Effects of photosynthesis on the accumulation of Mn and Fe by *Phaeocystis* colonies

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Abstract

The significance of Mn and Fe accumulation by *Phaeocystis* colonies and its control by photosynthesis were investigated by performing incubation assays with radiotracers (^{54}Mn, ^{59}Fe and ^{14}C). Experiments were conducted on pure cultures of *Phaeocystis globosa* and on natural communities collected during a *P. pouchetii* bloom in the Balsfjord (subarctic Norwegian fjord) and a *P. globosa* bloom in the Southern Bight of the North Sea. Results indicate significant accumulation of Mn and Fe in the cultured colonies, as previously shown for Mn. Most of the Mn and Fe accumulation occurred in the mucilaginous matrix of the colonies, and the intracellular assimilation represented only ~10% of the total uptake of these trace elements. These experiments demonstrated that photosynthesis largely governed the uptake of Mn by the colonies but only slightly affected the accumulation of Fe. The positive linear relationships observed for the Balsfjord samples between the Mn uptake and the C fixation in the light suggests photosynthetic control of dissolved Mn removal to the *Phaeocystis* colonies. As had been predicted in earlier studies, the increase in pH and dissolved oxygen observed around and inside the colonies during the photosynthetic activity of the cells could significantly decrease Mn solubility and enhance Mn oxidation rate. However, these changes would not affect significantly the precipitation of Fe according to the thermodynamic considerations. In the highly turbid waters of the North Sea, the removal of Mn and Fe is increased by both inorganic and organic suspended particles, with no significant effect of photosynthesis on the overall uptake.

Phaeocystis is an ecologically important bloom-forming alga. It has a worldwide distribution and develops massive blooms in its colonial form in nutrient-rich marine systems of all latitudes, e.g., in the Southern Ocean (Smith et al. 1999), the Arctic waters (Wassmann et al. 1990), and in the coastal waters of the North Sea (Cadée and Hegeman 1986; Lancelot and Mathot 1987). Because of its abundance and its complex polymorphic life cycle and associated peculiar physiology, this phytoplankter induces dramatic changes in the structure and functioning of the pelagic and benthic ecosystems (Pieters et al. 1980; Lancelot and Rousseau 1994). It has, therefore, a determinant role in the carbon cycle.

The life cycle of *Phaeocystis* is complex and not fully understood. It exhibits an alternation between free-living flagellated cells of 4.5–8 μm in diameter and spherical colonies of up to a few millimeters in diameter (Rousseau et al. 1994). Colonial cells synthesize exopolymers, mainly polysaccharides (Janse et al. 1996), which form a gel when in contact with seawater (van Boekel 1992). A thin but tough semipermeable ''skin'' containing amino groups separates the colonies from the external medium and has small pores between 1 and 4.4 nm in diameter that are permeable to inorganic ions and small organic molecules (Hamm et al. 1999).

Several studies have shown the existence of microenvironments of O_2 and/or pH gradients associated with microbial mats (Epping et al. 1998), marine snow, fecal pellets (Alldredge and Cohen 1987), algal colonies, large algal cells, and phytoplankton aggregates (Richardson et al. 1988; Richardson and Stolzenbach 1995). These microenvironments are induced by either O_2 consumption combined with CO_2 production during respiration (Alldredge and Cohen 1987) or by O_2 production and CO_2 removal with subsequent increase of pH during photosynthesis (Richardson et al. 1988; Richardson and Stolzenbach 1995; Epping et al. 1998). It was also demonstrated that these changes in redox conditions during photosynthesis, in particular the increase of pH, could lead to the oxidation and precipitation of soluble Mn(II) in both marine and freshwater systems (Richardson et al. 1988; Richardson and Stolzenbach 1995; Epping et al. 1998).

Microenvironments with high pH (e.g., 9) and strong oversaturation in O_2 (180% with respect to the atmosphere) were observed by microelectrode measurements around and inside *Phaeocystis* colonies during the photosynthetic activity of the colonial cells at saturating light intensities (Lubbers et al. 1990; Ploug et al. 1999). Lubbers et al. (1990) predicted from calculations based on thermodynamic and kinetic considerations that such high oxygen, and in particular such high pH, would lead to enhanced precipitation of Mn oxyhydroxides. Surprisingly, dark respiration by *Phaeocystis* cells caused pH and oxygen concentrations to decrease only slightly at the surface and inside the colonies, compared with the bulk water (0.04 unit of pH, 6% in oxygen concentration; Ploug et al. 1999). However, such redox conditions would not induce significant redissolution of particulate Mn (Stumm and Morgan 1996). Hence, additionally to the intracellular physiological uptake of Mn, essential to the growth of all phytoplankton species (Sunda 1988/1989), *Phaeocystis* colonies could indirectly accumulate mucus-associated particulate Mn because of the photosynthetic activity of the cells. Accumulation of Mn by cultured *Phaeocystis* colonies has been observed by Davidson and Marchant (1987) and Lubbers et al. (1990) on *Phaeocystis* sp., originating from respectively the Southern Ocean and the North Sea and grown in highly Mn-enriched media (>500 nM). Consequently, the brownish color of colonies that was observed in Antarctic (Davidson and Marchant 1987) and North Sea waters (Bätje and Michaelis 1986) has been suggested to result from Mn precipitates associated with the colony. On the basis of these laboratory results, it was also hypothesized that the high pH and oxygen concentrations sustained around and inside the colonies could favor the precipitation and accumulation of Fe, which is also redox sensitive (Lancelot and Rousseau 1994). Freshly precipitated Mn and Fe oxyhydroxides would, in turn, allow adsorption of phosphate (Slomp and van Raaphorst 1993) and other trace metals such as Zn, Cd, and Co (Balistrieri and Murray 1983, 1986). However, none of these reactions have yet been demonstrated with *Phaeocystis* for trace metal concentrations observed in the field.

In the present study, we conducted radiotracer incubation experiments to investigate the mechanisms responsible for Fe and Mn accumulation by *Phaeocystis* colonies. Incubation experiments were performed on cultured *Phaeocystis globosa* colonies and on natural phytoplankton communities during *Phaeocystis* blooms of two different *Phaeocystis* species (*P. globosa* and *P. pouchetii*). Incubations of the natural communities were conducted at different light intensities and compared with carbon fixation. The *Phaeocystis* intracellular assimilation of Fe and Mn was investigated by application of the Ti-washing technique (Hudson and Morel 1989). The objective was to determine the relative importance of the different possible uptake processes of Mn and Fe by *Phaeocystis* colonies (intracellular and mucus-associated uptake dependent and independent on photosynthesis). The simultaneous use of ^{14}C and radiolabeled trace metals (^{54}Mn and ^{59}Fe), together with a specific inhibitor of the photosynthesis, allowed us to investigate the role of photosynthesis in the removal of dissolved Mn and Fe. This methodology would also provide experimental evidence to verify the transfer

mechanism proposed by Lubbers et al. (1990) for Mn. Fe was also studied, considering the increasing attention paid to this element, which might be limiting under certain conditions and because it is, like Mn, a redox sensitive element. The question whether *Phaeocystis* sp. colonies could play a key role in the biogeochemical cycles of Mn and Fe by accumulating these trace metals in association with the mucus during photosynthesis of the colonial cells was also addressed.

Materials and methods

Polycarbonate or high density polyethylene flasks and filtration devices were used for *Phaeocystis* cultures, seawater samplings, and incubations. 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 HNO_3, except for the nutrient samples, which were collected in 1 N HCl acid-cleaned polyethylene vials. They were thoroughly rinsed with ultrapure water (Barnstead Nanopure System) before use. All procedures prior to the incubations were carried out in a class-100 laminar flow bench.

Chemical and biological parameters—Dissolved Fe and Mn concentrations were measured on samples filtered with 0.1-μm Nuclepore polycarbonate membrane filters with use of Graphite furnace atomic absorption spectrometry (GFAAS; Perkin Elmer Zeeman 5100 PC) for concentrations >20 nM (Schoemann et al. 1998) and flow injection analysis (de Jong et al. 1998) for the lower concentrations. Particulate Mn, Fe, and Al concentrations were determined after strong acid digestion (12 N three times quartz distilled [3Q] HCl, 14 N 3Q-HNO_3, and 40% Merck Suprapur HF) at 100–120°C (Schoemann et al. 1998). The extracted Mn, Fe, and Al were measured by Flame AAS (Perkin Elmer 2380) or GFAAS, depending on the concentration levels. Total suspended matter and nutrients were determined as in Schoemann et al. (1998). Chlorophyll *a* retained on 0.8-μm Nuclepore membrane was measured with a Turner Designs AU-10 fluorometer after methanol extraction. Phytoplankton abundance was determined by inverted microscopy in samples fixed with a 1% lugol-glutaraldehyde solution after a 12-h sedimentation of 5–10-ml samples in Utermöhl chambers (Hasle 1978). The biomass of the cultured *P. globosa* was estimated by use of a carbon content of 122 pg C cell^{-1}, as estimated for cultured *P. globosa* colonies by van Rijssel et al. (1997). This carbon content corresponds to the cellular carbon content surrounded by its mucus layer. Microscopic observation of the shapes and dimensions of the colonies as in Rousseau et al. (1990) allowed the determination of the colonies volume per volume of culture medium. Oxygen saturation was calculated from the dissolved oxygen concentrations determined by the Winkler method, temperature, and salinity.

Phaeocystis culture—Polycarbonate 10-liter carboys (Nalgene) were used for growing *Phaeocystis*. The culture medium was prepared with offshore water of the North Sea. The seawater was filtered through 0.2-μm polycarbonate membranes (Nuclepore) and enriched with 50 μM nitrate,

Table 1. Physicochemical characterization of the Svartnes and Marsdiep stations.

	Svartnes Station 1996	Marsdiep Station 1994
Position	69°22′N, 19°07′E	53°02′N; 04°58′E
Mean water depth (m)	190	7
Physicochemical parameters during the spring bloom		
Temperature (°C)	(2.8–8.2)	(6.3–13.3)
Salinity	(27.9–33.6)	(23.7–29.5)
Particulate Al (nM)	(164–2.60 × 10³)	(2.14 × 10³–50.8 × 10³)
Prebloom winter nutrients (µM)		
$Si(OH)_4$	(5.5–6.3)	(21.4–56.4)
NO_3^-	(7.0–7.7)	(52.2–138.5)
NH_4^+	—	(5.3–9.7)
PO_4^{3-}	(0.50–0.62)	(0.73–1.55)
Chl *a* max (µg L⁻¹)	5.13	74.6

25 µM ammonium, 5 µM phosphate, 20 nM Fe, and 20 nM Mn. The other elements were added according to the methods of Veldhuis and Admiraal (1987). Seawater was autoclaved in the culture bottle, but the nutrient solutions were sterilized separately in the microwave oven. *P. globosa* was isolated in the Southern Bight of the North Sea (51°26′05″N, 02°48′50″E). Cultures were inoculated with a suspension of free-living cells of *Phaeocystis,* obtained after filtration on a 10-µm acid-cleaned (1 N HCl, 12 h) Nylon plankton net of an exponentially growing culture of *Phaeocystis* colonies. Sterilization techniques were applied to make bacterial biomass negligible in comparison with phytoplankton. *Phaeocystis* colonies were grown at 10°C and a 12:12 dark:light (DL) cycle under light intensity of 180 µmol quanta m⁻² s⁻¹, corresponding to saturating conditions (Ploug et al. 1999).

Field sampling—Natural samples were collected during *Phaeocystis* blooms: during spring 1994 in the coastal area of the Southern Bight of the North Sea (Marsdiep Station; 53°02′N, 04°58′E) and in the Balsfjord, a subarctic Norwegian fjord (Svartnes Station; 69°22′N, 19°07′E), during spring 1996. Relevant physical, chemical, and biological characteristics of these stations are given in Table 1. The strong tidal currents and wind, combined with shallow depths, induce strong vertical mixing and resuspension in the Southern Bight of the North Sea (Otto et al. 1990). In contrast, the Balsfjord is a deep basin of 57 km in length with little freshwater input from autumn to the middle of May and low stratification in April, which intensifies in May and June (Reigstad and Wassmann 1996). As is reflected by the particulate aluminum concentrations, the terrigenous inputs at the Svartnes Station are low, compared with those of the Marsdiep Station. The nutrient level is also significantly higher at the Marsdiep Station. Blooms are more intense at the eutrophicated Marsdiep Station than at the Svartnes Station. They corresponded respectively to a maximum of 74.6 µg Chl *a* L⁻¹ at the Marsdiep Station during spring 1994

and of 5.1 µg Chl *a* L⁻¹ at the Svartnes Station during spring 1996 (Schoemann et al. 1998; Schoemann 2000).

At the Marsdiep Station, the sample was collected in late April 1994, as described in Schoemann et al. (1998). At the Svartnes Station, samples were taken at the depth of the Chl *a* maximum (at the surface on 19 April 1996 and at 20-m water depth on 7 May 1996). Surface samples were taken directly in polyethylene bottles from a polyester boat, to avoid contamination from the ship. Sampling of deep water was conducted onboard the RV *Hyas* with acid-cleaned GoFlo bottles. All samples were taken from the upwind and upstream sides of either the ship or the jetty and directly carefully transferred into HDPE bottles.

Radiotracer experiments—Incubations with radiotracers (¹⁴C, ⁵⁴Mn, and ⁵⁹Fe) were performed in the presence and absence of a photosynthesis inhibitor (DCMU: [3-(3,4)-dichlorophenyl-(1,1) dimethylurea]). Time-course experiments were conducted at constant saturating light intensity (180 µmol quanta m⁻² s⁻¹) on *Phaeocystis* cultures. In a second set of experiments, the light dependence of Mn and Fe removal by natural *Phaeocystis* colonies was investigated in relation to ¹⁴C uptake. ¹⁴C bicarbonate samples were incubated for 3–4 h, to measure the photosynthetic carbon fixation and to minimize radiocarbon losses by respiration. Labeled Mn and Fe samples were incubated for longer periods of time (8 and 12 h for the Marsdiep and Balsfjord samples, respectively), to achieve better sensitivity.

Samples were maintained in the dark for at least 4 h before starting the radiotracer experiment, which allowed the cells to reach their basal metabolism. Subsamples of 100 ml in acid-cleaned polycarbonate bottles were spiked with 37 kBq ⁵⁴MnCl₂ and 185 kBq ⁵⁹FeCl₃. These spikes corresponded to an addition of <0.5% of the dissolved concentration of Mn and <2% of the dissolved concentration of Fe initially present in the sample. Parallel radiocarbon incubations of 100-ml samples were conducted with a final concentration of 100 µCi L⁻¹ sodium ¹⁴C bicarbonate. The labeled samples were incubated in an illuminated growth cabinet at relevant temperature (10°C for *Phaeocystis* culture and Marsdiep Station samples and 2°C and 3°C for the Svartnes Station samples, respectively). Light intensities were measured with a Biospherical Instruments QSL-100 light sensor and adjusted as required with neutral density Lee filters.

In order to block specifically the electron flux at the start of the photosystem II and hence to inhibit the production of oxygen, DCMU was added 2 h prior to the addition of the radioisotopes, to reach a final concentration of 10 µM. The efficiency of the inhibition was successfully tested by comparing the ¹⁴C bicarbonate incorporation during incubation experiment on cultured *Phaeocystis* in the presence and absence of DCMU.

After incubation, the ¹⁴C-labeled samples were gravity filtered, to avoid the squeezing and disruption of the *Phaeocystis* colonies. They were filtered on either 25-mm Whatman GF/F filters or 0.8-µm Nuclepore polycarbonate membrane filters of 47-mm diameter. Filters collected for ¹⁴C measurements were stored at −18°C until analysis. The ¹⁴C radioactivity was determined by liquid scintillation with a Packard (Tri-Carb 160 CA) analyzer. Counting errors were

<3%. The photosynthesis-irradiance curves were fitted and the photosynthetic parameters estimated with the method of Marquardt-Levenberg with use of Platt's equation (Platt et al. 1980).

All samples labeled with ^{59}Fe and/or ^{54}Mn were filtered on 0.8-μm Nuclepore polycarbonate membrane filters of 47 mm. Samples to measure the total uptake of labeled trace metal were gravity filtered. For the determination of the intracellular uptake of Fe and Mn, labeled samples were filtered in this case at vacuum pressure <0.3 atm, because only cells had to be kept intact. These filters were further washed with the inorganic reductant Ti(III) complexed by citrate and ethylenediaminetetraacetic acid to remove extracellular Mn and Fe, according to the method of Hudson and Morel (1989). This latter method was specifically developed for Fe and successfully applied for other trace metals (i.e., Mn; Hutchins et al. 1999*a*). The ^{59}Fe and ^{54}Mn filtrates were acidified to pH < 2. Filtrates and filters were kept at room temperature. The gamma emission of ^{59}Fe and ^{54}Mn radioactivity was measured with an HPGE Canberra GeLi detector (relative efficiency of 20%), coupled with a multichannel gamma spectrometer series 20 model 2802. The minimum number of counts was fixed at 1,000 to reduce the statistical counting error to <3%. Balsfjord ^{54}Mn samples were measured by liquid scintillation with a Packard (Tri-Carb 160 CA) analyzer. Counting errors were <3%. Counting of both filters and solutions showed that no significant amount of the radiotracers was adsorbed on the walls of the containers and the filtration device. Activities were corrected for radioactive decay, geometry of the vials, and quenching.

The relative uptake of the labeled trace metals used in the experiments was calculated on the basis of the activity of the particles collected on the filters divided by the total activity (filter + solution), expressed in parts per thousand (ppt). ^{54}Mn and ^{59}Fe transfer to the particles also includes the isotopic exchange of the radioactive nuclides with the exchangeable Mn and Fe stable isotopes associated with the particles (Herzl and Roevros 1998). Because surface adsorption is a very rapid process, most of the isotopic exchange is expected to take place within a few minutes after isotope addition. This process was evaluated by taking a first sample after 10 min of incubation. The uptake rates were then calculated from the slope of the linear regression of time-dependent curves for the fraction of radioisotope present in the particulate phase over the period from 10 min after isotope addition until the end of the experiment. The photosynthesis-dependent transfer of Mn and Fe to the *Phaeocystis* colonies was further estimated, under the assumption that isotopic exchange is independent of light and of DCMU. Molar uptake rates were then estimated from the initial dissolved concentrations of Mn and Fe. Turnover times of the dissolved Mn and Fe pools relative to particulate formation were calculated from the transfer rates of ^{54}Mn and ^{59}Fe as the time needed to remove all the added dissolved ^{54}Mn and ^{59}Fe.

Results

Kinetic experiment on cultured Phaeocystis *colonies*—A radiotracer time-course experiment with pure *Phaeocystis*

colonies incubated under saturating light conditions was first conducted to identify the different pathways of Mn and Fe transfer to and within the colonies. The exponentially growing culture of *Phaeocystis* colonies contained 17 μg Chl *a* L^{-1} and 4.37 × 10^7 cells L^{-1}. Initial concentrations of dissolved Mn and Fe were 22 and 40 nM, respectively, which is in agreement with those observed in the coastal zone of the North Sea (Schoemann et al. 1998). At time zero of the culture experiment, particulate Mn and Fe were 2.38 and 41.5 nM, respectively.

The carbon assimilation by *Phaeocystis* colonies (cells + mucus) remained linear under constant saturating light conditions over the 12 h of the experiment, proceeding at a rate of 7.5 μM h^{-1} ($n = 5$, $r^2 = 0.99$; Fig. 1a). The 24-h incubated sample was unfortunately lost during its processing. Both ^{14}C fixation measured in the presence of DCMU (Fig. 1a) and in the dark (not shown) reached <0.5% of the ^{14}C fixation during photosynthesis. This indicates that photosynthesis inhibition by DCMU addition was achieved.

Ten minutes after spiking, 2.1 ppt of the dissolved ^{54}Mn was transferred to *Phaeocystis* colonies (Fig. 1b). After this initial transfer, the uptake of ^{54}Mn by *Phaeocystis* proceeded linearly with time during the 24-h experiment in the absence or presence of DCMU (Fig. 1b). Photosynthesizing *Phaeocystis* took up ^{54}Mn at a rate of 2.1 ppt h^{-1} ($n = 6$, $r^2 = 0.99$). Clearly, this uptake was largely driven by photosynthesis (80%), because only 0.40 ppt h^{-1} of the dissolved ^{54}Mn ($n = 4$, $r^2 = 0.96$) was transferred to *Phaeocystis* when photosynthesis was inhibited (Fig. 1b). The intracellular uptake of ^{54}Mn also proceeded linearly with time during the 24-h period (Fig. 1b) at a rate of 0.18 ppt h^{-1} ($n = 5$, $r^2 = 0.86$), contributing to only 9% of the total Mn uptake rate.

In the case of Fe, a significant initial transfer of dissolved ^{59}Fe by *Phaeocystis* was observed (Fig. 1c). About 95 ppt of the ^{59}Fe added was sorbed to *Phaeocystis* during the first 10 min after the addition of the radionuclide. After this rapid transfer, the dissolved ^{59}Fe was taken up by *Phaeocystis* at a constant rate of 15 ppt h^{-1} ($n = 6$, $r^2 = 0.99$) during the 24-h incubation period (Fig. 1c). The uptake rate of dissolved ^{59}Fe was still as high as 11 ppt h^{-1} ($n = 4$, $r^2 = 0.96$) when photosynthesis was inhibited (Fig. 1c). The intracellular uptake of dissolved ^{59}Fe proceeded linearly at a rate of 1.4 ppt h^{-1} ($n = 5$, $r^2 = 0.96$; Fig. 1c) and corresponded to 10% of the total uptake.

Effect of light intensity on Mn uptake by natural communities of the Balsfjord—The role of *Phaeocystis* colonies in the transfer of dissolved Mn to particles in natural environments was investigated in the Balsfjord at the time of a *Phaeocystis* bloom. In particular, the light dependence of the Mn transfer from the dissolved phase to the particulate phase was tested on two samples described in Table 2. Chl *a* concentration was similar in both samples, but *Phaeocystis* cell abundance was two times higher on 7 May than on 19 April. On the basis of an average *Phaeocystis* cell content of 0.5 pg Chl *a* deduced from Rousseau et al. (1990), *Phaeocystis* was estimated to contribute to ~60% and 100% of the Chl *a* on 19 April and 7 May, respectively. Typical photosynthesis-light responses without photoinhibition, up to 270 μmol quanta m^{-2} s^{-1}, were observed (Fig. 2a,c). The max-
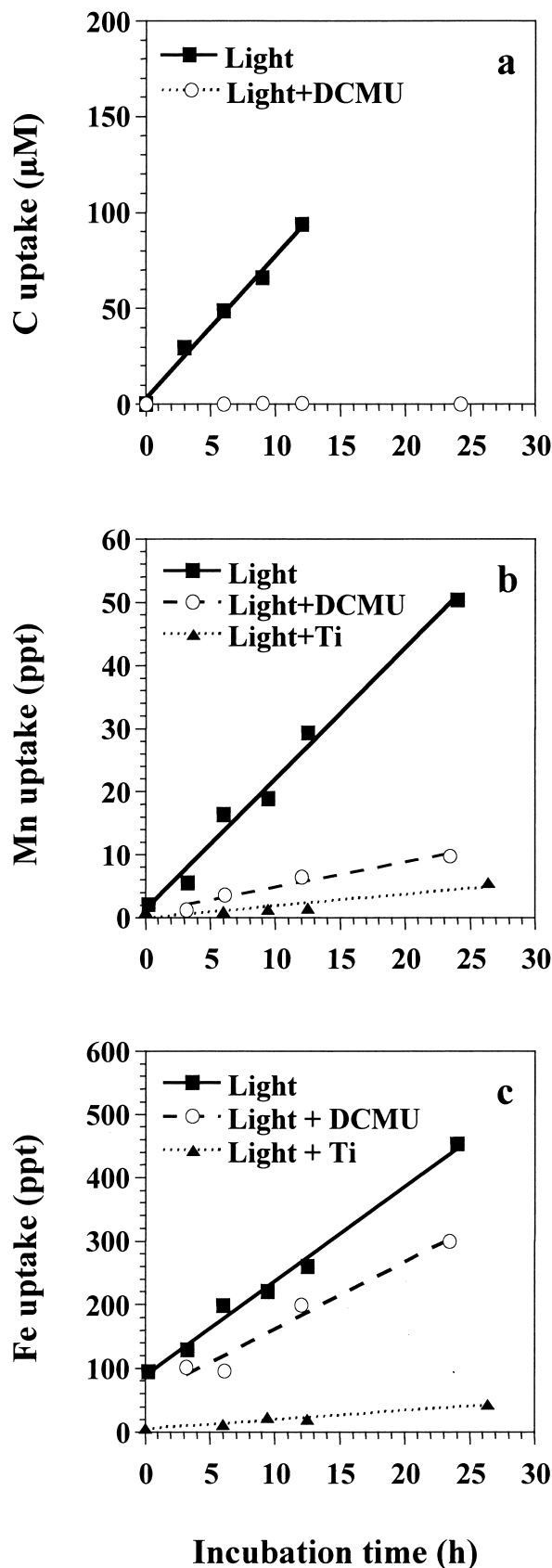
imum specific photosynthesis rate (P_{max}) was much higher on 19 April ($\sim 10.2 \pm 1.0$ mg C [mg Chl a]$^{-1}$ h^{-1}) than on 7 May ($\sim 0.78 \pm 0.06$ mg C [mg Chl a]$^{-1}$ h^{-1}). Such a difference corresponds to the large range of P_{max} (0.76–13 mg C [mg Chl a]$^{-1}$ h^{-1}) estimated for *Phaeocystis* colonies (Cota et al. 1994; Matrai et al. 1995), but this could also be due to the presence of other phytoplankton species with a higher photosynthetic capacity in the April sample. However, another possible explanation for the decrease of photosynthetic efficiency is the shortage of macronutrients or Fe, as is indicated by the low ambient concentrations of 7 May (Table 2). The light adaptation parameter (I_k) was higher for the sample collected at the surface on 19 April (~ 85 μmol quanta m^{-2} s^{-1}) than for the sample at 20 m depth from 7 May (~ 29 μmol quanta m^{-2} s^{-1}). This observation agrees with I_k values previously found for *P. pouchetii* (Verity et al. 1991; Matrai et al. 1995) and with a general better adaptation to low light intensities of phytoplankton communities collected at depth.

The uptake of ^{54}Mn in the dark was 0.22 ppt h^{-1} on 19 April and was 0.17 ppt h^{-1} on 7 May (data not shown). The ^{54}Mn uptake due to light (^{54}Mn in the dark is subtracted from the total ^{54}Mn uptake) was linearly dependent on the light for light intensities <100 μmol quanta m^{-2} s^{-1} and <40 μmol quanta m^{-2} s^{-1} on 19 April and 7 May, respectively (Fig. 2b,d). Surprisingly, ^{54}Mn light-dependent uptake measured in the 19 April sample at 270 μmol quanta m^{-2} s^{-1} was very low (0.08 ppt h^{-1}), which suggests some decrease in the Mn transfer to particles at high light intensity (Fig. 2b). Such a decrease at high light, however, was not observed for the deeper sample (Fig. 2d), which casts some doubt concerning the accuracy of this data point. With this exception, the light dependence of ^{54}Mn uptake mimicked that of ^{14}C, which suggests that Mn uptake is driven by photosynthesis.

Effect of light intensity on the uptake of Mn and Fe by natural communities of the Southern North Sea—The Fe and Mn tracer experiments were conducted on a sample collected on 29 April at the Marsdiep Station. The Chl a concentration was 20 μg L^{-1}, and *Phaeocystis* cell concentration was 2.4 $\times$ 10^7 cells L^{-1}, which represented $\sim$80% of the total phytoplankton abundance but $\sim$60% of the Chl a concentration. The oxygen saturation was 110%, demonstrating the dominance of the autotrophic activity (Table 2). For this experiment, three light intensities were tested, below and close to the saturating light of *Phaeocystis* colonies in the coastal North Sea as reported by Lancelot and Mathot (1987). Parallel experiments with the photosynthesis inhibitor DCMU were performed. Accordingly, the carbon uptake at light intensity close to saturation (121 μmol quanta m^{-2} s^{-1}) was higher (2.15 mg C [mg Chl a]$^{-1}$ h^{-1}) than that at 22 μmol quanta m^{-2} s^{-1} (0.35 mg C [mg Chl a]$^{-1}$ h^{-1}) and 46 μmol

$\leftarrow$

uptake with and without DCMU; (b) Mn total uptake with and without DCMU and Mn intracellular uptake obtained by Ti-washing; and (c) Fe total uptake with and without DCMU and Fe intracellular uptake obtained by Ti-washing.

Fig. 1. Time-course experiment on cultured *Phaeocystis* colonies incubated at constant saturating light intensity: (a) carbon

Incubation time (h)

Table 2. Characterization of the samples collected at the Svartnes and Marsdiep stations.

	Svartnes station		Marsdiep station
Phaeocystis species	*P. pouchetii*		*P. globosa*
Date of sampling	19 April 1996	7 May 1996	29 April 1994
Sampling depth	surface	−20 m	surface
Temperature (°C)	2	3	10
$Si(OH)_4$ (μM)	4.6	3.9	1.1
NO_3^- (μM)	0.5	0.1	49.3
PO_4^{3-} (μM)	0.27	0.17	0.11
Dissolved Mn (nM)	6.9	5.9	33
Dissolved Fe (nM)	25	4.2	73
Particulate Mn (nM)	4.7	1.21	100
Particulate Fe (nM)	161	95	7.57×10^3
Particulate Al (nM)	494	401	16.0×10^3
Suspended particulate matter (mg L^{-1})	4.4	3.6	60
Oxygen saturation (%)	105	109	110
Chl *a* (μg L^{-1})	2.09	2.11	20
Phaeocystis abundance (cells L^{-1})	2.68×10^6	5.17×10^6	2.40×10^7

quanta m^{-2} s^{-1} (0.44 mg C [mg Chl *a*]$^{-1}$ h^{-1}; Fig. 3a). As shown in Fig. 3a, the inhibition of photosynthesis by DCMU was complete.

Regardless the addition of DCMU, similar amounts of dissolved ^{54}Mn (7.9 ppt with and without DCMU) and of dissolved ^{59}Fe (157 and 160 ppt, respectively, with and without DCMU) were transferred to the particles during the 10 min after spiking (data not shown). Hence, DCMU did not interfere with the adsorption of dissolved ^{54}Mn onto particles, as was already shown by Rosson et al (1984), nor did it with the adsorption of dissolved ^{59}Fe.

The total uptake rates of ^{54}Mn and of ^{59}Fe were not affected by light or by photosynthesis (Fig. 3b,c). On the contrary, the intracellular assimilations of ^{54}Mn and ^{59}Fe were both ~3–4 times higher under light conditions (24–121 μmol quanta m^{-2} s^{-1}) than in the dark (Fig. 3d,e). However, these intracellular assimilations only represented 1%–3% and 4%–11% of the total uptake for ^{54}Mn and ^{59}Fe, respectively. Above 24 μmol quanta m^{-2} s^{-1}, the observed Mn and Fe intracellular uptake rates were constant (Fig. 3d,e) which contrasts with the observed photosynthesis-light relationship (Fig. 3a). This discrepancy suggests that photosynthesis and Mn and Fe intracellular uptake are not directly coupled. Rather, photosynthesis produces the minimum energy required for trace metal transfer inside the cell.

Discussion

Several authors have demonstrated the accumulation of Mn by *Phaeocystis* colonies grown in highly Mn-enriched media (>500 nM; Davidson and Marchant 1987; Lubbers et al. 1990). Lubbers et al. (1990) proposed that the Mn accumulation in the colonies could be indirectly due to photosynthesis. The photosynthetic activity of the colonial cells was shown to induce an increase in pH (e.g., up to 0.4 units) and in oxygen concentrations (e.g., from 310 to 530 μM) around and inside the *Phaeocystis* colonies (Lubbers et al. 1990; Ploug et al. 1999). These redox conditions were suggested to favor the precipitation and accumulation of Mn and Fe by the colonies (Lubbers et al. 1990; Lancelot and Rousseau 1994). In the field, Morris (1971, 1974) attributed the observed variations of the dissolved and particulate Mn in the Menai Straits to the accumulation of Mn by a concomitant bloom of *Phaeocystis* colonies. Additional investigations (Schoemann 2000) also showed that a *Phaeocystis* bloom in the Balsfjord could be responsible for the accumulation in the surface waters of particulate Mn and Fe as well.

The results on cultured *Phaeocystis* confirm the ability of the colonies to accumulate strongly Mn and show, moreover, the accumulation of Fe by the colonies. The total volume occupied by the *Phaeocystis* colonies was estimated to be 1.5×10^{-3} liter per liter of culture medium. Particulate Mn and Fe concentrations were 2.38 and 41.5 nM, respectively. Mn and Fe concentrations per volume of colonies (1.6 μM Mn and 28 μM Fe) were therefore, respectively, ~70 and 700 times higher than dissolved concentrations in the culture medium (22 nM Mn and 40 nM Fe). *Phaeocystis* colonies can thus accumulate Mn and Fe at concentrations observed during *Phaeocystis* blooms in the North Sea (Schoemann et al. 1998).

Partition of Mn and Fe between the dissolved and the particulate phases in Phaeocystis *culture and* Phaeocystis-*dominated ecosystems*—The uptake of radiotracers during incubation experiments can be quantified simply by a fast distribution coefficient K_d (Turner et al. 1992) represented by

$$K_d = \frac{(\text{dpm } g^{-1})_{\text{particulate}}}{(\text{dpm } g^{-1})_{\text{dissolved}}}. \tag{1}$$

This coefficient expresses the affinity of the metal isotope for the particulate phase. At saturating light intensity, the affinity of the Fe isotope for pure *Phaeocystis* colonies was one order of magnitude higher than that for Mn, which shows that the colonies are a better trap for Fe than for Mn (Table 3). The partition coefficients of the Mn isotope were similar in pure culture of *Phaeocystis* colonies and in the Balsfjord. By contrast, it was much higher at the Marsdiep Station (Table 3). This suggests that the uptake of Mn in the Balsfjord could have mainly been controlled by the *Phaeocystis* colonies, whereas other particles were responsible for the transfer of dissolved Mn to the particulate phase at the Marsdiep Station. In the case of Fe, similar partition coefficients were observed for both *Phaeocystis* culture and for the Marsdiep Station, although, in the latter area, particles were dominated by lithogenic material.

Very short turnover times of the dissolved Mn and Fe pools were estimated for the Marsdiep Station, compared
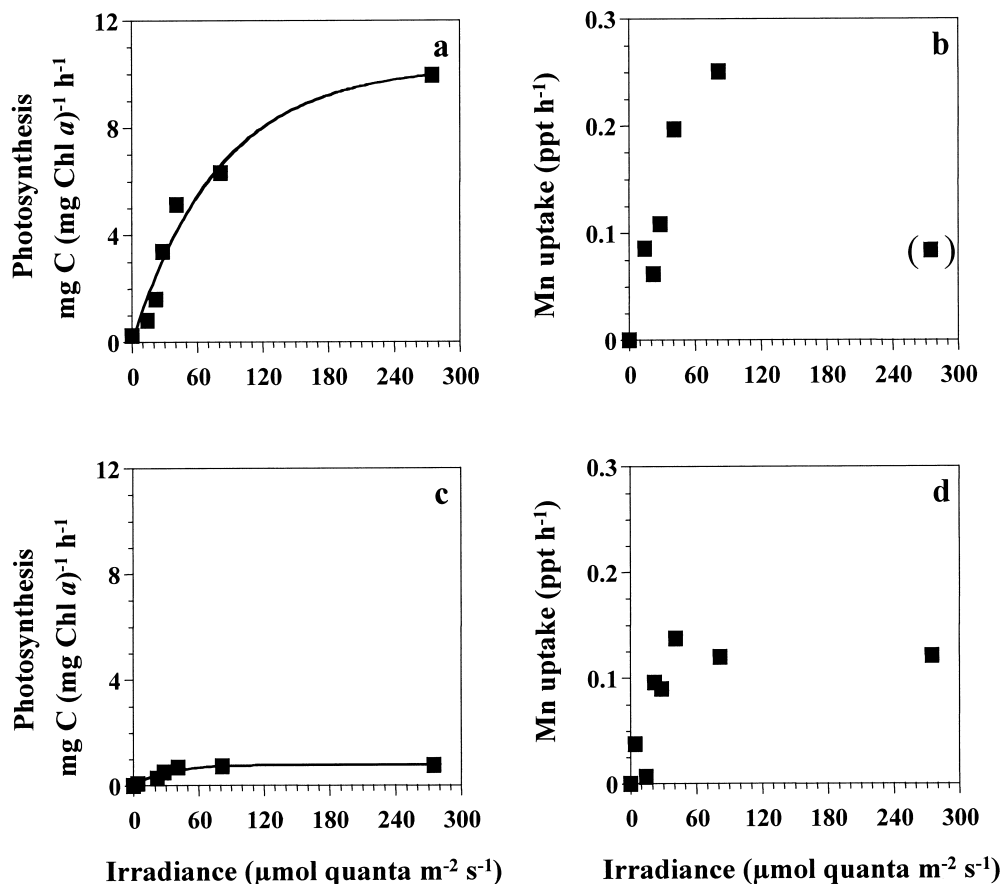
Fig. 2. Effect of light intensity on carbon fixation and on Mn uptake by natural phytoplankton communities collected in the Balsfjord during a *Phaeocystis* bloom (a and b) on 19 April and (c and d) on 7 May. Mn uptake in the dark has been subtracted from the total uptake.

with those obtained in culture and in the Balsfjord (Table 3). Sunda and Huntsman (1987) found similar short turnover times of dissolved Mn with respect to oxidation down to 0.7 d in a North Carolina estuary. This phenomenon was attributed to the high ratio of particulate to dissolved Mn and to bacterial mediation (Wollast et al. 1979; Sunda and Huntsman 1987). Without efficient surface catalysis or microbial mediation, the rate of Mn oxidation is extremely slow (Diem and Stumm 1984). This process is greatly accelerated by the presence of a solid phase capable to absorb Mn(II) and autocatalytically promotes the precipitation of the oxides (Stumm and Morgan 1996). The presence of suspended particles was also shown to increase significantly the rate of Fe precipitation (Aston and Chester 1973). However, high bacterial biomass is also generally observed at the Marsdiep Station in April–May (Brussaard et al. 1995). Several authors reported the presence of Fe- and Mn-depositing bacteria in coastal and open seawaters (Cowen and Silver 1984; Heldal et al. 1996). Therefore, bacterial-mediated oxidation could also have played a role on the removal of dissolved Mn and Fe. Both the presence of high concentration of suspended particles and that of bacteria could have had a significant role in the removal of dissolved Mn and Fe in our experiment conducted at the Marsdiep Station.

Several mechanisms can be invoked to explain the transfer of dissolved ^{54}Mn and ^{59}Fe to the particulate phase. The initial step in the uptake of radionuclides involves the rapid isotopic exchange with the stable elements at the surface of the solid phase (Herzl and Roevros 1998). The uptake of the radionuclides after this first step represents primarily the uptake by the particles via various mechanisms. For pure cultured *Phaeocystis* colonies, it includes Mn and Fe bound to the cell surfaces and intracellularly assimilated, adsorbed, and precipitated on or in the mucus layer. In addition, in the natural samples, it takes into account the uptake by other microorganisms and the removal by detrital organic and mineral particles.

The initial step is very rapid, and reversible. It can be expressed by the equilibrium relation,

$$*Me_{aq} + Me_s = Me_{aq} + *Me_s, \qquad (2)$$

$$K = \frac{(Me_{aq})(*Me_s)}{(*Me_{aq})(Me_s)}, \qquad (3)$$

where $*Me_{aq}$ and Me_{aq} represent the radioactive and the stable trace metal in the aqueous phase, respectively. The radioactive trace metal easily exchanged (after 10 min of incubation) with the stable isotope, Me_s, present at the adsorption sites at the surface of the solid is given by $*Me_s$. The isotopic exchange coefficient, $K,$ is very close to 1. This
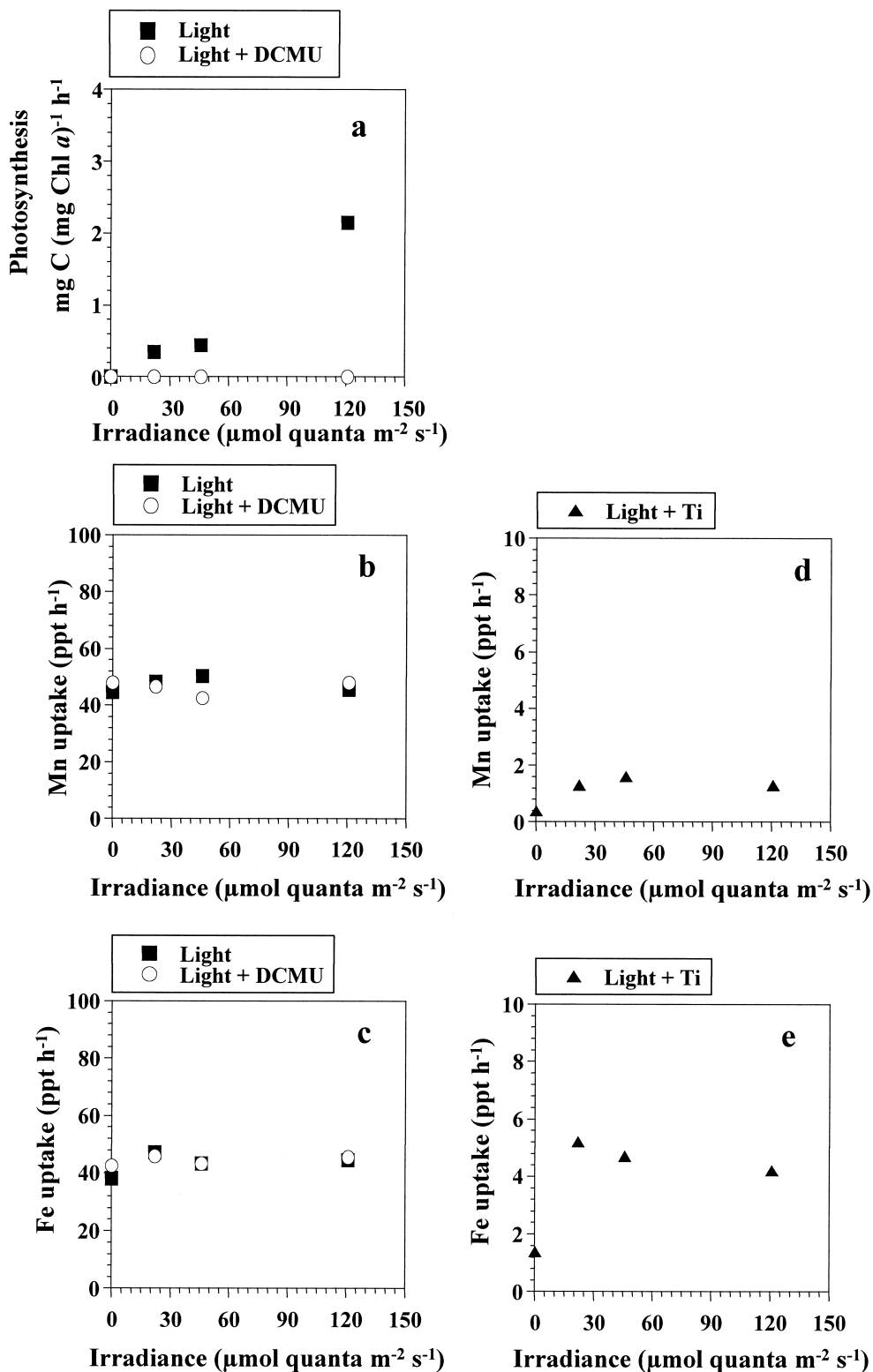
Fig. 3. Effect of light intensity on (a) total carbon fixation with and without DCMU, (b) total Mn uptake with and without DCMU, and (c) total Fe uptake with and without DCMU and the intracellular assimilation of (d) Mn and (e) Fe, by natural phytoplankton communities collected in the Southern Bight of the North Sea during a *Phaeocystis* bloom.

Table 3. Fast distribution coefficients of Mn and Fe for 12-h exposure, the concentrations of easily exchangeable particulate Mn and Fe, the exchangeable fraction, and the turnover times of Mn and Fe, estimated for *Phaeocystis* in culture and in natural *Phaeocystis* dominated environments at saturating light intensity.

	Culture	Svartnes station		Marsdiep station
Fast distribution coefficients (g/g)				
K_d Mn	2.0×10^3	1.3×10^3	1.1×10^3	2.7×10^4
K_d Fe	2.7×10^4	—	—	4.6×10^4
Exchangeable Mn and Fe				
Mn_s (nM)	0.046	<D.L	<D.L	0.26
Fe_s (nM)	4.2	—	—	14
Mn_s (%)	1.9	<D.L	<D.L	0.26
Fe_s (%)	10	—	—	0.18
Turnover time (d)				
Mn	20	88	134	0.83
Fe	3	—	—	0.88

relation allows the evaluation of the concentration of easily exchangeable metals present at the surface of the solid:

$$(Me_s) = (Me_{aq})\frac{(*Me_s)}{(*Me_{aq})}. \qquad (4)$$

The exchangeable fraction can then be obtained directly by dividing Me_s by the total concentration of the metal considered in the solid phase. Table 3 shows the concentration of rapidly exchangeable Mn and Fe both in culture and in natural environments. It is important first to underline the relatively large fraction of both Fe and Mn rapidly exchanged in the culture compared to the Marsdiep Station dominated by lithogenic particles. Furthermore, in the colonies, the fraction of rapidly exchangeable Fe is significantly higher than that for Mn.

Manganese is used primarily by phytoplankton as the primary electron acceptor in the oxidation of water in photosystem II and is present in some superoxide dismutases (Sunda 1988/1989). Iron is involved, among others, in photosynthetic as well as respiratory electron transfer proteins, in the synthesis of Chl *a* and in the reduction of nitrate (Sunda 1988/1989). The intracellular uptake rate of Fe in our *Phaeocystis* culture was estimated to be 1.3×10^{-18} mol cell^{-1} h^{-1}, an order of magnitude higher than that of Mn (9.0×10^{-20} mol cell^{-1} h^{-1}). This observation is in agreement with the higher Fe, compared with Mn, phytoplankton cell requirements (Raven 1990). About 1.0×10^{-18} mol Mn cell^{-1} h^{-1} and 1.4×10^{-17} mol Fe cell^{-1} h^{-1} were taken up totally by the *Phaeocystis* colonies (mucus + cells). This indicates that most of the total uptake of Mn and Fe (~90%) was associated to the mucus, whereas only ~10% was taken up intracellularly. Therefore, Mn and Fe accumulation by the colonies mainly occurs in the mucilaginous matrix of the colonies.

The observed difference of affinity of the colonies for the trace metals can furthermore be attributed to the chemical composition of the mucus together with the differences in reactivity, solubility, and complexation of Mn and Fe. The

mucus of *Phaeocystis* consists of more than eight different sugars and some carboxylated polysaccharides such as glucuronic acid (Janse et al. 1996), well known for binding metal cations (Decho 1990). Van Boekel (1992) showed that the gelling capacity of *Phaeocystis* mucus depends on Ca and Mg concentrations, which bind with the negatively charged groups of the exopolymers and serve as ionic bridges between the exopolymers. Dissolved Mn occurs primarily as free cation (Mn^{2+}; ~70%; Byrne et al. 1988) in seawater. Van Boekel (1992) suggested that other bivalent cations than Ca^{2+} and Mg^{2+}, like Mn^{2+}, might also be involved as ionic bridges between the mucus exopolymers. However, Brown and Lester (1982) showed a low tendency of Mn(II) to form organic complexes with exopolymers. Contrary to Mn, Fe has a strong ability to form organic complexes (Brown and Lester 1982).

In oxygenated seawater, Mn(II) is thermodynamically unstable. This leads to the precipitation of Mn(III and IV) oxides at however very slow rates (Stumm and Morgan 1996). In the case of Fe, the stable oxidation state in oxygenated seawater is Fe(III). Contrary to Mn, Fe is rapidly oxidized and precipitated in oxygenated environments. Iron, initially adsorbed at the surface of the colonies, can thus be easily precipitated and entrapped in the mucus.

Effect of photosynthesis—A contrasting effect of photosynthesis on the uptake of Mn compared with Fe is observed in our study on cultured *Phaeocystis*. The use of DCMU indicates that 80% of the uptake of Mn but only 30% of the uptake of Fe by *Phaeocystis* colonies is controlled by photosynthesis. Furthermore, the Ti-washing technique shows that only ~10% is intracellularly assimilated, and ~90% is taken up in the mucus. This demonstrates that the accumulation of Mn in the mucus is mostly due to photosynthesis.

The uptake of Mn was clearly light dependent in the Balsfjord during the bloom of *P. pouchetii*. Linear relationships were observed between Mn uptake, after subtracting that in the dark, and the photoassimilated carbon for the two experiments (Fig. 4), with the exception of Mn uptake at the highest light intensity in the experiment conducted on 19 April. The manganese to carbon molar ratio calculated from the slope of the linear regression of the light-dependent Mn uptake as a function of photoassimilated carbon was found to be 1.6 ± 0.1 μmol Mn mol^{-1} C ($r^2 = 0.90$, $n = 6$) for the experiment conducted on 19 April. On 7 May, when *Phaeocystis* colonies largely dominated the phytoplankton community, this Mn : C ratio was estimated to be 6.0 ± 0.5 μmol mol^{-1} ($r^2 = 0.81$, $n = 7$). These ratios are close to that of photosynthetically induced uptake (4.9 μmol Mn mol^{-1} C) observed in our culture experiment. The parallel light dependence of Mn and C uptake and the similar Mn : C ratios observed in the Balsfjord and in culture suggest that Mn cycling is mainly controlled by the photosynthesis of the *Phaeocystis* colonies present in this environment.

Photosynthesis may induce indirectly the precipitation of Mn by increasing the oxygen concentration and pH within and around the colony. The increase of either of these two parameters thermodynamically favors the oxidation-precipitation of Mn^{2+} and enhances the rate of these processes, as previously discussed by Lubbers et al. (1990). The main ef-
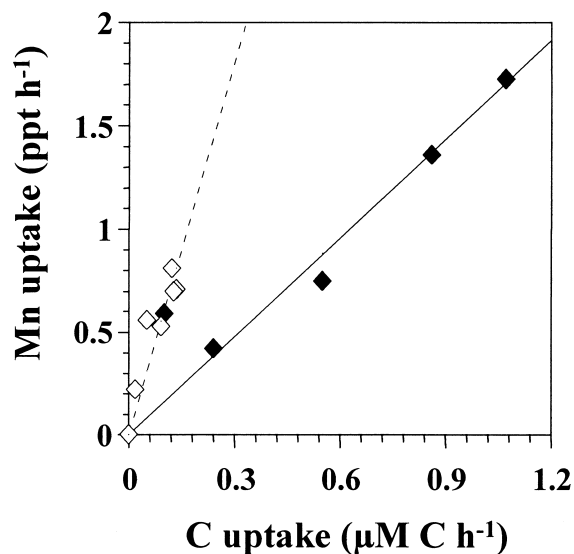
Fig. 4. Linear regressions between light-dependent Mn uptake (Mn uptake in the dark is subtracted) and carbon fixation during photosynthesis for the experiments conducted on 19 April (open diamonds; $y = 1.60x$, $r^2 = 0.90$, $P < 0.0001$, $n = 6$) and on 7 May (closed diamonds; $y = 6.04x$, $r^2 = 0.81$, $P < 0.0001$, $n = 7$) at the Svartnes Station.

fect is due to the change of pH, and there is only a slight influence of the increased oxygen concentration (Lubbers et al. 1990). It is interesting to note that, on the contrary, such a pH increase does not affect the Fe solubility (Liu and Millero 1999).

In the case of the Marsdiep Station, the effect of photosynthesis on the uptake of Mn and Fe by the *Phaeocystis* colonies is likely masked by the high concentration of suspended particles. Here, the photosynthesis of the *Phaeocystis* colonies does not influence significantly neither the Fe removal as observed in the culture nor the Mn transfer to the particles. In agreement with these results, Schoemann et al. (1998) showed no direct effect of *Phaeocystis* blooms on the Mn or Fe concentrations in the coastal waters of the North Sea during both 1993 and 1994.

Ecological and biogeochemical implications—The significant amount of Mn and Fe accumulated in the mucus of *Phaeocystis* may constitute an extracellular reservoir for these essential elements. In Fe or Mn limiting environments, where *Phaeocystis* colonies often dominate the phytoplankton community, e.g., the Southern Ocean, the Fe and Mn precipitates may become bioavailable via various pathways such as biological or photochemical reduction of the organically complexed Fe. The extracellular reservoir would only be beneficial for the *Phaeocystis* cells if they are able to use the accumulated Mn and Fe. Earlier studies suggested that only the dissolved inorganic pools of Mn and Fe were available to the phytoplankton (Hudson and Morel 1990).

It was previously hypothesized that decreases in pH and oxygen at the surface and inside the colonies, after the respiration of the cells in the dark, could allow the reduction of Mn and Fe and their subsequent availability to the cells

(Lancelot and Rousseau 1994). However, the observed decreases of pH (by 0.04 pH units) and of oxygen saturation (by 6%) in the dark (Ploug et al. 1999) are insufficient to reduce significantly Fe and Mn oxyhydroxides. Mechanisms such as physiological or photochemical reduction are more likely to render a portion of these Fe and Mn pools bioavailable. Eukaryotic phytoplankton may use cell-surface reductases to access organically bound Fe (Jones et al. 1987; Hutchins et al. 1999*b*). These reductases reduce the organic complexes, producing ligand-free Fe(II), which is then available to the cell. The availability of Fe could also be increased by rapid photochemically driven redox cycling between oxyhydroxides or organically complexed Fe(III) and inorganic Fe(II) (Finden et al. 1984; Wells and Mayer 1991). The organic content of the mucus may facilitate the photoreduction of solid phase Fe(III) to soluble Fe(II). Similar mechanisms may also be involved in the case of Mn and make it available to phytoplankton (Sunda and Huntsman 1994).

Conclusions

The success of *Phaeocystis* genera in marine systems has been attributed to its ability to form large mucilaginous colonies, which store energy, constitute a protection against grazers, and could also provide a reservoir for Mn and Fe (Davidson and Marchant 1987; Lancelot and Rousseau 1994). We showed by radioisotope incubation experiments that *Phaeocystis* colonies accumulate Mn and Fe in their mucus. Manganese uptake by the colonies is merely due to photosynthesis, presumably, as predicted by Lubbers et al. (1990) because of an increase of pH and dissolved oxygen concentration at the surface and inside the colonies. These chemical changes lead to a decrease of Mn solubility and induce an enhancement of the Mn oxidation rate. Iron accumulation results more likely from adsorption, complexation, and precipitation, which are less affected by photosynthesis. A linear positive relationship is observed in the Balsfjord between the Mn and C uptake in the light during a *Phaeocystis*-dominated spring bloom. This suggests the control of the Mn removal by the photosynthesis of the *Phaeocystis* colonies in this area. On the contrary, in regions of high turbidity, like in the Southern Bight of the North Sea, the transfer of dissolved Mn and Fe from the dissolved to the particulate phase are both independent of photosynthesis during the *Phaeocystis* blooms. Most of this transfer is likely to be controlled by adsorption and catalytic oxidation of the reduced metals related to the high-suspended load, which masked the effect of photosynthesis. The presence of Mn- and Fe-depositing bacteria can also promote the oxidation and precipitation of these metals. In areas where Mn and Fe concentrations may become limiting for phytoplankton growth, this accumulation in the mucus could constitute a supplementary reservoir of these elements to *Phaeocystis*. This mechanism could render this species more competitive with respect to other phytoplankton and therefore play a significant role in structuring the phytoplankton community and hence the associated food web.

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