Limnol. Oceanogr., 50(6), 2005, 1810–1819

Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system

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Abstract

The effect of atmospheric inputs on phytoplanktonic dynamics was investigated in the Mediterranean Sea during the season characterized by a stratified water column, low primary productivity, and low concentrations of nutrients ([nitrate] $<$ 50 nmol L^{-1}; [phosphate] = 20 nmol L^{-1}; [silicate] = 0.7 μmol L^{-1}). We report here data obtained during microcosm enrichment experiments performed on the natural assemblage using different combinations of realistic additions (Saharan dust, Fe, Fe + phosphate, and anthropogenic particles). Saharan dust and Fe + phosphate treatments significantly stimulated primary production. Anthropogenic particles and Fe + phosphate treatments increased the chlorophyll *a* concentrations, enhancing mainly the small cells (pico- and nanophytoplankton). The autotrophic community structure was significantly altered; for example, Fe and Fe + phosphate additions benefited prokaryotic populations, indicating possible nitrogen fixation. The colimitation of both phosphate and Fe was removed by these additions. Results emphasized the effect of Fe, although the ambient concentration was close to 1 nmol L^{-1}. The addition of dust benefited eukaryotic populations, which indicates that the dust was a possible source of nitrogen. An abiotic dissolution experiment of macronutrients attached to dust confirmed this hypothesis. The dissolution of Fe attached to the dust (0.23–0.61%) and to the anthropogenic particles (0.86–1.85%) was consistent with previous studies conducted under abiotic conditions. This result suggests that the possible enhancement of the dissolution processes caused by biological activity might have been balanced by Fe consumption by the biota and its adsorption on both mineral and organic particles.

Studying the key interactions between the ocean and the atmosphere is essential to understanding the present function of biogeochemical cycles in the ocean and to predict their evolution. Atmospheric deposition is now recognized as a significant source of external iron (Fe) and other nutrients for surface waters. Although the effect of Fe on productivity has been recognized in high-nutrient, low-chlorophyll (HNLC) regions (e.g., Martin et al. 1994), the ecological effects of atmospheric Fe and macronutrients in terms of species response and community structure in oligotrophic environments are poorly understood. Natural and anthropogenic changes in climate and global biogeochemistry can alter the atmospheric input of aerosols to the ocean. It is important to understand how these modifications cause changes in planktonic productivity and food web structure because they could result in altered carbon partitioning and biogenic air–sea gas fluxes.

The Mediterranean Sea is an oligotrophic quasi-enclosed basin receiving the highest rate of aeolian material deposition in the world (Guerzoni et al. 1999) in the form of strong pulses of mineral dust. In addition, it continuously receives anthropogenic aerosols from industrial and domestic activities from populated areas around the basin and other parts of Europe. This situation makes the Mediterranean Sea an excellent natural laboratory to study the biogeochemical effect of atmospheric inputs on the water column. In the open Mediterranean Sea during the season characterized by a stratified water column and a low primary productivity, the atmosphere becomes the main external source of nutrients for the mixed layer, the nutrients being depleted after the bloom. Therefore, this period of the year appears to be the ideal season for testing the fertilizing potential of the atmosphere to surface waters.

The aim of this study was to experimentally determine the effect of atmospheric deposition on biomass, community structure, and productivity of autotrophic communities during the stratified period. Different combinations of realistic additions were conducted on natural assemblages enclosed in microcosms to mimic the natural inputs of macro- and micronutrients from the atmosphere. These enrichment experiments were carried out to study the (co)limitations encountered in such low-nutrient, low-chlorophyll systems and to determine to what extent atmospheric inputs can relieve them. Another goal was to specify which phytoplanktonic community (pico-, nano-, or microphytoplankton) took the most advantage of these different inputs.

Materials and methods

Water collection and incubation—This research was carried out on board R/V *Téthys II* on 1 August 2003 in the northwestern Mediterranean Sea at the permanent time series DYFAMED site (43°25′N, 7°52′E, 50 km off Nice, France; Fig. 1). This open sea site (2,350 m depth) is protected from coastal inputs by the presence of the coastal Ligurian current.

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Acknowledgments

The work reported is part of the doctoral dissertation of S.B. (grant of the French Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche). It was supported by the European ADIOS Program (project EVK3-CT-2000-00035). We thank J. C. Marty, responsible for the French DYFAMED time series program, who facilitated this research, and the captain and crew of the *Téthys II* for their cooperative work at sea. We also thank O. Bonilla for counting bacteria and G. Sarthou, H. Claustre, and two anonymous reviewers for their helpful comments and suggestions. We thank K. Forbes for editing the manuscript.

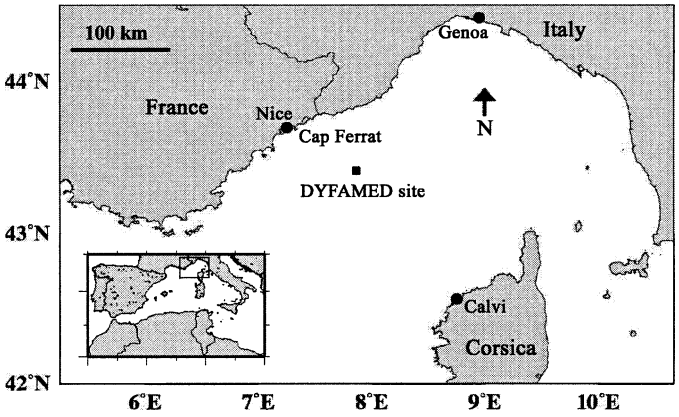

Fig. 1. Location of the DYFAMED time series station.

Seawater was collected at 10-m depths (above the thermocline) with a trace metal–clean Teflon pump system and was dispensed into acid-washed 4.5-liter transparent polycarbonate microcosms under a laminar flow hood. Filtered seawater (Sartorius Sartrobran-P-capsule 0.45-μm prefilter and 0.2-μm final filter) was also collected to analyze dissolved iron (DFe) concentration in surface seawater before the experiment.

The bottles were immediately amended with Saharan dust (0.25 mg L^{-1}), anthropogenic particles (0.01 mg L^{-1}), Fe (2.5 nmol L^{-1}), and Fe + phosphate (2.5 nmol L^{-1} Fe, 0.18 μmol L^{-1} phosphate). One unamended treatment was kept as a control. Each fertilization was performed in duplicate. The amount of Saharan dust and anthropogenic particles added was extremely low so that the realistic atmospheric inputs in the Mediterranean Sea could be reproduced. The concentrations of phosphate were similar to those encountered during the winter season in the mixed layer at the DYFAMED site according to Marty et al. (2002). For Fe, the addition corresponded to the concentration found in the mixed layer after a series of Saharan dust events (Guieu et al. 2002*a*). The dust we used was composed of fine fractions of surface soils collected in the Hoggar region (south Algeria) and was representative of the Saharan aerosol carried over the western Mediterranean Sea (Guieu et al. 2002*b*). A standard reference material of the National Institute of Standards and Technology named ''Urban particulate matter'' was used as a proxy for anthropogenic aerosol.

The bottles were capped, sealed with polyvinyl chloride tape, and incubated onshore in two outdoor tanks at ~50% ambient light level to reproduce the light conditions at the depth at which seawater was collected. A running seawater system continuously supplied water from the sea surface to maintain a constant temperature (25°C). For each experimental treatment, duplicates were discarded at three selected time points during the course of the experiment (T1 = 20 h; T2 = 44 h; T3 = 68 h) to minimize the risk of contamination from handling. Subsamples were used for the following measurements: chlorophyll *a* (Chl *a*); pigments analysis; primary production; bacterial abundances; DFe concentrations, which were only measured in the microcosms amended with Saharan dust; and anthropogenic particles to quantify Fe dissolution from these particles.

Dissolution experiment—Dissolution in seawater of Fe attached to the Saharan dust and anthropogenic particles used in this study was parameterized previously in Bonnet and Guieu (2004). To extend this knowledge, the dissolution processes of nitrate, phosphate, and silicate attached to these particles were also measured. Increasing amounts (eight concentrations between 0.01 and 10 mg L^{-1}) of Saharan dust and anthropogenic particles were added to natural filtered seawater (0.2 μm). After 7 d of incubation, the samples were passed through a 0.2-μm filter to determine the dissolved fraction of each nutrient. For details on methods and procedures, *see* Bonnet and Guieu (2004).

Analytical methods—Chl *a* and accessory pigments were measured by high-performance liquid chromatography. Samples (1 liter filtered through GF/F filters) were extracted in 3 mL of methanol and injected onto a reversed-phase chromatographic column and analyzed according to the protocol described by Claustre et al. (2004). Carbon assimilation was performed with the ^{14}C radiocarbon technique (Marty and Chiaverini 2002). Bacterial abundance was determined by epifluorescence microscopy as described in Noble and Fuhrman (1998). Duplicate samples of 0.8 mL were filtered onto 25-mm 0.02-μm filters (Anodisc, Whatmann) backed by wet 25-mm 0.45-μm prefilters (Millipore). Twenty randomly selected fields were counted so that there were at least 200 bacteria per filter. Nitrate, phosphate, and silicate in the bulk water used to perform the experiment were analyzed by the automated Technicon® Evolution II (Alliance Instruments) according to standard automated colorimetric methods (Tréguer and Le Corre 1975). Analytical precision for these measurements was 20 nmol L^{-1} (phosphate) and 50 nmol L^{-1} (nitrate and silicate). DFe concentrations were determined by flow injection analysis with chemiluminescence detection (Obata et al. 1993) on filtrate (0.2 μm) in each duplicate bottle amended with Saharan and anthropogenic particles. The samples were acidified with HCl Ultra Pure (Merck) at pH 2 and stored in the dark until analyzed. The detection limit was 20 pmol L^{-1} and the blank was 80 pmol L^{-1}. The accuracy of the method was accessed with NASS-5 reference seawater (3.58 ± 0.60 nmol L^{-1} certified value, 3.91 ± 0.04 nmol L^{-1} determined value, $n = 3$).

Results

Characteristics of the study site at the time of the experiment—During the mission, the water column was well stratified with a marked thermocline at 14 m depth. The surface waters were depleted in nutrients, with phosphate concentration close to the detection limit (20 nmol L^{-1}), nitrate concentrations of $<$50 nmol L^{-1}, and silicic acid concentrations of 0.7 μmol L^{-1}. The total Chl *a* (TChl *a*) concentration (Chl *a* + divinyl Chl *a*), a universal indicator of phytoplanktonic biomass, was 0.05 μg L^{-1} at 10 m depth, and phytoplanktonic biomass was dominated by picophytoplankton (41%) and nanophytoplankton (40%) communities. The contribution of microphytoplankton to the total phytoplankton biomass was relatively small (19%).

Total DFe concentration was 0.90 ± 0.02 nmol L^{-1} in the surface layer. The reason for this high DFe concentration

Table 1. Results of the statistical comparison between the control and each treatment at the end of the experiment (Mann–Whitney *U*-test) for Chl *a* and primary production. No data for primary production for addition with anthropogenic particles (samples lost).

	Fe	Dust	FeP	Anth
Chl *a*	NS	NS	S	S
Primary production	NS	S	S	Lost

NS, not significant (i.e, the difference between the two treatments is not statistically different); S, significant (i.e., the difference between the two treatments is statistically different); FeP, Fe + phosphate; Anth, anthropogenic particles.

(compared with <0.13 nmol L^{-1} for the same period in May 1995; Sarthou and Jeandel 2001) could not be explained by the small inputs from Saharan events that had occurred since the beginning of stratification: one Saharan event of low amplitude (0.288 g m^{-2}) had been recorded before the experiment at the Cap Ferrat atmospheric station (6 km from Nice, France), and three small events were recorded in Corsica, France, accounting for a total dust flux of 0.310 g m^{-2} (M.D. Loÿe-Pilot pers. comm.). Rather, this DFe concentration was attributed to the forest fires that occurred in the south of France continuously for 1 month before the experiment (Guieu et al. unpubl.).

Biological response during incubation—For each parameter measured, the statistical Mann–Whitney U-test (5% error rate) was used to determine whether concentrations in the bottles amended with nutrients were significantly different from those in the control bottles (Tables 1, 3).

Chl *a*: Additions of Fe + phosphate and anthropogenic particles had a significant effect on phytoplankton biomass over the duration of the experiment (Table 1; Fig. 2). It did not change significantly in the treatments amended with dust or Fe.

Primary production: Primary production was stimulated in most of the enriched bottles compared with the control over the incubation time (Fig. 3). The responses were significantly higher in the treatments amended with dust and Fe + phosphate than in the control: primary production integrated over the experimental period increased by 48% and 56%, respectively, compared with the unamended treatment. Production also increased after Fe addition, but in a lower proportion (+23%) that was not found to be statistically different from the unamended treatment. Primary production after the addition of anthropogenic particles could not be measured (because of human error, the samples were lost).

Phytoplankton community: Evolution of the phytoplankton community, divided into three representative size classes (Table 2) after the additions, was examined by pigment analysis (Fig. 4; Table 3). The biomass proportion of TChl *a* associated with picophytoplankton (<2 μm, BPpico), nanophytoplankton (2–20 μm, BPnano), and microphytoplankton (20–200 μm, BPmicro) was calculated as described by Vidussi et al. (2001) and revisited by Uitz et al. (unpubl.): BPpico = [0.86 (zea) + 1.01 (TChl *b*)]/DP, where DP is the sum of diagnostic pigments [DP = 0.86 (zea) + 1.01 (TChl *b*) + 0.60 (allo) + 1.27 (19′HF) + 0.35 (19′BF) + 1.41 (fuco) + 1.41 (peri)], BPnano = [0.60 (allo) + 1.27 (19′HF) + 0.35 (19′BF)]/DP, and BPmicro = [1.41 (fuco) + 1.41 (peri)]/DP (*see Table 2* for variable definitions).

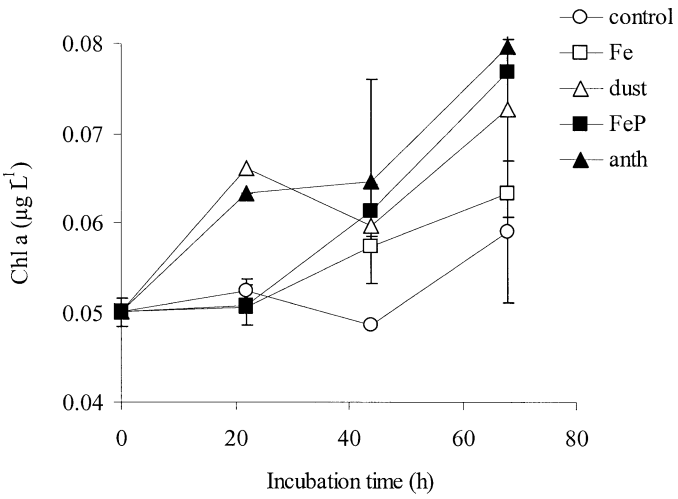

Fig. 2. Concentrations of Chl *a* in unamended and nutrient-amended treatments in the course of incubation. The error bars represent the standard deviation from duplicate incubations.

It should be noted that the range of variation of each pigment concentration was very narrow. The results obtained must therefore be considered with caution, especially for microphytoplankton, because the concentrations of fucoxanthin and peridinin measured were close to the average detection limit of the method.

Picophytoplankton revealed the most pronounced response to Fe additions between T0 and T3 (Fig. 4a); picophytoplankton dominated the entire community at the end of the experiment (Table 3); its proportion was significantly higher than in the control bottle at T3. Nanophytoplankton was stimulated in the treatments amended with Fe + phosphate and anthropogenic particles; their proportions were also significantly higher than in the unamended treatment at T3 (Table 3). The biomass proportion associated with mi-

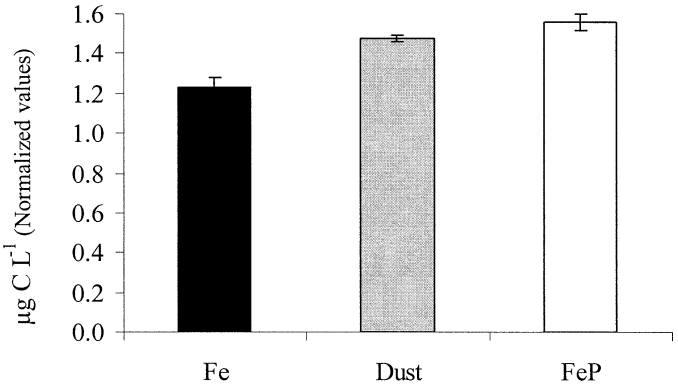

Fig. 3. Primary production integrated over the incubation period in nutrient-amended treatments and normalized to the control. The error bars represent the standard deviation from duplicate incubations.

Table 2. Biomarker pigments used in the present study, their abbreviations, taxonomic significance, and associated size class.

Diagnostic pigment	Abbreviation	Taxonomic significance	Size range (μm)	Phytoplankton size class
Fucoxanthin	fuco	Diatoms	>20	Microphytoplankton
Peridinin	peri	Dinoflagellates		
19′ Hexanoyloxyfucoxanthin	19′HF	Chromophytes nanoflagellates	2–20	Nanophytoplankton
19′ Butanoyloxyfucoxanthin	19′BF	Chromophytes nanoflagellates		
Alloxanthin	allo	Cryptophytes		
Chl *b* + divinyl-Chl *b*	TChl *b*	Green flagellates and prochlorophytes	<2	Picophytoplankton
Zeaxanthin	zea	Cyanobacteria and prochlorophytes		

crophytoplankton at T3 was not statistically different in the different treatments compared with the control.

Bacterial abundances: Bacterial abundances increased between T1 and T2 and decreased after T2 in all treatments and in the control bottle. Addition of anthropogenic particles to surface seawater had a slight positive effect on the bacterial abundances (+10% relative to the control between T1 and T2). The other treatments did not change bacterial abundances.

Potential release of Fe, nitrate, phosphate, and silicate from Saharan dust and anthropogenic particles—In this section, we examine the potential release of macronutrients nitrate, phosphate, and silicate and micronutrient Fe from the two types of particles that were used to simulate atmospheric input. For Fe, the numbers presented here represent the actual concentrations measured in the microcosm along the duration of the experiment (Table 4). These numbers will be compared in a future section to those obtained in abiotic conditions by Bonnet and Guieu (2004). For nitrate, phosphate, and silicate, we show in the following section the results obtained from the dissolution in abiotic conditions (Fig. 5a,b).

Iron: The values of DFe increased in the treatments amended with Saharan dust and anthropogenic particles (Table 4). Values of ΔDFe ($[DFe]_{\text{after introduction of particles}} - [DFe]_{\text{initial}}$) were used to calculate the percentage of Fe released by the particles. Considering an Fe content of 5% in the Saharan dust used in these experiments (Guieu et al. 2002*b*) and 3.91% in anthropogenic particles, the dissolution rates of Fe over the duration of the experiment were between 0.23% and 0.61% for the Saharan dust and between 0.86% and 1.85% for the anthropogenic particles (Table 4).

Nitrate: In our dissolution experiments conducted in abiotic conditions, the values of Δnitrate ($[\text{nitrate}]_{\text{after introduction of particles}} - [\text{nitrate}]_{\text{initial}}$) increased with increasing amounts of particles introduced and followed a linear relationship after the addition of Saharan dust (Fig. 5a) and anthropogenic particles (Fig. 5b) ($r^2 = 0.90$ and 0.96, respectively). ΔNitrate ranged from 0.5 to 1.2 μmol L^{-1} after Saharan dust addition and 0.15 to 5.4 μmol L^{-1} after urban particle addition. The introduction of 0.25 mg L^{-1} dust resulted in an increase in nitrate of 0.54 μmol L^{-1} in our microcosm, and the introduction of 0.01 mg L^{-1} anthropogenic particles resulted in an increase of 0.24 μmol L^{-1}.

Phosphate: From the same experiment, Δphosphate ($[\text{phosphate}]_{\text{after introduction of particles}} - [\text{phosphate}]_{\text{initial}}$) was below the detection limit (<20 nmol L^{-1}) after the introduction of Saharan dust. After addition of anthropogenic particles, Δphosphate was also below the detection limit for particle concentrations <1 mg L^{-1}. ΔPhosphate increased linearly for particle concentrations >1 mg L^{-1} ($r^2 = 0.99$) ranging from 0.13 to 1.98 μmol L^{-1} (Fig. 5b).

Silicate: ΔSilicate ($[\text{silicate}]_{\text{after introduction of particles}} - [\text{silicate}]_{\text{initial}}$) was below the detection limit (<50 nmol L^{-1}) after the introduction of Saharan dust. After addition of anthropogenic particles, Δsilicate was also below the detection limit for particle concentrations below 3 mg L^{-1} and increased linearly for particle concentrations above 3 mg L^{-1} ($r^2 = 0.99$) ranging from 0.22 to 0.65 μmol L^{-1} (Fig. 5b).

Discussion

Intensity of biological response—According to the productivity of the whole community, the magnitude of the biological response obtained after these atmospheric-like additions was significant in some of the treatments (+dust, +Fe, +phosphate). The mean Chl *a* concentration in these bottles remained low (~0.065 μg L^{-1}) and characteristic of the oligotrophic conditions encountered in the northwestern Mediterranean Sea during the summer season (Marty et al. 2002). To improve the understanding of this ecological effect, it is critical to relate this to the intensity of the fertilization conducted. Indeed, the key point of this experiment stems from the very low final particle or nutrient concentrations added to the natural assemblages to simulate those encountered in the natural environment under realistic conditions: the addition of 0.25 mg L^{-1} of Saharan dust in a 14-m mixed layer is equivalent to a medium-amplitude Saharan event (Ridame and Guieu 2002).

Our scientific approach was significantly different from the mesoscale Fe enrichments performed in HNLC waters, the aim of which was to prove the limiting character of Fe to primary production. In our experiment, we tried to mimic the natural deposition of mineral substances and anthropogenic particles to examine the capacity of a system, potentially limited by several nutrients, to react to a realistic atmospheric fertilization. To better understand the ecological response of the ecosystem, it is critical to examine which autotrophic community took the most advantage of the additions.

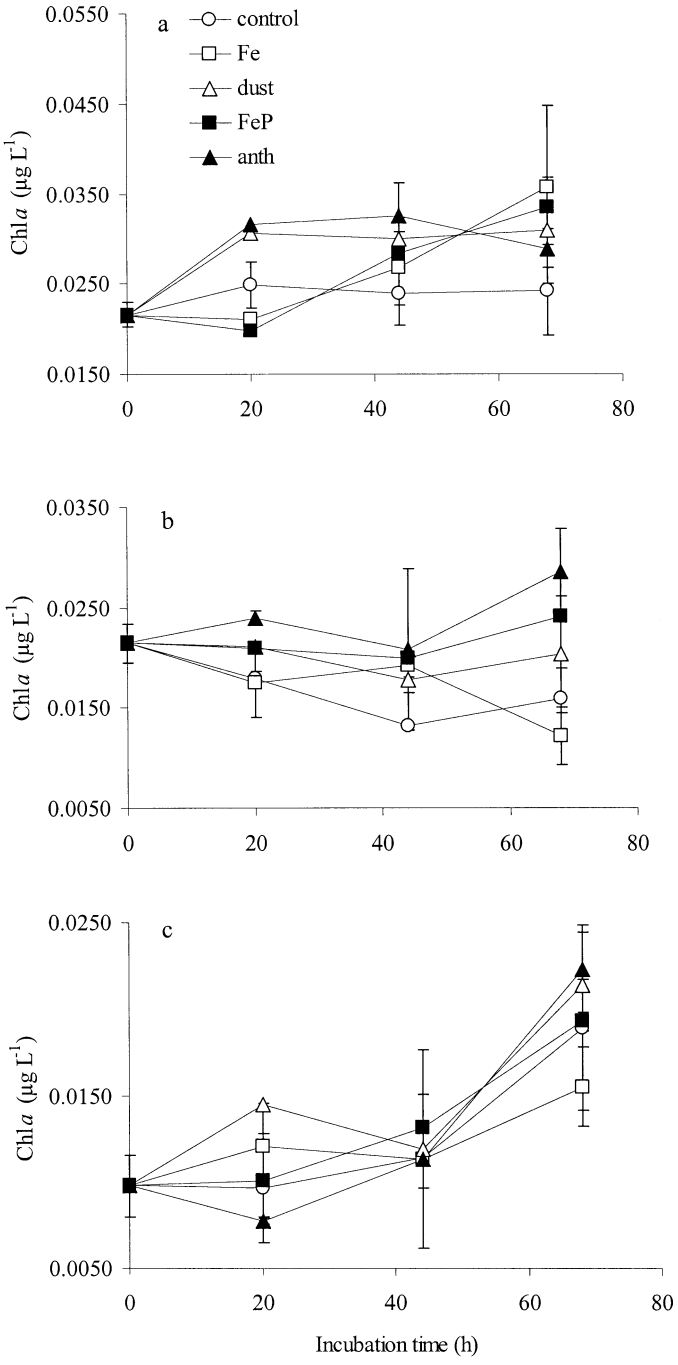

Fig. 4. Increase in Chl *a* concentrations with incubation time in the three different phytoplankton size classes—(a) picophytoplankton, (b) nanophytoplankton, and (c) microphytoplankton—derived from pigment analysis. The error bars represent the standard deviation from duplicate incubations. Note the different scales on the *y*-axis.

Effect on community structure—The absence of any major effect of the additions on bacterial abundance obtained in this experiment is probably due to the regulation of the bacterial biomass development by the heterotrophic nanoflagellates (Thingstad et al. 1998). Ridame (2001) also found that dust addition had no major effect on the bacterial abundance.

Table 3. Biomass proportion (BP) of TChl *a* associated with each size class of phytoplankton at the end of the incubation period (T3) in the control bottles and the nutrient-amended treatments.

	BP (%)		
	Picophyto-plankton	Nanophyto-plankton	Microphyto-plankton
Control	39–43	26–28	29–35
Fe	50–62*	14–25	24–25
Dust	42–43	25–30	27–33
FeP	40–48	28–34*	24–26
Anth	35–37	32–39	26–30

Anth, anthropogenic particles; FeP, Fe + phosphate.
* Values were statistically different from the control at T3.

However, she showed that bacterial production was clearly stimulated and proportional to the amount of dust introduced (increase of 70% relative to the control for a 10 mg L^{-1} addition after 48 h).

To understand how the additions affected the different communities, we estimated the part of TChl *a* associated with prokaryotic and eukaryotic populations. The prokaryotic proportion of TChl *a* was calculated as the sum of divinyl Chl *a* from prochlorophytes and Chl *a* from *Synechococcus* (calculated with Chl *a*:zeaxanthin = 1.65 (from Morel et al. 1993). It should be noted that we did not detect divinyl Chl *a* in our sample. Indeed, prochlorophytes are specifically abundant at depth at the end of the stratification period, usually in late fall (Marty et al. 2002). The eukaryotic proportion of TChl *a* was obtained by taking the difference of TChl *a* and prokaryotic Chl *a*. Despite the remaining ''oligotrophic conditions,'' we observed a shift of the communities in the amended bottles: at T3, the Chl *a* associated with eukaryotes increased in the treatment amended with dust (+12%), whereas prokaryotes increased in the bottles amended with Fe and Fe + phosphate (+27% and +16%, respectively). Because of the absence of prochlorophytes, the increase of prokaryotes is necessarily associated with cyanobacteria.

Nutrient supply and requirements—During the stratified period in the Mediterranean Sea, phosphate bioavailability appears to be the main limiting macronutrient for phytoplankton (Thingstad and Rassoulzadegan 1995) and bacterial production (Van Wambeke et al. 2002). However, the extremely low concentrations of nitrate (<50 nmol L^{-1}) in the surface waters before this experiment could suggest the existence of nitrate and phosphate colimitations and explain the low intensity of the responses observed. The role of iron could also be important because it has been known to play an important role in the nitrogen fixation process (*see*, e.g., Falkowski 1997). Although DFe concentration in the surface mixed layer was ''high'' (0.9 nmol L^{-1}) at the time of our experiment, this does not mean that a high fraction of this Fe was bioavailable. As pointed out previously (Blain et al. 2004; Mills et al. 2004) for studies conducted in an environment subjected to deposition of dust (which can have high dissolved Fe concentrations), the total dissolved concentration is a poor index of bioavailability. As emphasized

Table 4. Dfe concentrations and percent dissolved Fe (%Dis) associated with Saharan dust and anthropogenic particles for each replicate at each time point relative to the control before the experiment.

TO: 0.90 nmol L^{-1}	[DFe] (nmol L^{-1})	
	Dust	Anth.
T1	1.37±0.06 − %Dis = 0.23±0.06	1.06±0.01 − %Dis = 1.85±0.01
	1.52±0.01 − %Dis = 0.30±0.01	1.02±0.02 − %Dis = 1.35±0.02
T2	2.13±0.07 − %Dis = 0.61±0.07	0.98±0.03 − %Dis = 0,86±0.03
	1.57±0.08 − %Dis = 0.33±0.08	2.26 − %Dis = 16.73*
T3	1.26±0.05 − %Dis = 0.18±0.01	1.26±0.03 − %Dis = 4.31±0.03*
	1.98±0.12 − %Dis = 0.50±0.10	1.05±0.16 − %Dis = 1.72±0.16

* Contamination.

by Wu et al. (2001), concentrations as high as 1 nmol L^{-1} can present a small bioavailable fraction and can thus be somehow limiting.

The interpretation of the results of these experiments in a low-nutrient, low-chlorophyll system provided new insight into the nutrient supply, indirectly (by nitrogen fixation) and directly (from the particles introduced).

Nitrogen source: The addition of Fe and Fe + phosphate induced an increase of primary productivity (+27% and +56% over the 3-d incubation). This ability to grow without the addition of nitrogen could be explained by two mechanisms acting singly or together: efficient utilization of (undetectable) dissolved inorganic nitrogen or effective nitrogen fixation.

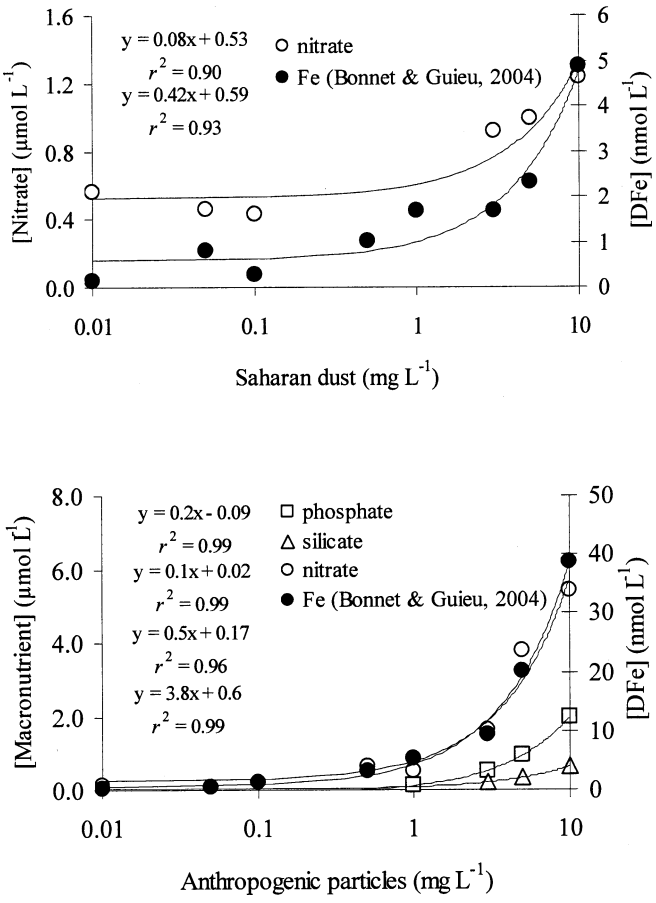

Fig. 5. Dissolution of nutrients from (a) Saharan dust and (b) anthropogenic particles in seawater from in vitro experiments.

The Chl *a* increases measured after Fe and Fe + phosphate additions were 0.027 and 0.013 μg L^{-1}, respectively. With a C : Chl *a* ratio of 6.4 mol C (g Chl *a*)$^{-1}$ and a N : C ratio of 16 : 106, the predicted uptake of nitrate would be 13 and 26 nmol L^{-1}, which is undetectable by our analyzer (detection limit = 50 nmol L^{-1}). The utilization of nitrate, ammonium, or both is thus a possible explanation for the observed Chl *a* increase in these treatments.

It is supposed that such a system is based on regeneration processes during the stratification period, but the only data on nitrogen regeneration available in the Mediterranean Sea were established in a coastal zone (Gulf of Lion): a high ammonia regeneration rate (up to 220 nmol L^{-1} d^{-1}) was found to be sufficient to sustain the ammonia plankton demand (Diaz and Raimbault 2000). Even if such a regeneration process is suspected to be lower in the open site where the experiment was performed, regenerated sources of nitrogen cannot thus be excluded.

Nitrogen fixation also has to be considered a possible source of nitrogen. Béthoux and Copin-Montégut (1986) made the hypothesis of strong nitrogen fixation processes to explain the net balance of nitrogen at the scale of the Mediterranean basin. Recent work with measurements of natural isotopic ratios confirmed this hypothesis in the oriental basin (Sachs and Repeta 1999) and in the occidental basin (Kerhervé et al. 2001). The filamentous cyanobacterium *Trichodesmium* is assumed to be the predominant oceanic N_2-fixing microorganism (Capone et al. 1997), but it is not abundant in the Mediterranean Sea (Béthoux and Copin-Montegut 1986). The role of unicellular cyanobacteria in N_2 fixation is taken into less consideration, although it is now recognized that these microorganisms express nitrogenase (Zehr et al. 2001). These N_2-fixing unicellular cyanobacteria are widely distributed in marine environments at concentrations of up to 1,000 cells mL^{-1} (Neveux et al. 1999) and potentially have a significant role in nitrogen dynamics in the world ocean.

As we have shown before, picophytoplanktonic populations grew significantly in the Fe treatment and profited the most from this Fe addition. The increase of cyanobacteria proportion in this treatment (+27% compared with the con-

trol) might suggest nitrogen fixation. But phosphate concentrations in these bottles were at the classical colorimetric detection limit (20 nmol L^{-1}), with only a part of it being bioavailable orthophosphate. However, Moutin et al. (2002) showed that phosphate concentrations below 5 nmol L^{-1} were sufficient to sustain cyanobacteria in the Mediterranean Sea. Indeed, these cells have a high affinity for orthophosphate and significantly higher maximum uptake rates than heterotrophic bacteria or eukaryotic algae. This result could explain the ability of small cells such as cyanobacteria to grow in the +Fe treatment that was N- and P-deplete. The high availability of phosphate in the treatment amended with Fe + phosphate (0.18 μmol L^{-1}) might explain the higher response obtained compared with the +Fe treatment. This hypothesis is consistent with the results obtained by Mills et al. (2004) in the eastern tropical North Atlantic, where nutrient addition bioassays were performed: the addition of Fe + phosphate resulted in a two- to threefold enhancement of N_2 fixation rate relative to the control, which suggests that N_2 fixation was colimited by both phosphate and Fe.

Our experiment suggests that nitrogen fixation might exist, at least during the season characterized by low productivity and stratified waters, and that this process would be enhanced by the atmospheric inputs of Fe and phosphate.

Nutrient supply by Saharan dust and anthropogenic particles: The lower proportion of cyanobacteria and the higher proportion of eukaryotic populations in the bottles amended with Saharan dust might indicate that dust provided a nitrogen source. The linear law obtained during the dissolution experiment of macronutrients attached to dust allowed us to determine that the input of nitrate from dust fertilization of the natural assemblage (0.25 mg L^{-1}) was 0.54 μmol L^{-1}. Di'Tullio and Laws (1991) observed that an increase of surface nitrate in the North Pacific Central Gyre (0.2 μmol L^{-1}) along with an increase of DFe after a dust deposition event was sufficient to stimulate autotrophic growth (+40%); the integrated N assimilation rates associated with submicron populations increased by 72% after the event.

Typical values of the half saturation constant for growth (Ks) nitrate for natural phytoplankton populations have been reported to be ~1.5 μmol L^{-1} in coastal waters (Fisher et al. 1988) and <0.6 μmol L^{-1} in the Oyashio Region (off Japan; Shiomoto et al. 1994). However, high DFe concentrations in the dust treatment might have increased the nitrate uptake capacity: the amount of DFe provided by the 0.25 mg L^{-1} dust addition was 0.74 nmol L^{-1} (on average over the incubation period) and resulted in a total DFe of 1.64 nmol L^{-1}. Indeed, Fe is a cofactor in the enzymes nitrate and nitrite reductase (Raven 1976), which are essential for nitrate uptake in phytoplankton. Franck et al. (2003) demonstrated that a 10 nmol L^{-1} Fe addition significantly increased nitrate maximum potential uptake rate (Vmax) in the California upwelling region (by 2.1 to 2.7 times). The same authors also reported that addition of Fe and Zn decreased Ks nitrate by 63%.

Because Δphosphate after the introduction of dust could not be measured (because it was below the detection limit of the analyzer), it was calculated according to the equation of Ridame and Guieu (2002), who parameterized the dissolution processes of phosphate associated with the same Saharan dust used in this experiment. The addition of 0.25 mg L^{-1} of dust to the natural assemblage corresponded to a phosphate input of 2 nmol L^{-1}, and the resultant phosphate concentration in this treatment was 22 nmol L^{-1}. As reported before, concentrations below 5 nmol L^{-1} are sufficient to sustain small cells (Moutin et al. 2002).

Finally, nutrient availability was profoundly altered in the treatment amended with dust. By providing a significant source of Fe (Guieu et al. 2002*b*), phosphorus (Ridame and Guieu 2002), and nitrate (this study), dust allowed an increase of 48% of the primary productivity, relieving the ongoing colimitations. The occurrence of Saharan events at the end of the spring bloom in the Mediterranean Sea is suspected to have a stronger effect on the photoautotrophic communities. Indeed, the surface mixed layer can become Fe-deplete (DFe < 0.1 nmol L^{-1}) in that period in the absence of any Saharan event (Sarthou and Jeandel 2001). By providing a significant source of Fe, phosphate, and nitrate to a partially nutrient-deplete mixed layer, a Saharan dust deposition event occurring during April or May might reinforce or allow the bloom to last for a larger period of time.

We obtained the same type of result after the addition of anthropogenic particles. We saw that these particles can provide a significant source of nitrate (the addition of 0.01 mg L^{-1} corresponding to an increase of +0.24 μmol L^{-1}, according to the linear law established in the dissolution experiment). This anthropogenic source of nitrogen, coming from the combustion of fuels and the use of fertilizers, is subject to future changes, both in amount and geographical distribution, depending on population and industrial growth in various regions. Delivery of atmospheric nitrogen to coastal regions in Europe and North America is estimated to have increased by 50–200% during the past 50 yr (Paerl 1995). In the open ocean, atmospheric nitrogen accounts, at present, for only a small percentage of the annual new nitrogen delivered to the surface (Spokes et al. 2000). If the human-derived nitrogen species increase in the future, it could increase phytoplankton production, leading to changes in CO_2 exchange and the emission of other climatically important trace gases. In nitrogen-poor regions, where nitrogen fixation by marine cyanobacteria acts to relieve nitrogen stress, such inputs of anthropogenic nitrogen could have a significant biogeochemical effect. Anthropogenic particles can also provide a source of Fe (+0.16 nmol L^{-1}) and silicate (+0.02 μmol L^{-1} calculated from the linear law). They can also be a source of phosphate, but the low amount of particles introduced in this treatment did not result in an increase in phosphate concentrations.

Availability of Fe provided by Saharan dust and anthropogenic particles—The percent dissolution of Fe attached to the Saharan particles found in this experiment is consistent with those obtained by Bonnet and Guieu (2004) with the use of the same Saharan dust at the same particle concentrations but in abiotic conditions (0.2-μm filtered seawater). With the same dust, Blain et al. (2004) and Mills et al. (2004) also found, in biotic conditions, low dissolution rates in agreement with those obtained by Bonnet and Guieu (2004) (Fig. 6a). The DFe concentrations obtained after the

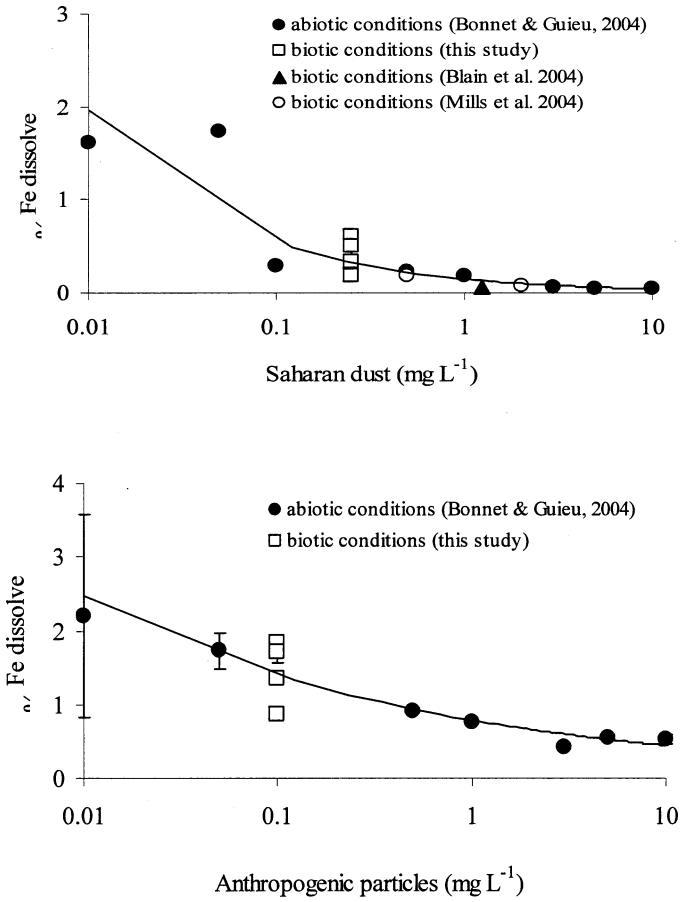

Fig. 6. Percentage of Fe in the dissolved form as a function of the amount of (a) Saharan dust and (b) anthropogenic particles introduced in seawater.

addition of 0.01 mg L^{-1} urban particles are also consistent with those obtained by Bonnet and Guieu (2004) (Fig. 6b). The processes acting as a DFe sink (biological uptake, scavenging on lithogenic and biogenic particles) and those acting as a DFe source (dissolution processes, release of organic ligands by the biota) might have been balanced in the experiment that was conducted on the natural assemblage.

Sink of DFe: The consumption of Fe by the cells constitutes the first process that removes Fe from the dissolved phase, but it is usually very small; it was found to be 0.04 nmol in the experiment conducted with the same Saharan dust by Blain et al. (2004). Moreover, sequestration of DFe by adsorption processes on particulate matter could also contribute to the removal of DFe in these treatments. When aerosol particles enter the seawater, two physicochemical processes occur simultaneously: dissolution of Fe from the aerosol particles and adsorption of DFe at the surface of these particles. Considering the adsorption capacity of 27 nmol mg^{-1} mineral particles (Zhuang and Duce 1993), the theoretical concentration of DFe, if the scavenging processes did not exist, would be ~15 times higher than what was actually measured in this experiment. Additionally, because of the presence of biogenic particulate matter in this experiment, the scavenging processes might have been more important than in the abiotic experiment. Indeed, the surface of algae provides various types of surface groups on which metal ions can be adsorbed (carboxyl, amino groups, and sulfide groups in proteins at the cell surface; Sigg 1998). Moreover, phytoplanktonic and bacterial exudates might have enriched the pool of transparent exopolymeric particles (particles formed abiotically by coagulation of colloidal organic matter and substances exuded by phytoplankton and bacteria) in the microcosms. These particles (1–100 μm) also have a large complexing capacity of DFe because of their richness of anionic polysaccharides. Beauvais (2003) showed that these particles can adsorb up to 20 nmol of Fe in natural conditions per liter of coastal water.

Source of DFe: The main source of DFe comes from the abiotic release of Fe attached to the particles. These dissolution processes might have been enhanced in the experiment containing the natural assemblage by the biological activity (dissolution in acidic zooplankton guts; Moore et al. 1984) or by bacteria fixation on the particles (Ridame 2001), but it is difficult to quantify these processes. The presence of the biological compartment in this experiment might have contributed to a reduction in the precipitation processes of DFe. It is known that binding organic ligands increases Fe solubility in seawater and could play an important role in Fe bioavailability (Sunda et al. 1991). These organic ligands include siderophores produced by microorganisms in response to Fe stress (Granger and Price 1999) and the release of other intracellular material (e.g., porphyrin complexes; Hutchins et al. 1999) generated by zooplankton degradation.

The role of the food web in these dissolution and scavenging processes is a relevant issue regarding the fate of atmospheric Fe and its bioavailability in the ocean and has to be more precisely defined in future experiments.

This experiment indicates that nutrients supplied by the atmosphere can have a clear biogeochemical effect on the autotrophic communities in a low-nutrient, low-chlorophyll system such as the Mediterranean Sea. Despite the remaining oligotrophic conditions, the realistic additions of dust and Fe + phosphate stimulated primary production, and addition of anthropogenic particles increased the Chl *a* concentrations, enhancing mainly the small cells (pico- and nanophytoplankton). Modification of the eukaryotic and prokaryotic proportions according to the nature of fertilization could result in different adaptations concerning nitrogen acquisition by the cells, for which the role of Fe is always a key factor in nitrogen fixation processes and nitrate uptake. To our knowledge, the release of nitrate by desert dust in seawater was never before witnessed.

The atmospheric supply of nutrients in oligotrophic regions during the stratified period has a significant effect on the phytoplanktonic dynamics. Because of their extent, they have a clear biogeochemical significance at the global scale. Emerson et al. (1997) estimated that more than half of the C export from the photic layer occurs in oligotrophic environments; the design of this experiment did not allow an accurate estimate of carbon export, but our results should prompt consideration of this aspect in further research concerning the fate of desert dust in low-nutrient low-chlorophyll areas.

In general, the highest atmospheric concentrations of dust over marine areas are found in the northern hemisphere (Duce and Tindale 1991). However, the pattern and magnitude of delivery of dust varies dramatically with season, vegetation, and soil aridity in the source area (Mahowald et al. 1999). It also depends on natural climate variability, human land disturbance, local- and regional-scale weather, and global atmospheric circulation. In the future, if climate change should result in elevated temperatures, it has been suggested that subsequent increases in aridity will lead to enhanced dust input (Falkowski et al. 1998). Regarding anthropogenic particles, model projections suggest that this source of nutrients might be three to four times higher in 2020 (relative to 1980) in the coastal ocean and somewhat lower in the open ocean (Galloway et al. 1994). Changes in atmospheric inputs will likely affect phytoplankton processes and, in turn, alter the exchange of climatically important trace gases between the atmosphere and ocean, thus providing potential feedbacks.

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Received: 5 February 2005
Accepted: 10 July 2005
Amended: 21 July 2005