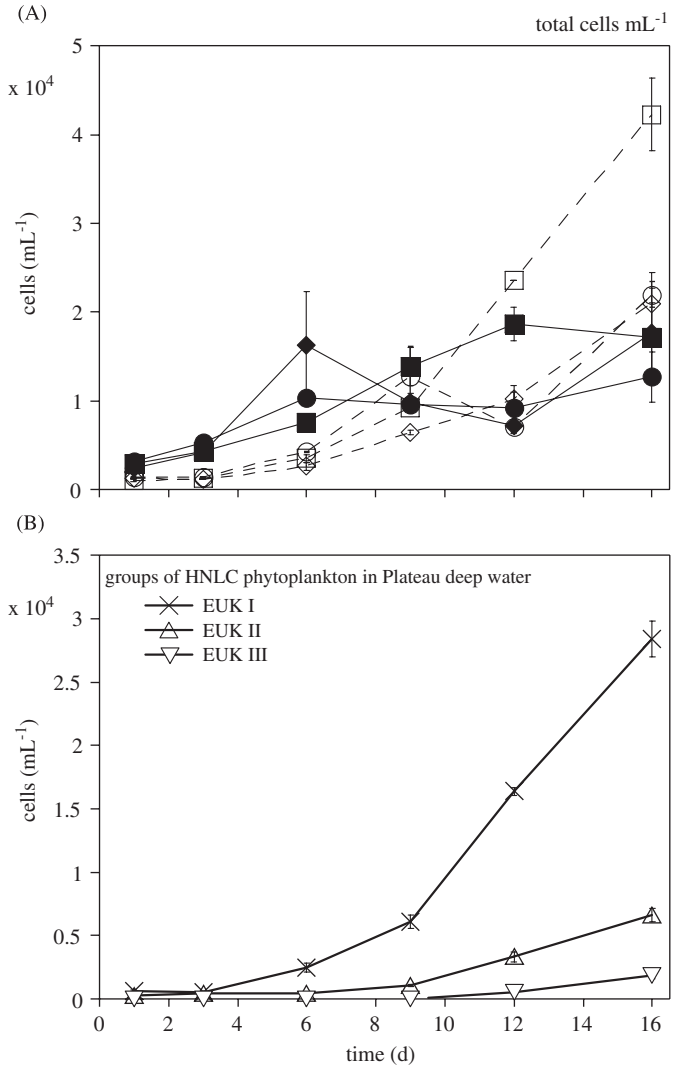

Fig. 2. (A) Average ($n = 5$) total phytoplankton cell numbers (cell size $<30\,\mu m$, mL^{-1}) versus time (d) of Plateau and HNLC phytoplankton in Plateau surface, Plateau deep or HNLC surface water. Symbols as in Fig. 1A. (B) Cell numbers (mL^{-1}) of dominant groups of HNLC phytoplankton (EUK I–III) in Plateau deep water versus time (d).

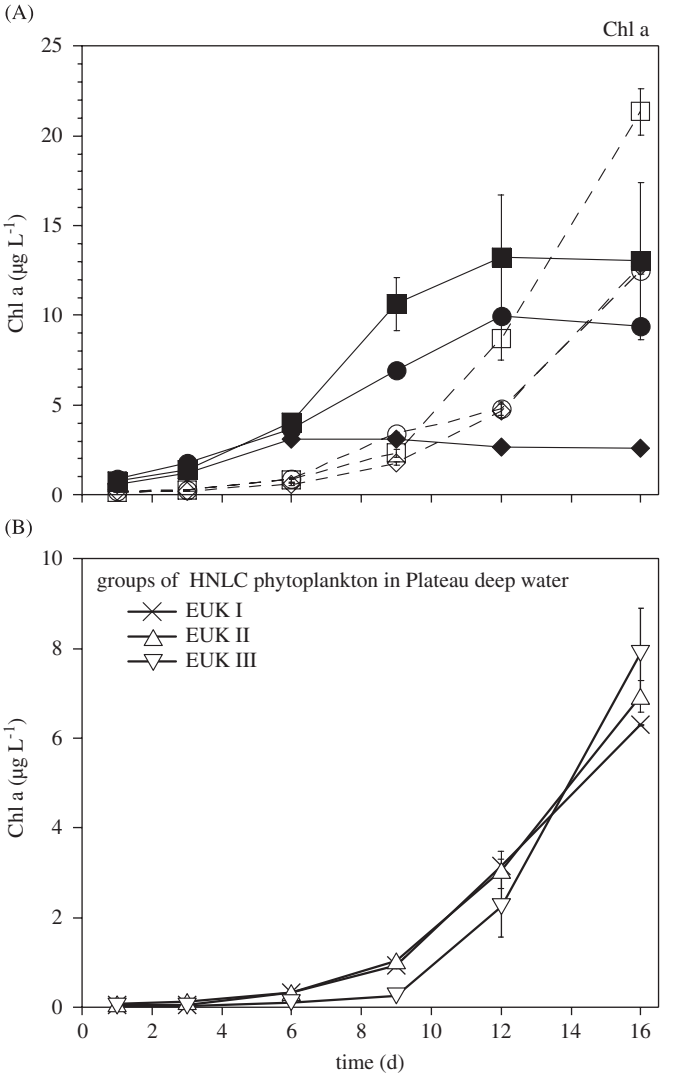

Fig. 3. (A) Average ($n = 5$) calculated total Chl *a* ($\mu g\,L^{-1}$, for calculation, see Section 2) in time (d) during the Plateau and HNLC phytoplankton translocation experiments in Plateau surface, Plateau deep or HNLC surface water. Symbols as in Fig. 1A. (B) Chl *a* ($\mu g\,L^{-1}$) of dominant groups of HNLC phytoplankton (EUK I–III) in Plateau deep water versus time (d).

of $3779\,\mu g\,C\,L^{-1}$ was observed (Fig. 4A, Table 2). This latter concentration was well above the maximum diatom carbon biomass measured in the field of $80\,\mu g\,C\,L^{-1}$ (Armand et al., 2008) at station A3. The increase in phytoplankton carbon was predominantly caused by the group EUK III (no data shown). In the experiments with HNLC phytoplankton, the largest increase of $1257\,\mu g\,C\,L^{-1}$ was measured after transfer into Plateau deep water, predominantly caused by EUK III (Fig. 4B). A much smaller, but equal, increase of HNLC phytoplankton was measured in C after transfer into HNLC surface or Plateau surface water (about $400\,\mu g\,L^{-1}$, Fig. 4A, Table 2). Armand et al. (2008) report a maximum diatom carbon biomass at C11 of $20\,\mu g\,L^{-1}$. Chl *a* and carbon concentrations (both based of FCM data and therefore restricted to phytoplankton $<30\,\mu m$) varied during the experiment, and therefore also the C:Chl *a* ratio changed (Fig. 5): after 16 days of incubation C:Chl *a* decreased slightly to about 50 for HNLC phytoplankton and increased substantially to 180–290 for Plateau phytoplankton.

The average bacterial abundance during the translocation experiment showed little variation during the first 9 days (Fig. 6A). The initial bacterial abundance in the experiments matched well with that found in the field (Brussaard et al., 2008), indicating that filtration did not remove the bacteria. After 9 days, bacterial abundances in the Plateau phytoplankton treatments increased eightfold in the Plateau deep water and threefold in the Plateau surface treatment as compared to the initial numbers (Table 2). For the experiments with HNLC phytoplankton lower absolute increases in bacterial abundance than in the Plateau phytoplankton experiments were observed. In the HNLC phytoplankton experiments threefold and twofold

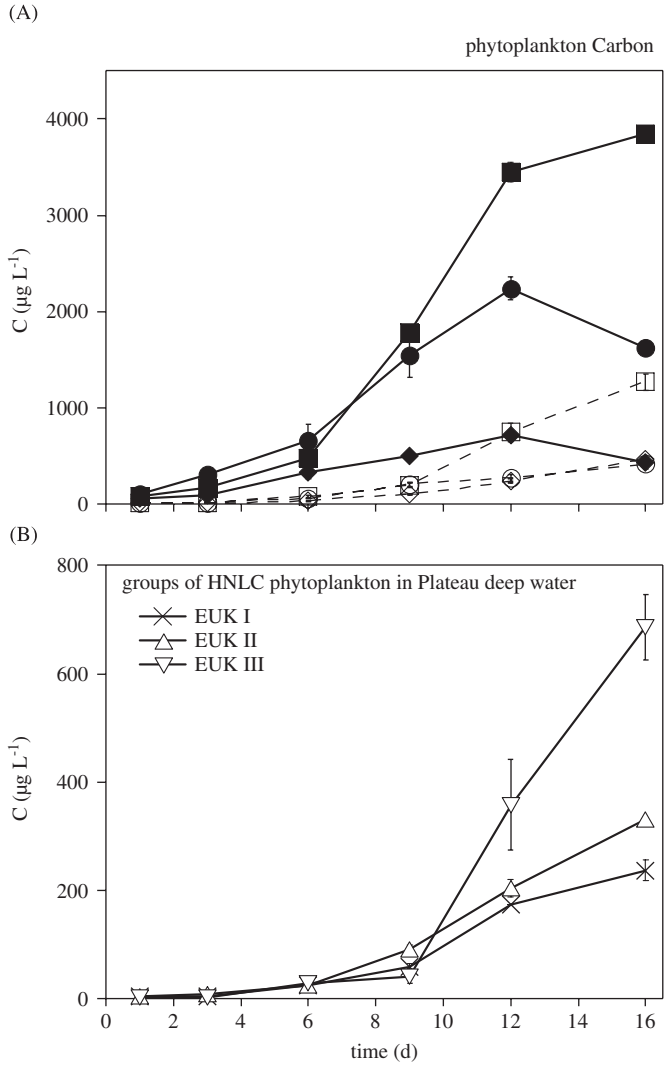

Fig. 4. (A) Average ($n = 5$) calculated total phytoplankton carbon ($\mu g\,L^{-1}$, for calculation, see Section 2) versus time (d) during the Plateau and HNLC phytoplankton translocation experiments in Plateau surface, Plateau deep or HNLC surface water. Symbols as in Fig. 1A. (B) Cellular carbon ($\mu g\,L^{-1}$) of dominant groups of HNLC phytoplankton (EUK I–III) in Plateau deep water versus time (d).

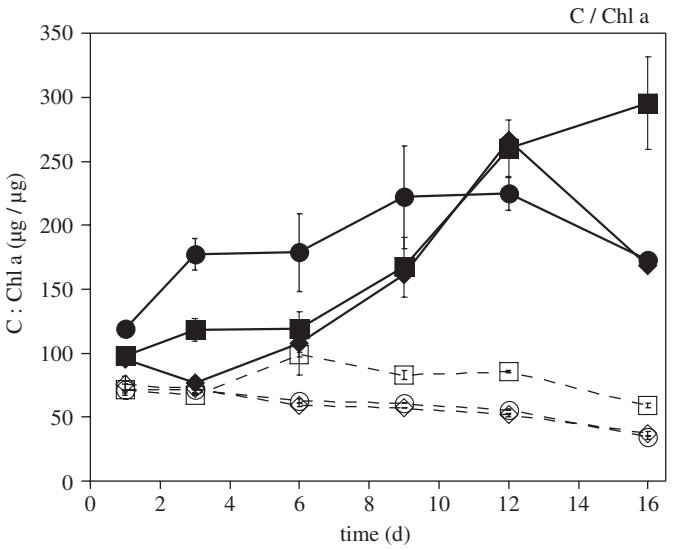

Fig. 5. Average ($n = 5$) C:Chl *a* ($\mu g\,\mu g^{-1}$) versus time (d) during the Plateau and HNLC phytoplankton translocation experiments in Plateau surface, Plateau deep or HNLC surface water. Symbols as in Fig. 1A.

increases as compared to the beginning were observed in Plateau surface and HNLC surface treatments, respectively (Table 2).

The virus abundances showed comparable dynamics to that of the bacteria; increasing only after 9 days and mostly in the Plateau water treatments (Fig. 6B, Table 2). Initial virus numbers in Plateau phytoplankton experiments were about twice those of the HNLC phytoplankton experiments (Fig. 6B), matching the observation in the field (Brussaard et al., 2008). From Day 9 onwards, virus numbers in Plateau phytoplankton experiments increased sevenfold (deep treatment), whereas virus numbers in HNLC phytoplankton experiments showed only moderate increases (threefold increase) as compared to the beginning. Interestingly, in the HNLC phytoplankton translocation experiments only with Plateau surface or Plateau deep water, enhanced abundances of putative algal viruses, i.e. viruses whose flow cytometric signature resembled other known algal viruses (Fig. 7A and B), with maximum increases in the Plateau deep water treatment (10.5%, Table 2). The latter was accompanied by increased abundance of the $<30\,\mu m$ phytoplankton.

3.2. Single-species experiment

The small diatom *C. brevis* was clearly not affected by the origin of the water: inoculation in Plateau surface, Plateau deep or HNLC surface water resulted in similar growth characteristics (Fig. 8A) and photosynthetic efficiency (Table 3). Generally, growth rates were relatively low in this species; under non-deplete conditions typical growth rates of 0.35–0.4 d^{-1} are possible (Timmermans et al., 2001). The measurements of F_v/F_m indicated that the algae were functioning well, and again irrespective of the watertype used. Cell size of *C. brevis* did not change in response to the three different watertypes (no data shown).

The large diatoms species grew well and showed the highest F_v/F_m when incubated in Plateau deep water (Fig. 8B–D, Table 3). In *F. kerguelensis* the highest growth rates and F_v/F_m were measured in Plateau deep water (Table 3, Fig. 8B). The number of cells per chain were on average the smallest in Plateau surface water (12 ± 3), and the highest in Plateau deep water (29 ± 6). The cell size of individual cell of *F. kerguelensis* did not alter ($62 \pm 6\,\mu m$). *Thalassiosira* sp. growth rates and F_v/F_m in Plateau surface and HNLC surface water were only slightly lower than in Plateau deep water (Table 3, Fig. 8C). The number of cells per chain (average 7 ± 2), nor the size of the individual cells ($76 \pm 5\,\mu m$) changed in Plateau surface, Plateau deep or HNLC surface water. Finally, in *C. dichaeta* growth rates and F_v/F_m were slightly higher in Plateau deep water

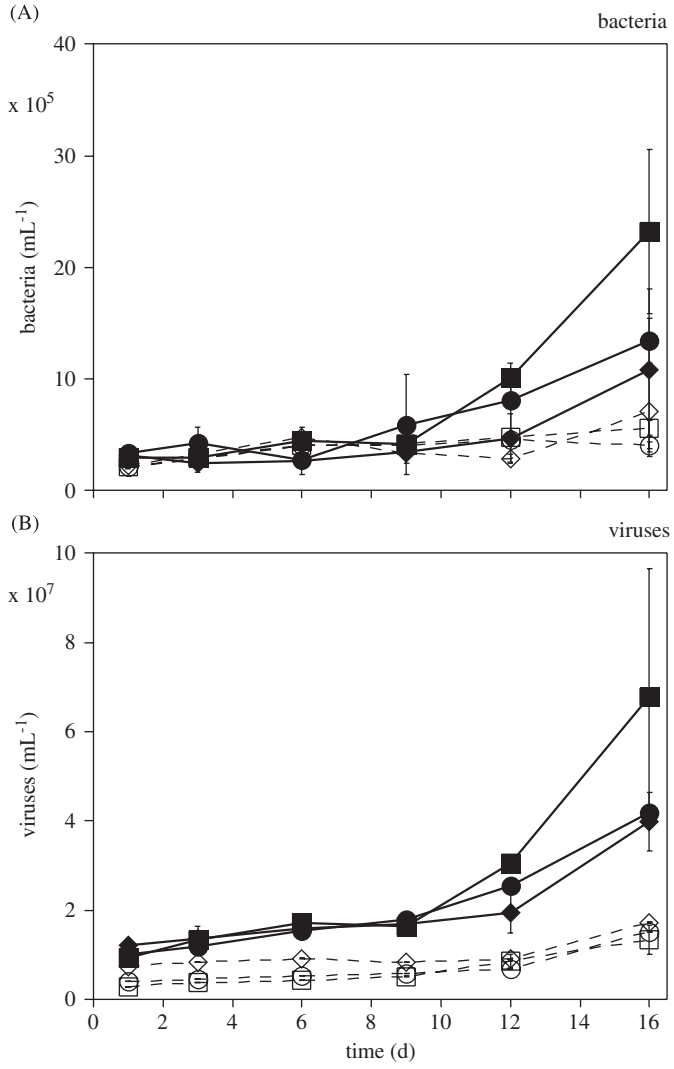

Fig. 6. Average ($n=2$) of (A) bacterial abundance and (B) virus abundance (both in mL^{-1}) during Plateau and HNLC phytoplankton translocation experiments in Plateau surface, Plateau deep or HNLC surface water versus time (d). Symbols as in Fig. 1A.

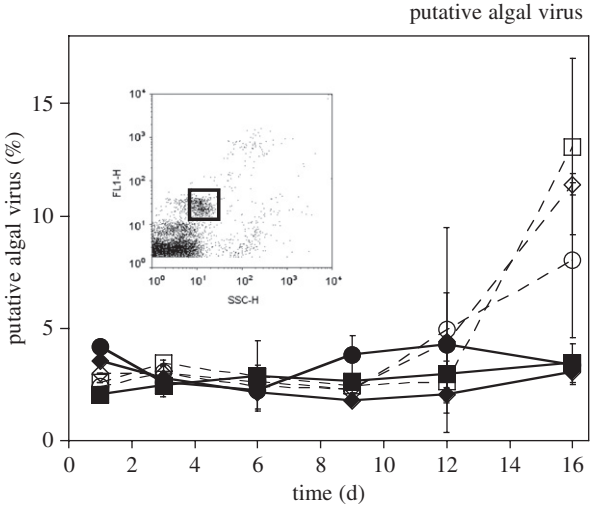

Fig. 7. Average ($n=2$) percentage of putative algal virus versus time (d) during Plateau and HNLC phytoplankton translocation experiments in Plateau surface, Plateau deep or HNLC surface water. Symbols as in Fig. 1A. *Inset*: Putative algal virus in HNLC phytoplankton translocation experiment at $t=16$ days.

(Table 3, Fig. 8D). The number of cells per chain in Plateau deep and HNLC surface water (6 ± 1) was only somewhat higher than in Plateau surface water (4 ± 2). The size of individual cells varied minimally, with the smallest cells in Plateau surface and HNLC surface water ($72\pm2\,\mu m$) and the largest cells in Plateau deep water ($83\pm5\,\mu m$). In the additional experiments (in the home laboratory) with *F. kerguelensis* and *C. dichaeta*, in which DFB was added to Plateau deep water, reduction of growth rates as well as F_v/F_m, to values comparable as measured in Plateau surface and HNLC surface water (Table 3).

4. Discussion

The translocation of natural phytoplankton assemblages and single-species cultures into the different water types collected on and around the Kerguelen Plateau demonstrated that Fe-rich deep water stimulated the phytoplankton more than (Fe-poor) surface water from the Plateau or open HNLC ocean. It was observed that numerical and biochemical changes occurred in particular in the phytoplankton upon amendment with Fe-rich water: cell abundance, chlorophyll autofluorescence, photosynthetic efficiency, Chl *a* and cellular C increased. As such these experiments provide evidence that the Kerguelen Plateau is a potentially important source of Fe in this naturally enriched environment. This is in line with the field observations (Blain et al., 2007), evidencing that the occurrence of the Kerguelen bloom is contingent on persistent iron fertilization, and its duration due to the concomitant supply of macronutrients from surrounding HNLC waters and from below. We can, however, not completely rule out that other factors, e.g., other trace metals or dissolved (in)organic carbon concentrations can have modifying effects in our translocation experiments. The changes in cell carbon concentration supported size-related effects, indicative that the larger-sized phytoplankton (>8 μm diameter, mainly diatoms), generally accepted as the main drivers of the biological carbon pump (Buesseler, 1998) benefited the most of the Fe enrichment (cf. Veldhuis and Timmermans, 2007).

The conditions encountered during the KEOPS cruise were very suitable for the present type of experiments: waters over the Kerguelen Plateau, relatively high in Chl *a* concentration, could easily be compared to typical HNLC waters in the adjacent open Southern Ocean. The deliberate choice was made to do translocation experiments, i.e. to work with natural, filtered, plankton-free seawater collected at the different contrasting sites and use phytoplankton as indicators of the change in environmental conditions, in order to quantify the effects of Fe. Plateau deep water stimulated the phytoplankton more than Plateau surface or HNLC surface water, indicative of

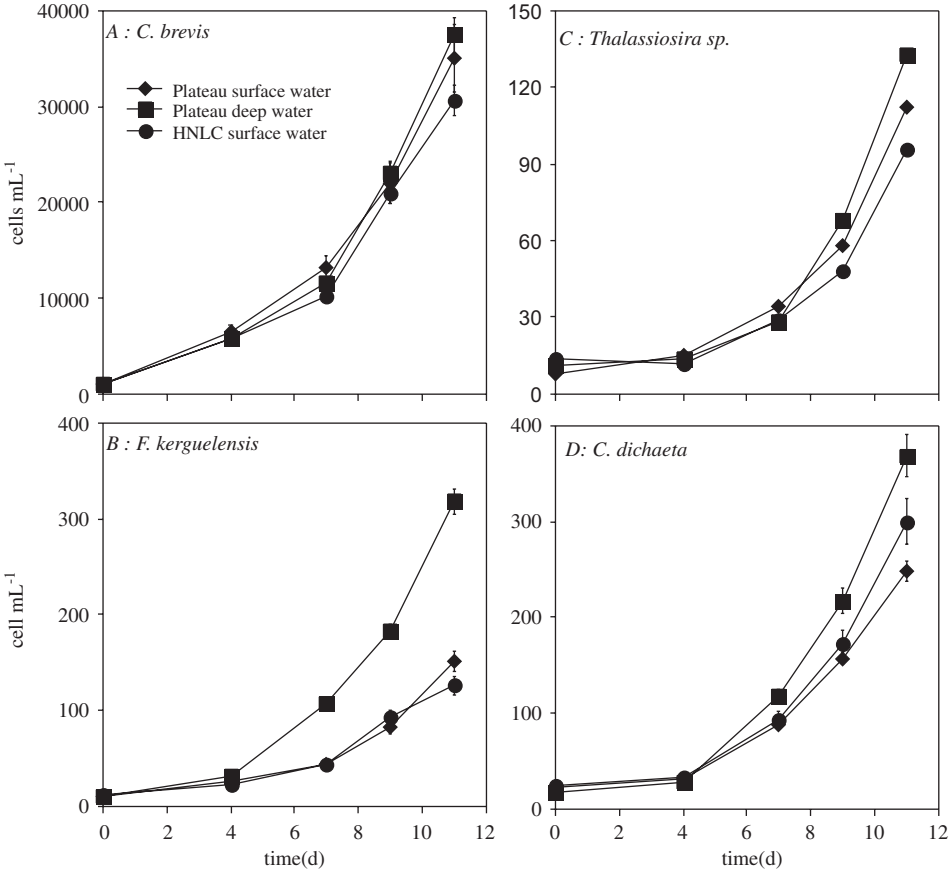

Fig. 8. Average cell numbers (mL^{-1}, ±S.D., $n = 3$) of *C. brevis* (A), *F. kerguelensis* (B), *Thalassiosira* sp. (C) and *C. dichaeta* (D) versus time (d) in Plateau surface, Plateau deep or HNLC surface water. Note the differences in scale of the *y*-axes.

Table 3
Growth rates and F_v/F_m (d^{-1} or a.u., respectively, average $n = 3$, Day 4–11) of *C. brevis*, *F. kerguelensis*, *Thalassiosira* sp. and *C. dichaeta* in Plateau surface, Plateau deep or HNLC surface water

	Plateau surface	Plateau deep	HNLC surface	Plateau deep + DFB[a]
Growth rate				
C. brevis	0.24±0.01	0.27±0.02	0.23±0.04	–
F. kerguelensis	0.23±0.03	0.29±0.03	0.22±0.05	0.22±0.03
Thalassiosira sp.	0.31±0.02	0.35±0.03	0.31±0.03	–
C. dichaeta	0.26±0.03	0.32±0.05	0.30±0.01	0.25±0.02
F_v/F_m				
C. brevis	0.50±0.03	0.51±0.02	0.49±0.02	–
F. kerguelensis	0.38±0.04	0.49±0.07	0.37±0.04	0.40±0.02
Thalassiosira sp.	0.48±0.04	0.51±0.03	0.48±0.04	–
C. dichaeta	0.45±0.06	0.49±0.06	0.40±0.02	0.43±0.05

[a]Additional experiment with 1 nM DFB (final concentration).

a higher Fe bioavailability in waters collected close to the sediment. This general finding still left the possibility open that not only elevated Fe (Plateau deep water only), but also higher silicic acid concentrations (Plateau deep and HNLC surface water) stimulated the phytoplankton, as the phytoplankton community was dominated by diatoms. The conditions as found over the Plateau were indicative of a large diatom bloom in its last stage (post bloom conditions) of development, with low ambient levels of silicic acid. The diatoms in this naturally Fe-enriched region were found to have high affinity for silicic acid which allowed them to persist at low Si concentrations (Mosseri et al., 2008). Moreover, since Fe–silicic acid co-limitation in the Southern Ocean has been demonstrated (Franck et al., 2000; Boyd, 2002; Coale et al., 2004), it can be assumed that the relative low concentrations of silicic acid in Plateau surface water may have affected the diatoms as well, next to Fe. The results from especially

the HNLC phytoplankton translocation experiments with Plateau deep and HNLC surface water (only differing in DFe) in combination with the additional experiments with single-species cultures of Southern Ocean diatoms (selectively removing only Fe by DFB), however, confirmed that in particular Fe originating from the Plateau was the principal factor limiting phytoplankton growth, corroborating the main findings in the SOFEX experiments (Coale et al., 2004). The report that the input of DFe from aerosols coming from the Kerguelen Islands was minimal (F. Dulac, personal communication), further emphasizes the importance of the Plateau sediment as a source of Fe fertilizing surface phytoplankton.

The results as obtained from the translocation experiments indicated furthermore that "just" chemical measurements of DFe and macronutrient concentrations may not be the ideal way to study effects on the microbial foodweb. In surface waters DFe concentrations were the same at the Plateau and at the HNLC site. Either bioavailability of the 0.09 nM DFe at the Plateau was different from that in the HNLC ocean (see Fe-complexation by organic ligands; Gerringa et al., 2008) or, alternatively, the flux of Fe at the Plateau may have been higher (Blain et al., 2007; Park et al., 2008). Recently, a debate has started on the FeL model, indicating that not Fe' (all inorganic Fe species), but FeL, is determining Fe bioavailability (Shaked et al., 2005; Salmon et al., 2006). The differences in the organic complexation of Fe as observed during KEOPS (Gerringa et al., 2008) seem to support this idea. As the debate continues, the phytoplankton species remain in our view the ultimate indicators determining the bioavailability of Fe, integrating all relevant chemical and biological parameters. Under conditions where manipulation of the real ecosystem is impossible, the translocation experiments thus can (simply) mimic the flux of Fe and follow the responses on the microbial foodweb. Some issues still remain to be addressed however, such as, e.g., the relative importance and feasibility of Fe flux from below (see Blain et al., 2007, 2008b; Park et al., 2008), the regeneration rate of DFe in surface water (see Sarthou et al., 2008; Brussaard et al., 2008) or the possibility of an adapted phytoplankton population with high affinity for Fe (just as for silicic acid, see Mosseri et al., 2008).

In the translocations with HNLC phytoplankton the responses generally were pronounced: in Plateau deep water the measured biological parameters consistently peaked at the end of the experimental period, with lower responses in Plateau surface and HNLC surface water. Generally, the increase started only after Day 6, indicative of the low biomass with which the HNLC phytoplankton experiments started. The HNLC phytoplankton was clearly numerically dominated by relative small diatoms ($<10\,\mu m$; cf. Armand et al., 2008), which were, because of their surface-to-volume ratio, expected to be hardly affected by low-Fe concentrations (Timmermans et al., 2001). Still, addition of Fe-rich Plateau deep water caused them to perform better. In the analyses of the individual phytoplankton groups, especially the cell carbon concentration of the larger-sized group increased (diatoms 8–30 μm diameter, EUK III, Fig. 4B). But of course we cannot rule out the possibility that also the smaller-sized phytoplankton was stimulated by Fe, but grazing matched an increase in biomass. The stimulation of larger-sized phytoplankton is in line with artificial mesoscale in situ Fe-enrichment experiments in the Southern Ocean, where the relative large diatoms always showed the clearest response (de Baar et al., 1995; Timmermans et al., 2004; Veldhuis and Timmermans, 2007). As confirmed by microscopical observations, large diatoms ($>30\,\mu m$) played a minor role in the HNLC phytoplankton assemblage from the beginning of the experiment (Armand et al., 2008) and even addition of Fe-rich Plateau deep water did not change this. During the HNLC phytoplankton experiments, Chl *a* concentrations increased more than the cellular carbon concentration, resulting in lower C:Chl *a* ratios towards the end of the experiments. This implies that the phytoplankton cells also used the elevated Fe availability to increase their chlorophyll content (and photosynthetic efficiency, F_v/F_m) (Behrenfeld and Kolber, 1999; Geider and LaRoche, 1994), resulting in an overall better growth and physiological health of the phytoplankton especially in the Plateau deep water.

In the Plateau phytoplankton translocation experiments similar trends as in the HNLC phytoplankton experiments were observed in the biological parameters, but they were less pronounced. This was most likely due to the fact that the initial biomass was already relatively high at the start of the experiment and due to the fact that the phytoplankton community was in its last stage of development (Mosseri et al., 2008; Armand et al., 2008). As with the HNLC phytoplankton experiments, the clearest size-related effects of the different watertypes were seen in the cellular carbon concentrations: transfer into Plateau deep water caused especially the EUK III group (diatoms, esd 8–30 μm) to increase. The microscopic (and macroscopic) observations of a bulk excess biomass formation by *Phaeocystis* in both the Plateau deep and surface treatment at the end of the experiments were not evidenced in all analysis. For the FCM measurements this can be explained by the size of the colonies (not detected by the FCM), but the PAM fluorometer measurements should have included *Phaeocystis*. The initial biomass was high already at the start of the Plateau phytoplankton experiments, and towards the end of the experimental period total Chl *a* and C concentrations leveled off. This could be due to limitation by macro- and/or micronutrients, as it appears that only a continuous flux of Fe and other major nutrients, most notably silicic acid, is necessary to sustain the long lasting phytoplankton bloom over the Plateau (Blain et al., 2007, 2008a). In the translocation experiments this flux was absent. Moreover, grazing or viral lysis may also have controlled algal growth. Brussaard et al. (2008) reported microzooplankton to be a substantial mortality agent. Excluding the microscopic findings, both Plateau deep and

HNLC surface water caused the Plateau phytoplankton to perform better, either fueling the phytoplankton with Fe and silicic acid or just silicic acid, respectively. Based on the Chl *a* and phytoplankton carbon concentrations, the combination of Fe and silicic acid (Plateau deep water) resulted in the best growth and physiological conditions of the phytoplankton. But in comparison to the HNLC phytoplankton, the overall response of the Plateau phytoplankton was more moderate.

In the both Plateau and HNLC phytoplankton experiments, increases of virus abundance were accompanied by increases in bacterial abundance, as indicated by a constant virus-to-bacteria ratio (approximately 30). Clearly, the bacterial abundances were the highest in the Plateau phytoplankton experiments, most likely explained by the higher biomass, and subsequent higher concentration of organic substrate that must have been present in these experiments. The fact that bacterial numbers still increased at the end of the Plateau phytoplankton experiments with Plateau surface and HNLC surface water (where phytoplankton C decreased) indicated that ongoing excretion of organic substrate by the phytoplankton played a role. The Plateau phytoplankton seemed to increase its carbon per cell more than the Chl *a* concentration in the course of the experiments, resulting in enhanced C:Chl *a* ratio, that were substantially higher than those of the HNLC phytoplankton. This increase may be accompanied by enhanced excretion of organic substances, which could be substrate for bacteria. But in general, Fe appeared of minor importance: the bacterioplankton followed the development of algal biomass and the virioplankton that of the bacterioplankton. Putative algal viruses were observed only in the HNLC phytoplankton experiments. The flow cytometric signature of these viruses resembled other known algal viruses (Brussaard, 2004b), suggesting that the numerically second most phytoplankton were their potential host. The increase in these viruses accompanied the strong increase in total phytoplankton cell numbers at the end of the HNLC phytoplankton experiments. Despite the notable increase in abundance of putative algal viruses, the effect of viral lysis on the standing stock of the phytoplankton was minimal ($<3\%$, assuming a burst size of 600). Viral lysis of phytoplankton was detected at station C11 (open ocean) selectively for the 2–4 μm group, but at low rate ($0.1\,d^{-1}$, Brussaard et al., 2008).

The stimulatory effects of the Fe- and silicic acid-rich deep water were grossly confirmed in the single-species experiments. The large diatoms performed best in terms of growth rates and photosynthetic efficiency after incubation in the Fe-rich Plateau deep water. Obviously, these species were the most sensitive to increased Fe availability. The responses in terms of number of cells per chain and size of individual cells varied per species without any clear relation with the treatment. The growth rates were comparable to those previously reported under Fe-deplete and Fe-replete conditions in natural Southern Ocean water (Timmermans et al., 2001, 2004). In Plateau surface water not only the indigenous diatoms (Mosseri et al., 2008), but also the large diatoms were still able to grow, in spite of Si concentrations close to or below their half saturation values (Sarthou et al., 2005). Similarly, photosynthetic efficiencies were hardly affected by the low silicic acid concentrations in the surface water incubations. The small diatom *C. brevis* was not affected by any of the watertypes used, confirming the field conditions and laboratory study (Timmermans et al., 2001) where these small diatoms grew well, also under low-Fe conditions. The additional experiments with *F. kerguelensis* and *C. dichaeta* incubated in Plateau deep water in which the Fe selectively was bound by DFB further clarified the dominant role of Fe.

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