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Degree of oligotrophy controls the response of microbial plankton to Saharan dust

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Abstract

To determine the effects of Saharan dust on the abundance, biomass, community structure, and metabolic activity of oceanic microbial plankton, we conducted eight bioassay experiments between ca. 30°N and 30°S in the central Atlantic Ocean. We found that, although bulk abundance and biomass tended to remain unchanged, different groups of phytoplankton and bacterioplankton responded differently to Saharan dust addition. The predominant type of metabolic response depended on the ecosystem's degree of oligotrophy and was modulated by competition for nutrients between phytoplankton and heterotrophic bacteria. The relative increase in bacterial production, which was the dominant response to dust addition in ultraoligotrophic environments, became larger with increasing oligotrophy. In contrast, primary production, which was stimulated only in the least oligotrophic waters, became less responsive to dust as the ecosystem's degree of oligotrophy increased. Given the divergent consequences of a predominantly bacterial vs. phytoplanktonic response, dust inputs can, depending on the ecosystem's degree of oligotrophy, stimulate or weaken biological CO_2 drawdown. Thus, the biogeochemical implications of changing dust fluxes might not be universal, but variable through both space and time.

Eolian dust fluxes from arid regions represent a source of nutrients for the open ocean and can have important effects on marine biogeochemical cycles and the functioning of the earth system (Jickells et al. 2005; Mahowald et al. 2009). Observational and experimental studies have shown that enhanced dust inputs can stimulate phytoplankton biomass, primary production (PP), and N_2 fixation (Young et al. 1991; Bishop et al. 2002; Bonnet et al. 2005), potentially increasing the efficiency of the biological pump in the sequestration of atmospheric CO_2. However, dust inputs can also cause a direct (e.g., not mediated by phytoplankton) increase in the abundance and metabolic rates of heterotrophic bacteria (Herut et al. 2005; Pulido-Villena et al. 2008; Reche et al. 2009), which are often nutrient-limited in ultraoligotrophic waters (Rivkin and Anderson 1997; Caron et al. 2000; Mills et al. 2008). In fact, it has been shown that bacteria can outcompete phytoplankton during nutrient addition experiments (Joint et al. 2002; Thingstad et al. 2005; Mills et al. 2008), effectively preventing an increase in PP in response to nutrient enrichment. If this is also the case after a dust deposition event, even if bacterial biomass increases, there will be no immediate effect on biological drawdown of CO_2 because bacterial production (BP) is fueled by organic carbon already present in the system. An additional biogeochemical implication stems from the fact that nutrient limitation of bacterial metabolism can contribute to the accumulation of dissolved organic carbon (DOC) in the surface layers of oligotrophic regions during the stratification period (Thingstad et al. 1997). Considering that DOC export through winter mixing is a major process of biogenic carbon export in the open ocean (Carlson et al. 1994; Emerson et al. 1997), alleviation of bacterial nutrient limitation by dust could enhance surface DOC consumption and cause a reduction in the downward flux of organic carbon (Rivkin and Anderson 1997).

Given the divergent ecological and biogeochemical implications involved, it is important to determine the effects of dust inputs on both the autotrophic and the heterotrophic components of the microbial plankton community. This determination needs to be conducted over a large geographical range, because the biological response to nutrient inputs is likely to depend on the environmental conditions and the initial composition and physiological state of the various microbial groups. Few studies have addressed the effects of dust inputs on phytoplankton and heterotrophic bacteria concurrently, and those that have were based on single observations and experiments conducted in one particular location (Herut et al. 2005). As a result, the factors that control the magnitude and dominant type of microbial response to dust in the open ocean are currently unknown.

Here we report on the results of eight bioassay experiments, conducted over a latitudinal range spanning > 6000 km in the central Atlantic Ocean, during which the response of microbial plankton to realistic additions of Saharan dust was comprehensively assessed in terms of

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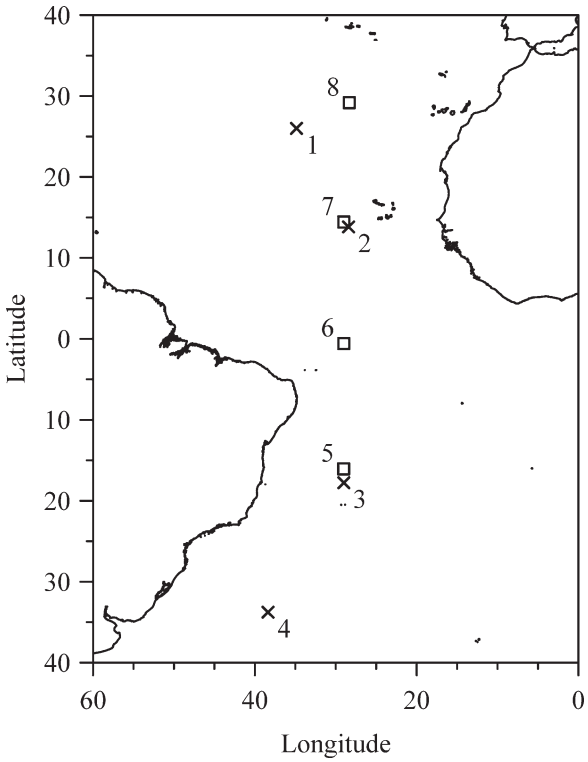

Fig. 1. Location of the dust addition experiments conducted during the 2007 (crosses, experiments 1–4) and 2008 cruises (squares, experiments 5–8).

abundance, community structure, and metabolic activity. The sampled stations, although corresponding always to broadly oligotrophic settings, covered a wide range of conditions in terms of hydrography and expected nutrient availability. Therefore, our experimental approach allowed us to identify general patterns in the microbial response to dust inputs along a gradient of oligotrophy.

Methods

Sampling and experimental setup—We conducted eight dust addition experiments during the *Trichodesmium* and N_2 fixation in the tropical Atlantic (TRYNITROP) cruises, which took place on board RV *Hespérides* in the central Atlantic Ocean during 16 November–16 December 2007 and 08 April–06 May 2008 (Fig. 1, Table 1). At each station, six 4-liter, acid-cleaned polycarbonate bottles were individually filled (true replicates) with unfiltered surface seawater. Water collection was conducted on board a Zodiac to minimize contamination from the ship's hull. Once back on board, dust was individually added to three microcosms at a final concentration of 2 mg L^{-1}, while the remaining three microcosms served as controls. The dust concentration used simulates the dust enrichment caused by an event of high deposition (20 g m^{-2}) in a 10-m-deep water layer (Mills et al. 2004; Pulido-Villena et al. 2008). Observations (Lawrence and Neff 2009) and models (Mahowald et al. 2005) show that annual deposition rates in the eastern North Atlantic (roughly between 5–25°N and east of 30°W) are in the range 10 to 50 g m^{-2} yr^{-1}. However, Saharan dust deposition tends to occur in pulses, and a single event can sometimes account for 40–80% of the annual flux (Guerzoni et al. 1997). One of these events, therefore, could well cause an enrichment of 2 mg L^{-1} in the upper 10-m layer of the eastern North Atlantic. In the case of the South Atlantic, the dust concentration used is likely to exceed the magnitude of any natural inputs, as this region experiences a lower rate of atmospheric deposition. However, we decided to add the same amount of dust in all experiments because one of our main objectives was to ascertain how different communities respond to the same perturbation.

In experiments 1–4, conducted in 2007, the added dust was Saharan soil, whereas in experiments 5–6, conducted in 2008, the added dust was atmospherically processed dust collected during a Saharan dust storm. After adding the dust, bottles were placed inside an on-deck incubator filled with running seawater pumped from the sea surface. The irradiance received by the microcosms was attenuated to 80% using a neutral density screen. After 48 h, subsamples were collected from each microcosm for the determination of nutrient concentration, picoplankton abundance, chlorophyll *a* concentration, and bacterial diversity. In addition, subsamples were also collected for the determination

Table 1. Properties of the stations where the dust addition experiments were conducted and initial characteristics of the water samples used. na, not available.

	Experiment number							
	1	2	3	4	5	6	7	8
Latitude	26.0°N	13.8°N	17.8°S	33.8°S	16.1°S	0.6°S	14.4°N	29.2°N
Longitude	34.8°W	28.4°W	29.0°W	38.4°W	29.0°W	29.0°W	29.0°W	28.3°W
Sampling date (2007–2008)	21 Nov	26 Nov	04 Dec	10 Dec	17 Apr	21 Apr	25 Apr	29 Apr
Surface temperature (°C)	24.6	26.3	24.9	19.4	28.0	28.7	24.3	20.6
1% PAR depth (m)	118	70	171	81	151	66	85	123
18°C isotherm depth (m)	215	67	218	45	204	74	98	178
Nitracline depth (m)	na	55	120	72	148	50	42	152
Surface NO_3 (nmol L^{-1})	na	150	105	109	34	35	56	127
Surface NH_4 (nmol L^{-1})	na	14	na	na	17	14	11	13
Surface PO_4 (μmol L^{-1})	na	0.04	0.11	0.09	0.13	na	0.02	0.02
Surface Chl *a* (μg L^{-1})	0.11	0.21	0.08	0.13	0.10	0.21	0.22	0.10
% Chl *a*<2 μm	55	75	57	57	55	54	64	57

of bacterial production, primary production, N_2 fixation, and community respiration.

Total Fe and Al in dust—For the Saharan soil added in experiments 1–4, 10 mg of sample were weighed into a Teflon microwave digestion bomb. Three milliliters of concentrated subboiling distilled HNO_3, 3 mL of concentrated subboiling distilled HCl, and 2.5 mL of concentrated HF (Romil SpA grade) were added to the sample. The bomb was sealed and digested in an Anton Parr MF100 microwave digestion system at 175°C for 60 min. The resulting digest was transferred to an open 30-mL Teflon digestion bomb and evaporated on a hot plate at 140°C until ca. 0.5 mL was remaining. At this stage 0.25 mL of $HClO_4$ (Romil SpA grade) was added and the temperature increased to 170°C. The sample was evaporated to near dryness before a further 0.25 mL of $HClO_4$ was added and the sample evaporated to complete dryness. The residue was redissolved in 20 mL of 0.3 mol L^{-1} subboiling distilled HNO_3 and analyzed by inductively coupled plasma mass spectroscopy. The analysis was performed in triplicate and two aliquots of a certified reference material and one reagent blank were processed similarly. Recoveries of Fe and Al in the reference material were 99% ± 1% and 101% ± 2%, respectively, and the reagent blank was negligible. For the dust added in experiments 5–8, an equivalent total acid digestion procedure was used with atomic absorption spectroscopy (Murphy 1985).

Total N and organic C in dust—For all dust samples, four aliquots of ca. 100 mg were placed in clean glass vials. Approximately 2 mL of concentrated HCl (AnalaR grade) were added to two of these aliquots, which were then left overnight for any carbonate to dissolve. The overlying solution was carefully removed by pipette and replaced with ultrapure water and left for several hours to settle. This was repeated five to six times until the overlying solution had neutral pH. All four aliquots were dried at 70°C before analysis for total C and N using a Carlo Erba EA 1108 elemental analyzer. The system and calculations used are described elsewhere (Wilkinson 1991). A reference material (low organic content soil standard OAS, B2152, elemental microanalysis) was used to verify the calibration of the instrument (total C: certified 1.52% ± 0.02%, measured 1.50% ± 0.03%; total N: certified 0.13% ± 0.02%, measured 0.14% ± 0.01%).

Dust composition—The dust added in experiments 1–4, conducted in 2007, consisted of the fine fraction (< 20 μm) of soil collected in Mali, which was obtained by dry hand-sieving through successive polyethylene meshes (100 μm and 20 μm). This is the finest fraction that is possible to obtain by dry-sieving and thus as close as possible to the size distribution of atmospheric dust particles. Particle size distributions on dust samples prepared in a similar way by Guieu et al. (2002) showed that the size distributions of the sieved particles were broadly comparable with those of mineral dust particles in collected rain samples, although with a slightly larger modal size (~ 10–20 μm for sieved dust compared with ~ 5–10 μm for dust in rain samples). Total Fe and Al (weight : weight) in the dust were determined as 3.67% ± 0.10% Fe and 6.46% ± 0.23% Al, which are within the range typically found in Saharan soils and aerosols (Guieu et al. 2002). The material also contained 1.51% ± 0.11% organic C, within the range of 1–3% previously reported for Saharan aerosols (Tomadin et al. 1984; Eglinton et al. 2002), and 0.15% ± 0.02% of total N, also close to previously reported values (ca. 0.1%) of total N in Saharan aerosols (Eglinton et al. 2002). The dust added in experiments 5–8, conducted in 2008, was atmospherically processed dust collected on board RV *Discovery* during a Saharan dust storm in the Gibraltar strait region (36.8°N, 9.1°W) on 09–10 April 1982. This material contained 4.5% Fe and 7.7% Al (Murphy 1985). The organic C content of this dust was determined as 1.86% ± 0.08% and the total N was 0.11% ± 0.02%.

Hydrography, irradiance, and nutrients—Vertical profiles of temperature (0–300 m) were obtained with a conductivity, temperature, and salinity SBE911 plus probe attached to a rosette equipped with Niskin bottles. The vertical distribution of photosynthetically active irradiance (PAR, 400 to 700 nm) was measured with a Satlantic Ocean Color Profiler 100FF radiometer. For the determination of phosphate concentration, samples were stored frozen at −20°C until analysis in the laboratory ashore, which was conducted with a Technicon segmented-flow autoanalyzer and using standard colorimetric protocols. The concentration of nitrate, nitrite, and ammonium was determined on board on fresh samples with a Technicon segmented-flow autoanalyzer and using modified colorimetric protocols that allow achievement of a detection limit of 2 nmol L^{-1} (Raimbault et al. 1990; Kerouel and Aminot 1997). Nitracline depth was taken as the first depth where nitrate concentration was ≥ 200 nmol L^{-1}.

Chlorophyll a (Chl a) concentration—Size-fractionated Chl *a* concentration was determined fluorometrically using the nonacidification technique (Welschmeyer 1994). Samples (250-mL) were sequentially filtered through 2- and 0.2-μm polycarbonate filters and pigments were extracted in 90% acetone at −20°C overnight. Fluorescence was measured on a Turner Designs 700 fluorometer, which had been calibrated with pure Chl *a*. Total Chl *a* concentration was determined as the sum of the two size-fractionated values.

Picoplankton abundance—The abundance of *Synechococcus*, *Prochlorococcus*, picoeukaryotes, and heterotrophic bacteria was determined on board on 0.6-mL fresh and 0.4-mL frozen samples (autotrophic and heterotrophic groups, respectively) using a Becton Dickinson FACSCalibur flow cytometer (Calvo-Díaz and Morán 2009). Samples for heterotrophic bacteria were preserved with 1% paraformaldehyde + 0.05% glutaraldehyde and frozen at −80°C until analysis within the same day. Before analysis, heterotrophic bacteria were stained with 2.5 mmol L^{-1} SybrGreen deoxyribonucleic acid fluorochrome. Picoplankton groups were identified on the basis of their fluorescence and light side scatter (SSC) signatures.

Synechococcus and *Prochlorococcus* cyanobacteria and eukaryotic cells were identified in plots of SSC vs. red fluorescence (FL3, > 650 nm) and orange fluorescence (FL2, 585 nm) vs. FL3, whereas heterotrophic bacteria were distinguished by their green fluorescence (FL1, 530 nm) after SybrGreen staining.

Trichodesmium *spp. abundance*—The ship's nontoxic water supply was used to determine the surface abundance of *Trichodesmium* trichomes. Water was collected from ca. 5-m depth by a Teflon pump and carried to the laboratories through epoxide-free silicone pipes. At each sampling site, between 50 and 130 liters of seawater were filtered through a 40-μm nylon mesh. Particles were then transferred to a 100-mL glass bottle by gently rinsing the mesh with 0.2-μm filtered seawater. Samples were preserved in Lugol's solution and stored in the dark until analysis in the laboratory. After allowing the samples to settle in sedimentation chambers, counting of trichomes was carried out with a Nikon Diaphot microscope.

Bacterial community composition—The in situ abundance of different bacterial populations was determined using catalyzed reported deposition–fluorescence in situ hybridization (CARD-FISH), using the procedures described in Teira et al. (2008). We used oligonucleotide probes specific for the domain Eubacteria (EUB338, EUB338II, and EUB338III), the Gammaproteobacteria (GAM42a) subclasses, the Bacteroidetes group (CF319a), the *Roseobacter* lineage (Ros537), and the SAR11 cluster (SAR11-441R). Samples from each microcosm (10–15 mL in volume) were fixed with 0.2-μm filtered paraformaldehyde (1% final concentration) and subsequently stored at 4°C in the dark for 12–18 h. Thereafter, each sample was filtered through a 0.2-μm polycarbonate filter (Millipore, 25-mm filter diameter) supported by a cellulose nitrate filter (Millipore, 0.45 μm), washed twice with Milli-Q water, dried, and stored in a microfuge vial at −20°C until further processing in the laboratory. Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated with lysozyme. Filters were cut into sections and hybridized at 35°C with horseradish peroxidase (HRP)-labeled oligonucleotide probes during a minimum of 2–4 h. Tyramide-Alexa488 was used for signal amplification (30–40 min). We used 55% formamide for all probes except for SAR11-441R (45% formamide). The hybridization for all the probes was done overnight and cells were counterstained with a 4′,6-diamidino-2-phenylindole (DAPI) mix (5.5 parts of Citifluor, 1 part of Vectashield, and 0.5 parts of phosphate-buffered saline solution with DAPI [final concentration 1 μg mL^{-1}]). The slides were examined with an epifluorescence microscope equipped with a 100-W Hg lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample. For each microscope field, two different categories were enumerated: total DAPI-stained cells and cells stained with the specific probe. Negative control counts (hybridization with HRP-Non338) averaged 0.25% and were always below 1.0% of DAPI-stained cells. The counting error, expressed as the percentage of standard error between replicates (100 × SE/mean) was < 2% for DAPI counts and < 10% for FISH counts.

Primary production—Size-fractionated PP was measured with the ^{14}C-uptake technique (Marañón et al. 2001). For each microcosm, 100-mL polystyrene bottles (two light and one dark bottles) were filled with seawater, supplemented with 15 μCi (555 kBq) of $NaH^{14}CO_3$, and incubated for 24 h on deck under the same light and temperature conditions. After the incubation, samples were sequentially filtered through 2-μm and 0.2-μm pore size polycarbonate filters under low vacuum pressure. Filters were exposed to concentrated HCl fumes for 10 h to remove inorganic ^{14}C. Finally, liquid scintillation cocktail was added to the filters and the radioactivity present on each sample was measured on a Wallac scintillation counter. For the calculation of carbon fixation rates, and to correct for the nonphotosynthetic fixation of ^{14}C, the radioactivity measured in the dark samples was subtracted from that measured in the light bottles.

N_2 fixation—N_2 fixation was measured with the $^{15}N_2$ uptake technique (Montoya et al. 1996). For each microcosm, one acid-washed, clear polycarbonate bottle (2 liters in volume) was filled with seawater and supplemented with 2 mL of $^{15}N_2$ (98 atom%, SerCon). After incubation on deck for 24 h, the whole volume was filtered through a 25-mm GF/F filter. Afterward, filters were dried at 40°C for 24 h and then stored until pelletization in tin capsules. ^{15}N atom% in particulate organic matter was measured with an elemental analyzer combined with a continuous-flow stable isotope mass spectrometer (FlashEA112 + Deltaplus, ThermoFinnigan), using an acetanilide standard as reference.

Bacterial production—BP was measured with the [^{3}H]-Leu uptake method (Smith and Azam 1992). Samples were incubated on deck for 2 h under the same light and temperature conditions experienced by the microcosms. Empirical Leu-to-C conversion factors (CF) were determined on a total of 15 stations during the two cruises, following the methods detailed elsewhere (Calvo-Diaz and Morán 2009), and ranged between 0.2 and 0.8 kg C mol Leu^{-1}. An empirical calibration between SSC and cell diameter (Zubkov et al. 1998) was performed with water from the same stations to estimate bacterial biovolume (BB). We used an allometric relationship (Gundersen et al. 2002) for converting BB to cellular carbon content (CCC): CCC (fg C cell^{-1}) = $108.8 \times BB^{0.898}$. The CFs obtained at the station where the dust addition experiments were performed (or at the nearest available station) were used to calculate bacterial biomass production rates from Leu uptake rates.

Respiration—In experiments 1–4, conducted during the 2007 cruise, community repiration (CR) was estimated using in vivo electron transport system (ETS) activity rates (Martínez-García et al. 2009). In vivo ETS activity rates were measured using the INT method, which is based on the reduction of the tetrazolium salt 2-(*p*-iodophenyl)-3-(*p*-

nitrophenyl)-5-phenyltetrazolium chloride (INT) to INT-formazan (INT-F) by ETS dehydrogenase enzymes. Briefly, three 250-mL dark bottles were filled from each microcosm bottle. One bottle was immediately fixed by adding 2% (v:v) formaldehyde and served as a control. After INT addition, samples were incubated in the dark during 4 h in the same on-deck incubators where the microcosms were kept. After incubation, samples were fixed and then filtered through 0.2-μm pore size polycarbonate filters. The reduced INT-F was extracted from the filters using propanol and its concentration determined colorimetrically on a Ultra Violet-2401 PC Shimadzu spectrophotometer.

In experiments 5–8, conducted during the 2008 cruise, CR rates were measured with the O_2 consumption method. Six water samples were collected from each microcosm through silicon tubing into dark, calibrated borosilicate glass bottles with a nominal volume of 120 mL. Three bottles were incubated in the dark for 24 h in the same on-deck incubator used for the other experiments. The other three samples were fixed immediately to determine the initial oxygen concentrations. Measurements of dissolved oxygen were made with an automated Winkler titration system using a potentiometric end-point detector. Detailed methods and error quantification for the O_2 evolution method are described elsewhere (Mouriño-Carballido and Mcgillicuddy 2006). Previous measurements in coastal and oceanic environments had shown a highly significant relationship ($r^2 = 0.80$, $p < 0.001$, $n = 72$) between the ETS activity measured by the INT method and that measured by the O_2 evolution method (Martínez-García et al. 2009). To transform ETS activity rates (ETS, μmol INT-F L^{-1} d^{-1}) into oxygen consumption rates (R, μmol O_2 L^{-1} d^{-1}) a R:ETS ratio of 12.8 is used (Martínez-García et al. 2009).

Calculation of dust-induced responses—To compare the effect that dust addition had on the different standing stocks and rates, we calculated the relative change (%) for each variable as $100 \times (D - C)/C$, where D and C are the mean value of the variable in the dust and the control treatments, respectively. We used the standard deviation as an indicator of uncertainty in our measurements. For each variable, we calculated the standard deviation of the relative change by propagating the standard deviation of the measurements in the control and the dust treatments. The differences between treatments for each variable were analyzed using the Student's *t*-test for comparisons of means.

Results

Initial conditions—All experiments were conducted in oligotrophic locations of the central Atlantic Ocean (Fig. 1), characterized by warm (> 19°C) surface waters, strong thermal stratification, and low nitrate (34–150 nmol L^{-1}), ammonium (11–17 nmol L^{-1}), phosphate (0.02–0.13 μmol L^{-1}), and Chl *a* concentrations (0.08–0.22 μg L^{-1}) in the upper mixed layer (Table 1, Fig. 2). However, the degree of stratification and, consequently, oligotrophy varied among experiments, because the cruise track crossed a wide latitudinal range and also because experiments took place in two contrasting seasons. This varying degree of stratification and oligotrophy can be quantified using as indicators the depths of the euphotic layer (1% PAR level), the 18°C isotherm, and the nitracline (Fig. 2). In our study, all these depths covaried (Pearson's $r \geq 0.83$ and $p \leq 0.02$ for all paired variables). Thus, experiments 2 (13.8°N), 4 (33.8°S), 6 (0.6°S), and 7 (14.4°N) took place in less oligotrophic settings, as indicated by the shallower depths of the euphotic layer (≤ 85 m), 18°C isotherm (≤ 98 m), and nitracline (≤ 72 m). In contrast, experiments 3 (17.8°S) and 5 (16.1°S) corresponded to the most oligotrophic locations, as suggested by their very deep (≥ 151 m) euphotic layers, indicative of a very low concentration of suspended particles in the upper water column. These stations also had a deep nitracline (≥ 120 m) and 18°C isotherm (≥ 204 m).

Changes in standing stocks—The concentration of nitrate, ammonium, and phosphate in the dust treatments did not differ from that in the controls after 48 h (data not shown). Given the contents of N (0.10–0.15%) and P (0.08–0.14%) in Saharan dust and their solubility in seawater (≤ 30%) (Guieu et al. 2002; Ridame and Guieu 2002; Herut et al. 2005), we estimate that our dust additions (2 mg L^{-1}) caused only nanomolar increases in dissolved inorganic N and phosphate concentration (up to approximately 65 and 30 nmol L^{-1} for N and P, respectively). Considering the typical kinetics of nutrient release observed during dust dissolution experiments (Ridame and Guieu 2002), it is likely that these increases occurred during the first hours of incubation and were countered by rapid uptake by the microbial community. Similar nutrient enrichments have been calculated for moderate to intense natural events of dust deposition (Ridame and Guieu 2002; Herut et al. 2005).

Total Chl *a* concentration, which is a proxy for total phytoplankton biomass, showed modest relative changes in response to dust, with values typically ranging between −25% and 25% (Fig. 3A). Picophytoplankton (0.2–2 μm) Chl *a* concentration was more responsive, with a 40% decrease in experiment 3 and a 40% increase in experiments 4 and 7 (Fig. 3B). Among the photosynthetic picophytoplankton, *Prochlorococcus* was always numerically dominant, with initial abundances of up to 7×10^5 cells mL^{-1}, whereas the highest *Synechococcus* abundance was $\sim 3 \times 10^4$ cells mL^{-1} (Table 2). Initial picoeukaryote abundances ranged between 600 and 4000 cells mL^{-1}. These picophytoplankton groups displayed contrasting patterns in their overall response to dust addition (Fig. 3C–E). *Synechococcus* and *Prochlorococcus* showed marked decreases of their abundance in the presence of dust (Fig. 3C,D). These decreases were statistically significant (*t*-test, $p < 0.05$) in experiments 1, 4, and 5 for *Synechococcus* and experiments 2 and 4 for *Prochlorococcus*. In several experiments that had low initial numbers of *Synechococcus* and *Prochlorococcus*, these genera were virtually absent from the dust treatments at the end of the experiment. In contrast, the overall response of the picoeukaryotes to dust addition

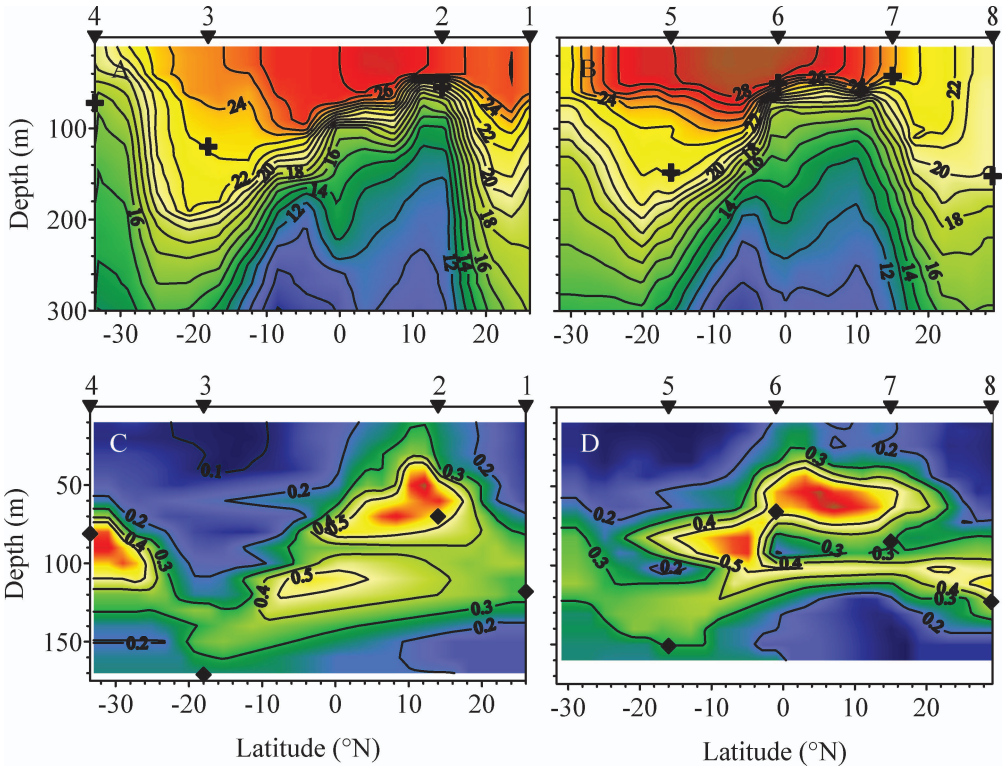

Fig. 2. Vertical and latitudinal distribution of (A, B) temperature (°C) and (C, D) chlorophyll *a* concentration (mg m^{-3}) during the (A, C) 2007 and (B, D) 2008 cruises. Triangles indicate the location of each bioassay experiment. Crosses and diamonds indicate the depth of the nitracline and the euphotic layer, respectively, at the locations where the experiments were conducted.

tended to be positive, particularly in less oligotrophic stations (experiments 2, 4, 6, and 7), where the relative change in picoeukaryote abundance reached 50–200%, although this increase was statistically significant only in experiments 4 and 7 (Fig. 3E). In the most oligotrophic settings (experiments 3 and 5), picoeukaryote abundance decreased moderately or remained unchanged.

The initial abundance of heterotrophic bacteria in our experiments ranged between 4 and 9 × 10^5 cells mL^{-1} (Table 2). Among the identified eubacteria, SAR11 was always the most abundant group, with abundances in the range 7–26 × 10^4 cells mL^{-1}. Bacteroidetes and Gammaproteobacteria often had similar abundances, within the range 2–20 × 10^4 cells mL^{-1}, whereas *Roseobacter* tended to be the least abundant group. The relative changes in total abundance of heterotrophic bacteria remained, in most cases, unimportant (Fig. 1F). Total bacterial biomass was also rather stable, as it was strongly correlated to bacterial abundance (Pearson's $r = 0.85$, $p < 0.01$). However, this relative constancy in total bacterial abundance and biomass masked some noticeable changes in the abundance of specific groups of eubacteria in response to dust addition (Fig. 4). *Roseobacter*, and especially Gammaproteobacteria, showed a tendency toward positive relative changes in most experiments, reaching values of up to 100–200% (Fig. 4A,D). There was considerable variability between replicates in the abundance of each bacterial group, and as a result differences were not significant in many experiments. The increases in Gammaproteobacteria in response to dust were statistically significant in experiments 2 and 3. In contrast, SAR11 had mostly negative responses (Fig. 4B), which were statistically significant in experiments 3, 4, 5, and 8 (*t*-test, $p < 0.05$). The Bacteroidetes group did not show any consistent trend toward positive or negative responses (Fig. 4C).

Changes in metabolic rates—Heterotroph-dominated processes such as BP and CR tended to show larger responses to dust addition than autotrophic processes, such as PP and N_2 fixation (Fig. 5). PP, which ranged between 0.5 and 4 μg C L^{-1} d^{-1}, responded positively or showed no response to dust addition in seven of eight experiments (Fig. 5A). The highest positive responses (30–100%) were observed in experiments 2, 4, and 6, which, as explained above, had been identified as having relatively less oligotrophic initial conditions. The dust-induced increase in PP was statistically significant in experiments 2 and 4 (*t*-test, $p < 0.05$). N_2 fixation, which ranged between 1 and 35 ng N L^{-1} d^{-1}, did not show a consistent trend toward either increased or decreased values in response to dust. The relative response of N_2 fixation varied between −70% and 140% (Fig. 5B). The filamentous diazotroph *Trichodesmium* spp. was present only in the locations of experiments 1, 2, 6, and 7, with abundances ranging between 60 and 260 trichomes L^{-1}. In these experiments, no dust-induced stimulation of N_2 fixation was detected.

BP, which ranged from 0.1 to 6 μg C L^{-1} d^{-1}, showed large positive responses (≥ 300%) in experiments 3, 5, and

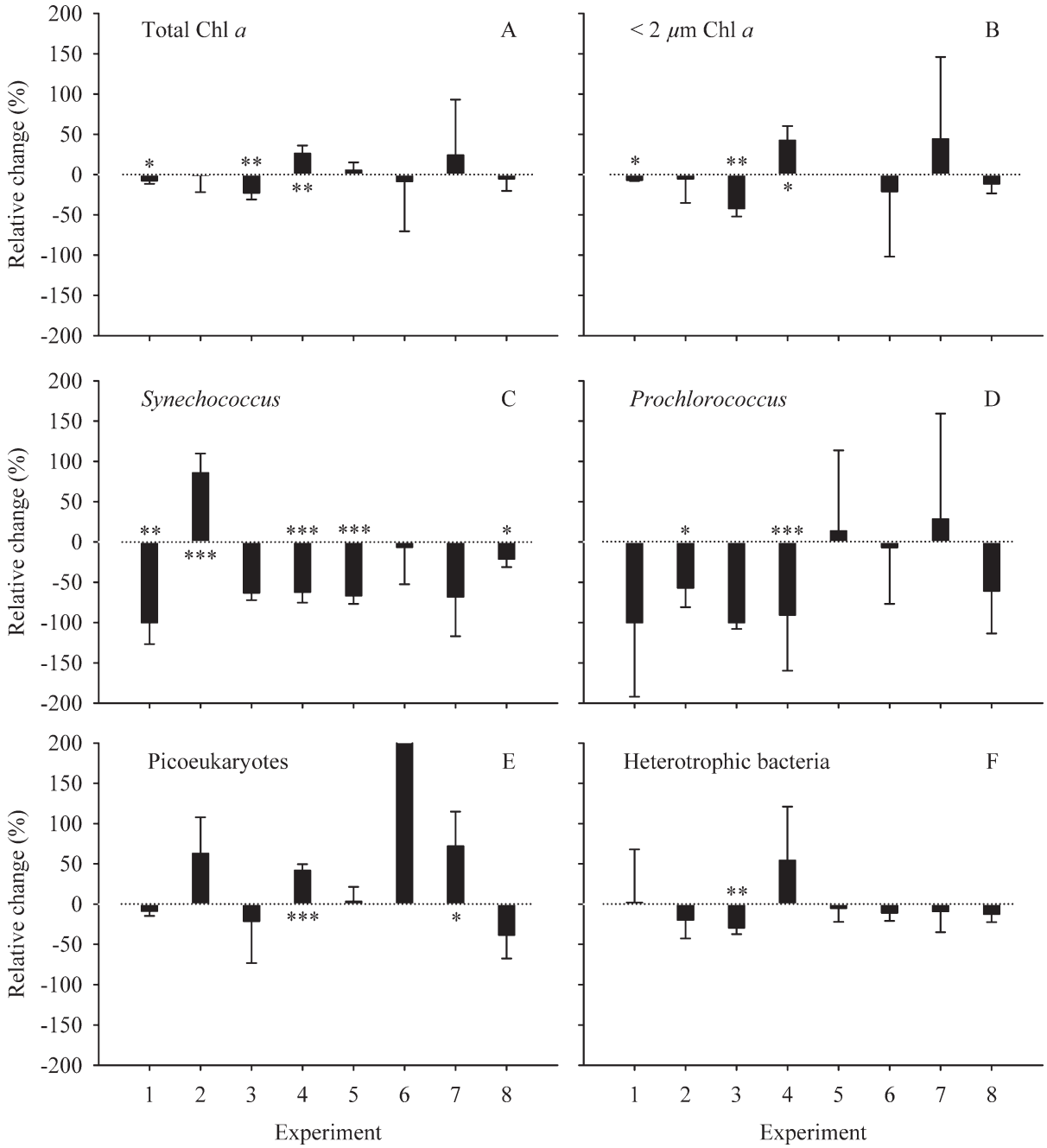

Fig. 3. Relative change (%) in (A) total chlorophyll *a* concentration, (B) chlorophyll *a* concentration in the < 2 μm-size fraction, and the abundance of (C) *Synechococcus*, (D) *Prochlorococcus*, (E) picoeukaryotes, and (F) heterotrophic bacteria in dust-amended bottles as compared with the control bottles for each experiment. Relative change in a given variable was calculated as 100 × ([mean in dust treatment − mean in control]/mean in control). Error bars indicate 1 SD. Asterisks denote the existence of significant differences in the mean of each variable between the control and the treatment bottles (*t*-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

8 (Fig. 5C). In these experiments, the differences in BP between the dust-amended bottles and the control were statistically significant (*t*-test, $p < 0.05$). The initial conditions of experiments 3, 5, and 8 suggested a strong degree of oligotrophy (Table 1). In contrast, in experiments 4 and 6, whose initial conditions suggested only a moderate degree of oligotrophy (Table 1), BP decreased significantly in the presence of dust. The exception to this pattern was experiment 7, which had also been identified as a less oligotrophic site but showed a significant increase in BP in response to dust.

Using our estimates of the amount of N and P released from the dust, we have calculated the increase in biomass production that could be sustained by these nutrient inputs. Assuming a C : N molar ratio of 5–7, the N released from the dust (~ 65 nmol L^{-1}) could sustain a total increase in C biomass of approximately 3.9–5.5 μg C L^{-1}. The increases in PP observed in response to dust (in experiments 2, 4, and

Table 2. Initial picoplankton abundances (10^3 cells mL^{-1}) in the seawater used for the dust addition experiments.

	Experiment number							
	1	2	3	4	5	6	7	8
Synechococcus	3.3	28.3	1.5	3.4	0.5	4.7	4.8	4.9
Prochlorococcus	95.7	697.2	22.2	10.0	50.5	43.6	153.9	60.0
Picoeukaryotes	1.0	4.0	0.6	1.6	1.2	1.0	0.7	0.8
Heterotrophic bacteria	861	889	493	430	468	926	668	492
Roseobacter	26.4	78.4	26.7	64.8	86.1	11.0	38.4	18.1
SAR11	190	168	66	264	206	97	122	88
Bacteroidetes	61	107	43	85	202	70	104	44
Gammaproteobacteria	48.7	22.1	29.6	73.9	107.9	49.5	142.1	41.6

6) were in the range 1–1.5 μg C L^{-1} d^{-1}. The increases in BP in response to dust (in experiments 2, 3, 5, 7, and 8) were in the range 0.2–1.8 μg C L^{-1} d^{-1}. Therefore, in all cases the N released from the dust would be sufficient to explain the measured increases in PP and BP. The low N : P ratio of the dust means that P would be in excess.

CR, which ranged between 0.5 and 3 μmol O_2 L^{-1} d^{-1} in the controls, showed substantial (> 100%) increases in the dust treatments of experiments 2, 3, and 8 (Fig. 5D), reaching a response value > 1000% in experiment 3, which showed also the highest positive response in BP. The increases in CR observed in experiments 2 and 3 were statistically significant (*t*-test, $p < 0.05$). A negative response in CR was observed in experiments 4, 6, and 7.

General patterns in the response to dust addition—By representing the values of relative change obtained in all experiments, it is possible to identify broad patterns in the response of each variable to dust addition, regarding both magnitude and sign (Fig. 6). Chl *a* concentration and the abundance of heterotrophic bacteria were the least responsive variables, with mean, absolute values of relative

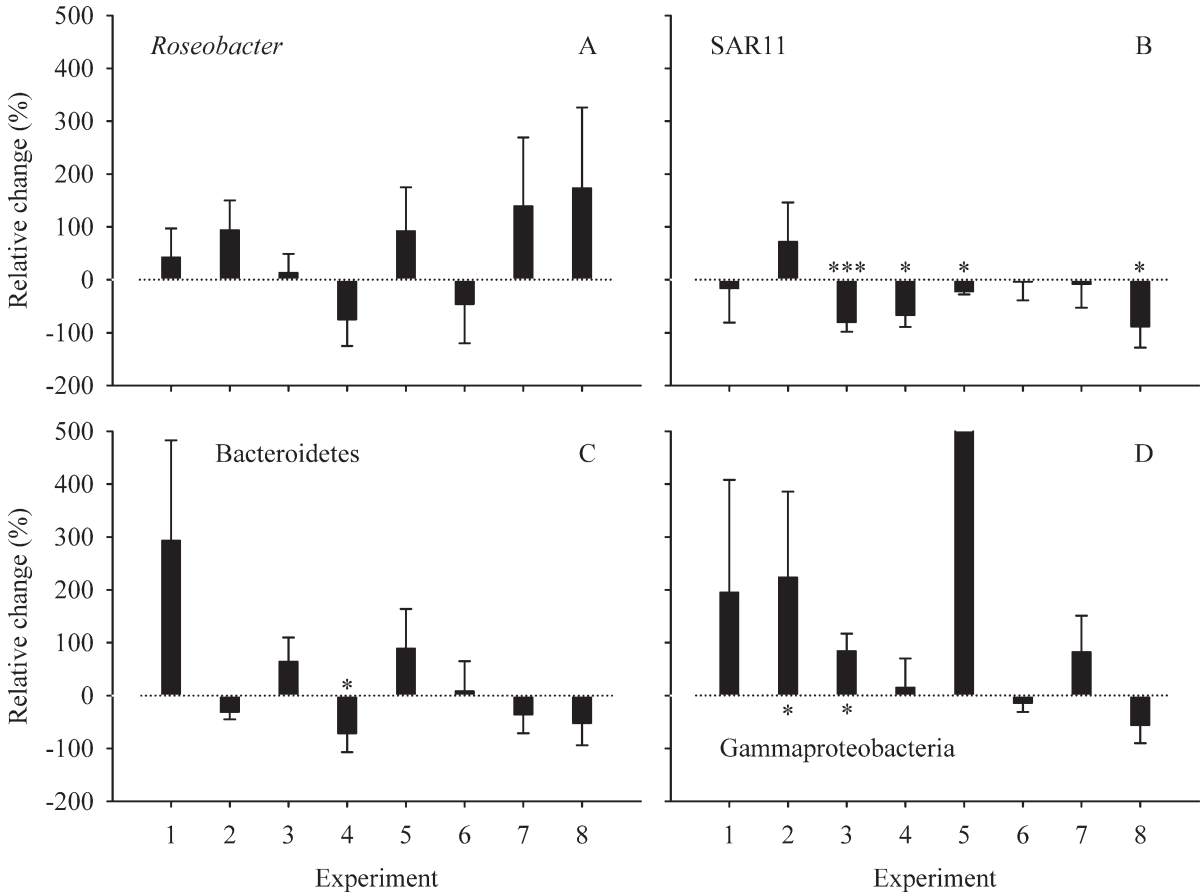

Fig. 4. Relative change (%) in the abundance of (A) *Roseobacter*, (B) SAR11, (C) Bacteroidetes, and (D) Gammaproteobacteria in dust-amended bottles as compared with the control bottles for each experiment. Error bars indicate 1 SD. In experiment 5, the relative change and SD for Gammaproteobacteria were 684 and 492, respectively. Asterisks denote the existence of significant differences between control and treatment (*t*-test; * $p < 0.05$; *** $p < 0.001$).

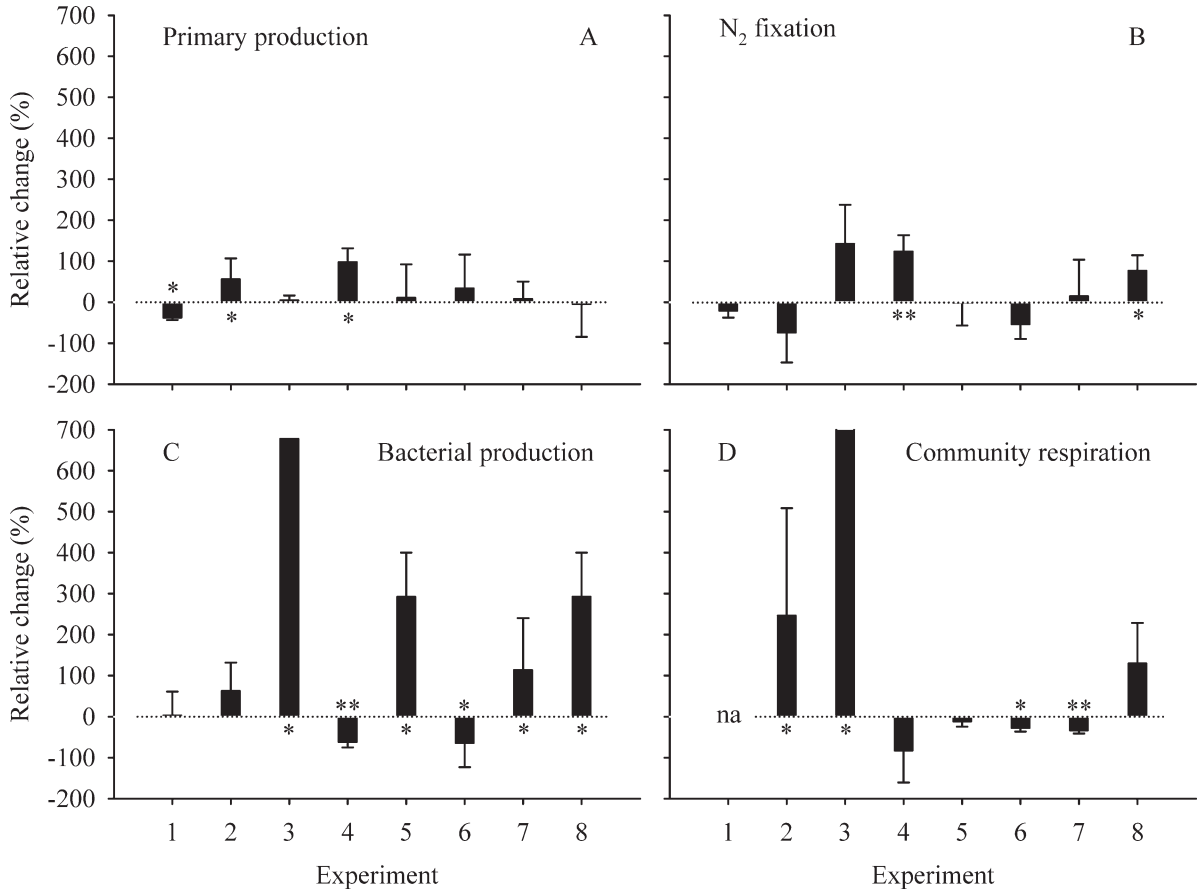

Fig. 5. Relative change (%) in (A) primary production, (B) N_2 fixation, (C) bacterial production, and (D) community respiration in dust-amended bottles as compared with the control bottles for each experiment. Error bars indicate 1 SD. In experiment 3, SD for the relative change in bacterial production was 534, and the relative change and SD for community respiration were 1225 and 458, respectively. Asterisks denote the existence of significant differences between control and treatment (*t*-test; * $p < 0.05$; ** $p < 0.01$). na, not available.

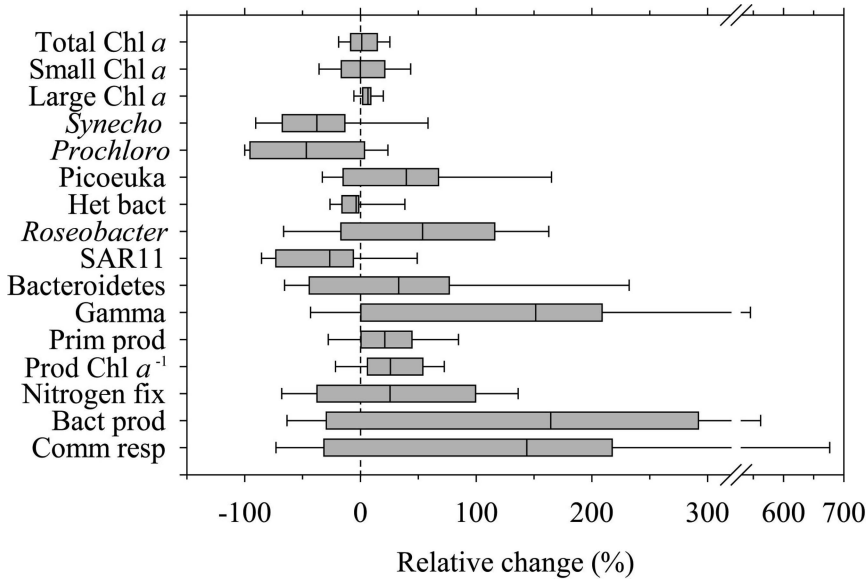

Fig. 6. Box–whisker plots showing the relative change of different variables in response to dust addition in all experiments combined. For each variable, boxes enclose the 25th and 75th percentiles, bars enclose the 5th and 95th percentiles, and the vertical continuous line is the mean.

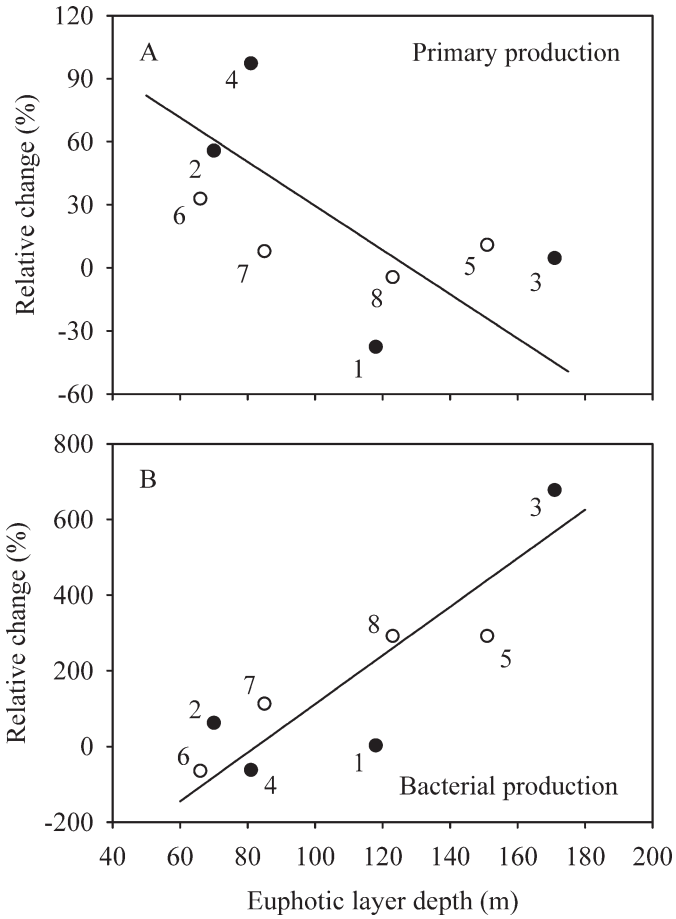

Fig. 7. Relationship between euphotic layer depth and the relative change in (A) primary production and (B) bacterial production. Labels next to each symbol indicate experiment number, and closed and open circles correspond to experiments conducted in 2007 and 2008, respectively. Shown are the reduced major axis regression lines for all data combined. *See* Table 3 for statistical parameters.

change below 6%. The relative change in the abundance of *Synechococcus* and *Prochlorococcus* was more variable, but tended to be negative, with mean values of −38% and −47%, respectively. In contrast, the response in the abundance of the picoeukaryotes tended to be positive and took a mean value of 40%. Among the identified groups of heterotrophic bacteria, *Roseobacter* and Gammaproteobacteria showed a tendency toward positive responses (mean values of 54% and 151%, respectively), whereas the opposite was true for the SAR11 cluster (mean response value of −26%). PP, Chl *a*-specific PP, and N_2 fixation tended to show moderate and positive responses, with mean relative change values near 25%. Finally, BP and CR showed the largest positive responses to dust addition, with mean relative change values around 150%.

Our experiments were conducted at locations that differed in their degree of oligotrophy, which in case 1 waters can be quantified by using the depth of the euphotic layer as a proxy (Kirk 1994). We plotted the relative change in PP and BP against the depth of the euphotic layer to ascertain the relationship between the degree of oligotrophy and the metabolic response of phytoplankton and bacterioplankton to dust addition (Fig. 7, Table 3). The relative change in PP in response to dust decreased significantly with the euphotic layer depth and only in relatively less oligotrophic conditions the photoautotrophs were able to respond to dust addition with enhanced rates of C fixation (Fig. 7A). By contrast, the relative change in BP increased significantly with the euphotic layer depth, which means that bacterial metabolism tends to be more stimulated by dust addition in the most oligotrophic environments (Fig. 7B). Also, BP decreased after dust addition in the less oligotrophic stations (experiments 4 and 6). These opposite patterns in the dust-induced responses of PP and BP across a gradient of oligotrophy were observed in each of the two sets of experiments conducted in 2007 and 2008. In addition, the opposite patterns in the response of PP and BP were found also when other variables (such as the nitracline depth and the depth of the 18°C isotherm) were used as indicators of degree of stratification and oligotrophy (data not shown).

Discussion

Our set of eight bioassay experiments, conducted over a basin-wide geographical range, allowed us to identify consistent patterns in the response of oceanic microbial plankton abundance, diversity, and metabolism to Saharan dust inputs. The patterns we observed can be summarized as follows: (1) dust addition causes larger changes in metabolic rates than in total biomass; (2) although bulk abundance and biomass tend to remain unchanged, different groups of phytoplankton and bacterioplankton respond differently to dust addition; (3) heterotroph-dominated metabolic rates are more responsive to dust inputs than autotrophic metabolic rates; and (4) the predominant type of metabolic response depends on the

Table 3. Parameters of the reduced major axis linear regression between the euphotic layer depth (independent variable) and the dust-induced relative change in primary production and bacterial production (dependent variables).

Rate	Data set	Equation	r^2	n	p
Primary production	2007	$y=-1.3x+119.6$	0.40	4	0.37
Primary production	2008	$y=-0.4x+38.1$	0.37	4	0.38
Primary production	All experiments	$y=-1.0x+134.5$	0.29	8	0.12
Bacterial production	2007	$y=7.5x-548.0$	0.75	4	0.13
Bacterial production	2008	$y=4.5x-283.7$	0.86	4	0.07
Bacterial production	All experiments	$y=6.4x-529.9$	0.74	8	0.006

ecosystem's degree of oligotrophy. These patterns result from observations conducted over large spatial scales and during contrasting seasons. The biological responses were essentially the same in 2007 and 2008, despite the fact that two different kinds of materials, Saharan soil and collected aerosols, were used on each set of experiments. Previous studies (Guieu et al. 2002; Ridame and Guieu 2002; Pulido-Villena et al. 2008) and our own analyses (*see* section *Dust composition* in Methods) show very similar contents of macronutrients, organic C, and Fe in Saharan soils and aerosols collected during Saharan dust storms.

Biomass and community structure—The standing stocks of microbial plankton tended to show much smaller responses to dust addition than metabolic rates such as PP and BP. This pattern has been observed previously during nutrient addition experiments (Mills et al. 2008), dust addition experiments (Bonnet et al. 2005), and natural events of dust deposition (Herut et al. 2005), and highlights the strong trophic control exerted by protist grazers on both bacteria and phytoplankton in the open ocean (Zubkov et al. 2000; Calbet and Landry 2004). The uncoupling between standing stocks and metabolic rates has been noted in the Atlantic subtropical gyres, where a 20-fold range of variability in PP rates, associated with a gradient in vertical nutrient supply, was accompanied only by a modest threefold range in chlorophyll biomass (Marañón et al. 2003). Our results suggest that the standing stocks of microbial plankton in low-nutrient, low-chlorophyll waters are unlikely to increase noticeably in response to dust deposition unless very intense and sustained events take place.

The decrease of *Synechococcus* and *Prochlorococcus* in the dust-amended treatments was unexpected, because natural populations of these genera have been shown to increase their abundance in response to the addition of inorganic nutrients (Davey et al. 2008; Moore et al. 2008; Paytan et al. 2009). We suggest that the predominantly negative response of *Synechococcus* and *Prochlorococcus* in our experiments may have been due to a toxic effect caused by the dust. Some Saharan aerosols, presumably due to their elevated Cu content, have toxic effects on *Synechococcus* (Paytan et al. 2009). The toxic effect of Cu on natural populations of *Prochlorococcus* has also been shown (Mann et al. 2002). In addition, the abundance of this genus decreased sharply both in vitro during dust addition experiments and in situ after a Saharan dust storm (Herut et al. 2005). Another mechanism that could have caused a decrease in the abundance of *Synechococcus* and *Prochlorococcus* in the dust-amended microcosms is the iron-mediated stimulation of specific prokayotic or viral pathogens. Irrespective of the underlying mechanism, our observations do suggest an overall negative effect of Saharan dust on picoplanktonic cyanobacteria, which are the main contributors to PP in the oligotrophic ocean. In contrast, the picoeukaryotes responded positively to dust addition in most of the experiments, suggesting that any possible toxic effect was overcome by the increased availability of nutrients released from the dust. This result agrees with the observation that the picoeukaryote-to-cyanobacteria biomass ratio tends to increase during conditions of enhanced nutrient supply, both in open ocean (Zubkov et al. 1998; Durand et al. 2001) and coastal environments (Calvo-Díaz and Morán 2006).

We also observed changes in the composition of the heterotrophic bacterial assemblage in response to dust addition, even though total abundance remained relatively constant. These changes were consistent with the known distribution and physiological properties of the different identified phylogenetic groups. Thus, the abundance of *Roseobacter*, which is associated with enhanced resource availability in meso- to eutrophic environments (Buchan et al. 2005), tended to increase in the dust-amended microcosms. Gammaproteobacteria, which are able to respond to increased nutrient availability (Horňák et al. 2006), also showed increased abundances in most of the dust treatments. By contrast, SAR11, a particularly abundant lineage in oligotrophic areas of the open ocean (Morris et al. 2002; Malmstrom et al. 2004), responded negatively to the dust. Our results thus suggest that community structure of both phytoplankton and bacterioplankton may be affected by dust inputs even if bulk abundance and biomass remain unchanged. To the extent that these taxonomic groups may be associated with different metabolic processes and differ in their growth and loss rates, these changes can have implications for the production and fate of biogenic materials in the upper ocean.

Metabolic rates—The largest metabolic responses to dust were measured in heterotroph-dominated processes such as BP and CR. We found five instances where the relative change in response to dust exceeded a value of 200% for BP and CR, whereas PP never increased by more than 100%. The higher ability of heterotrophic bacteria to increase their productivity in response to experimental dust additions has been observed in the eastern Mediterranean, where 20-fold and 5-fold increases in BP and PP, respectively, were reported (Herut et al. 2005). Similarly, the combined addition of nitrate, ammonia, and phosphate (at a final concentration of 1, 1, and 0.2 μmol L^{-1}, respectively) to surface water collected in the subtropical North Atlantic resulted in 11- to 35-fold increases in BP, whereas PP increased only by a factor of 3 (Mills et al. 2008; Moore et al. 2008). It has been shown that in situ bacterial abundance and metabolic rates increase in response to Saharan dust inputs, both in the western Mediterranean Sea (Pulido-Villena et al. 2008) and in an oligotrophic mountain lake (Reche et al. 2009), whereas phytoplankton standing stocks remain unchanged. Compared with autotrophic phytoplankton, heterotrophic bacterioplankton are characterized by lower C : N and C : P ratios (Chrzanowski et al. 1996; Vrede et al. 2002), which imply that they have higher demands for nutrients. Because of their smaller size and higher surface-to-volume ratios, prokaryotic heterotrophs can acquire inorganic nutrients more effectively when ambient concentrations are extremely low, thus limiting the extent to which phytoplankton can benefit from nutrient pulses (Cotner and Biddanda 2002; Joint et al. 2002). We cannot rule out the possibility that the comparatively small response of

phytoplankton production was due to the existence of a lag phase, such that the duration of our experiments (72 h) may have been too short to observe an increase in C fixation after the dust addition. However, there is evidence indicating that open-ocean photosynthetic picoplankton can respond to transient nutrient increases in a matter of hours (Glover et al. 2007). In conclusion, we suggest that the most common effect of dust inputs over the oligotrophic areas of the Atlantic Ocean, where Fe is not the primary limiting nutrient either for PP (Mills et al. 2004; Davey et al. 2008; Moore et al. 2008) or for BP (Mills et al. 2008), may not be necessarily an increase of C fixation, but an enhancement of bacterial metabolism caused by the inorganic nutrients released from the dust.

N_2 fixation is strongly dependent on Fe availability (Falkowski 1997; Karl et al. 2002) and eolian dust is the primary vector for Fe input into the open ocean (Jickells et al. 2005). In addition, N_2 fixation colimitation by P and Fe has been demonstrated in the tropical Atlantic Ocean (Mills et al. 2004). In this same study, it was also shown that Saharan dust, added at a final concentration of 2 mg L^{-1}, induced a two- to fourfold increase in N_2 fixation rates in three experiments. In our experiments, however, we only found stimulation of N_2 fixation by dust addition on three occasions and in the absence of *Trichodesmium* spp. filaments, which suggests that unicellular diazotrophs must have been involved in the detected response. *Trichodesmium* spp. are characterized by slow growth and metabolic rates, and therefore it is possible that our 72-h incubation period was not sufficient to detect a response in terms of enhanced N_2 fixation rates. In any event, our results do suggest that the degree of nutrient limitation of community diazotrophy in the tropical Atlantic is variable both geographically and temporally.

The sign and magnitude of the metabolic response to dust in terms of primary and bacterial productivity depended on the ecosystem's degree of oligotrophy. The response of PP to dust tended to decrease with increasing oligotrophy, whereas the response of BP showed the opposite pattern. Given the experimental evidence that both PP and BP are colimited by N and P, it has been suggested that phytoplankton and bacteria compete for inorganic nutrients in the subtropical North Atlantic (Mills et al. 2008; Moore et al. 2008). Our observation of an inverse pattern in the dust-induced response of BP and PP depending on the ecosystem's nutritional status supports the view that competition for nutrients between phytoplankton and bacteria is indeed widespread throughout the tropical and subtropical Atlantic Ocean. We suggest that, in ultraoligotrophic conditions, the superior ability of heterotrophic bacteria to take up nutrients prevents phytoplankton from responding to the nutrients released from the dust. This scenario is similar to that encountered in the eastern Mediterranean Sea, where the in situ addition of P at a final concentration of 110 nmol L^{-1} caused a doubling of BP while Chl *a* decreased by 40% (Thingstad et al. 2005). In less oligotrophic conditions, however, bacteria may be less limited by inorganic nutrients, and phytoplankton would then be able to utilize the nutrients released from the dust, thus increasing their productivity.

Biogeochemical implications—Eolian dust fluxes have increased during the second half of the 20th century, partly as a result of land-use change, and some models predict that they may continue to rise in the 21st century (Jickells et al. 2005; Mahowald et al. 2009). Biogeochemical models tend to associate increased dust fluxes with ocean fertilization and a stronger biological pump (Martin 1990; Ridgwell et al. 2002; Jickells et al. 2005). The basis for this association is the Fe-mediated stimulation of PP observed during dust storms in oceanic regions that are strongly Fe limited (Bishop et al. 2002). However, our study shows that this fertilization effect is not universal and that additional interactions must be taken into account. In oligotrophic areas that are not strongly limited by Fe supply, dust inputs can have as their main effect an increase in BP and CR, rather than in phytoplankton productivity. In these cases, the response of the microbial community is likely to favor the microbial loop and the remineralization of DOC, thereby reducing atmopheric CO_2 drawdown and the potential for C export outside the euphotic zone (Rivkin and Anderson 1997; Thingstad et al. 1997). In our study, the balance between a predominantly autotrophic vs. heterotrophic response to enhanced dust fluxes depended on the ecosystem's degree of oligotrophy. If this pattern is confirmed widely, and given the current trend of ocean warming and the ensuing expansion of low-chlorophyll regions (Polovina et al. 2008), the phytoplankton response to eolian dust fluxes may weaken progressively, whereas that of bacterial metabolism may become stronger. To reproduce the biogeochemical feedbacks among climate change, eolian fluxes, and marine biota, models should incorporate the contrasting effects of dust on different microbial groups and metabolic rates, the competitive interactions between phytoplankton and heterotrophic bacteria, and the potentially divergent responses of communities inhabiting ecosystems with different degrees of oligotrophy.

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