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Effects of a dust deposition event on coastal marine microbial abundance and activity, bacterial community structure and ecosystem function

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The Mediterranean coast receives large inputs of dust with a potential fertilization effect. We evaluated the effect of a wet dust deposition event on microbial abundance and activity, community structure and metabolic balance. Dust collected during a dust storm event was added to a series of microcosms. We added a realistic concentration (0.05 g L^{-1}) based on the phosphorus concentration contained in the dust and, in addition, we included a P amended tank to distinguish the effect of dust from that of the phosphorus in the dust. We also included a higher dust concentration tank (0.5 g L^{-1}). Dust increased the initial water–phosphorus concentration by 0.3 μM and the dissolved organic carbon (DOC) concentration by 14 μM, and increased bacterial abundance (1.8-fold) and bacterial production (5-fold). At the end of the experiment, primary production and community respiration were stimulated by dust and by P, but the net result of the addition of low amounts of dust was an initial switch towards heterotrophy, whereas the net result of the high-dust (DH) additions and the P addition was a shift towards autotrophy. Bacterial community structure changed little between P and low dust, but these were very different from the control and the DH communities.

INTRODUCTION

Coastal microbial communities are subject to external forcing events, such as the inputs of allochthonous nutrients from rivers, coastal runoff, submarine groundwater discharge, resuspension of benthic particles etc. These allochthonous inputs contain organic and inorganic nutrients, which affect microbial diversity and function (Markaki *et al.*, 2003; Wassmann and Olli, 2004; Bonnet *et al.*, 2005; Pulido-Villena *et al.*, 2008). Among these sources of allochthonous nutrients to the coastal ocean, atmospheric transport is a particularly important type (Herut *et al.*, 1999). Wind-transported particles can settle by wet deposition associated with rain, and this has been shown to be an effective mechanism of dust deposition in the western Mediterranean Sea (Prodi and Fea, 1979; Loyë-Pilot and Martin, 1996; Guerzoni *et al.*, 1997; Guerzoni *et al.*, 1999; Ridame and Guieu, 2002). The Sahara and the Sahel are, by far, the largest sources of dust particles (Prospero and Lamb, 2003). Wet deposition events, or "dust rains" of Saharan origin (SD), in the coastal NW Mediterranean are common during late winter and early summer.

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Featured Article

The Mediterranean Sea, including the NW Mediterranean, is known to have photosynthetic and heterotrophic microbial communities which are P-limited, particularly during the summer season (Thingstad *et al.*, 1998; Pinhassi *et al.*, 2006) and, since SD has been shown to contain bioavailable phosphorus (Herut *et al.*, 2002), dust deposition events can stimulate the activity and the production of P-limited microorganisms. In particular, primary production (PP) has been shown to respond to dust inputs in the area (Lenes *et al.*, 2001; Ridame and Guieu, 2002; Bartoli *et al.*, 2005; Bonnet *et al.*, 2005; Herut *et al.*, 1999, 2002, 2005).

It has also been reported that heterotrophic prokaryote (i.e. bacterial) abundance and activity may also respond to dust enrichments (Herut *et al.*, 2005; Pulido-Villena *et al.*, 2008). One could speculate that the phosphorous contributed by deposition can stimulate algae, and bacteria may be stimulated as a consequence of the algal stimulation. However, SD contains nutrients other than P, particularly N and Fe (Jickells *et al.*, 1995), and is also known to be a source of organic carbon for aquatic ecosystems (Pulido-Villena *et al.*, 2008). Heterotrophic bacteria are often the best competitors for phosphorus uptake (Currie, 1984), and the simultaneous addition of DOC and P might shift the competition for P in favour of bacteria (Thingstad *et al.*, 1999). Thus, it could be hypothesized that the processes driven by heterotrophic prokaryotes would be more stimulated by a dust event than the autotrophic processes. Dust events could have the capacity to shift a system towards net heterotrophy by stimulating bacterial respiration more than PP. Bacterial respiration was stimulated by P additions in the Sargasso Sea (Obernosterer *et al.*, 2003), and the addition of DOC of probably low quality in the presence of enough inorganic P should presumably force bacteria to respire most of the low-quality C, thus greatly increasing community respiration (CR). In fact, Pulido-Villena *et al.* (2008) observed that a natural dust deposition event of 2.6 g m^{-2} induced a 1.5-fold net increase in bacterial abundance and a 2-fold increase in respiration, whereas experimental dust additions (between 2 and 20 g m^{-2}) also stimulated abundance and respiration (1.5- to 3-fold increases). These authors, however, did not specifically compare the heterotrophic stimulation caused by the dust with the simultaneous expected stimulation of PP (as found by others, e.g. Herut *et al.*, 2005).

Addition of P and DOC from the dust, and also the changes in photosynthetic microbial community structure caused by the fertilization (Pinhassi *et al.*, 2004), can affect the structure of the bacterial assemblages. It is particularly interesting to understand whether it is the P or the DOC component of the dust that mostly determines community structure, and whether there are phylotypes particularly stimulated by the dust pulses. In addition to the inputs of nutrients associated with dust, viable bacteria can be transported long distances (Kellogg and Griffin, 2006; Hervàs *et al.*, 2009), with little-known effects on the resident bacterial community structure.

We designed an experiment to (i) confirm the stimulation of PP and bacterial function caused by additions of dust collected in a wet deposition event to the P-limited oligotrophic plankton community and see whether this translates into microbial community metabolism shifts, (ii) separate the effects of the fertilization by the P contained in the dust, from the effects of the other components of the dust, particularly the bioavailable DOC and (iii) see whether dust fertilization would alter bacterial community structure in a way fundamentally different from the changes caused by P fertilization alone. We collected dust during a wet Sahara dust deposition event. After a drying–rewetting cycle, the dust was added to a series of microcosms at an "experimental" concentration of 0.05 g L^{-1}. We used two "controls" on top of a no-addition treatment: one with inorganic P alone at the same concentration as in the dust addition, and one with 10 times more dust to observe the effects of a deposition pulse of the same large magnitude as found sporadically in the Mediterranean (Loyë-Pilot and Martin, 1996). Furthermore, as the dust depositions in this area are due to storms (Ridame and Guieu, 2002), we added a turbulence treatment to discriminate the effects of the associated water movement from the effects of dust alone.

METHOD

Experiment setting

The experiment was carried out with surface water collected in Blanes Bay, NW Mediterranean (The Blanes Bay Microbial Observatory, 41°40′0″N, 2°48′0″E), on 16 May 2006. Eight tanks of 15 L were used for the experiment filled with 150 μm filtered seawater. Of the eight tanks, four were established as controls (C), four received a low dust (DL) concentration (0.05 g L^{-1}), two received a high dust (DH) concentration (0.5 g L^{-1}), and two received an inorganic phosphorus addition (P). Half of the tanks were incubated with turbulence, and the other half without. Tank replicability was achieved using two replicates per condition in half of the treatments, since space constraints limited the full

replication in all treatments. At the end of the experiment, 66% of the volume still remained in the tanks.

Saharan dust was collected on 21 February 2004 in Villefranche-sur-Mer, France (43°42′18″N, 7°18′45″E), during an intense dust storm. A hard plastic tray was exposed 1 m above the ground to the rain (ca. 24 h). The tray had 4 cm high side walls and the dust accumulated at the bottom of the tray. Several hours after the rain had stopped, the supernatant was poured off and the dust was transferred with a plastic spatula into an acid-washed Nalgene high-density polyethylene bottle. The dust was then dried at 60°C. The dry dust was ground (Agatha mortar) before a single initial addition to the experimental tanks. Saharan dust deposition events carry between 5 and 8000 mg of dust per litre (Ridame and Guieu, 2002). A 0.05 g L^{-1} dust addition released 0.38 ± 0.08 μmol PO_4^{3-} L^{-1}, and this value was used as our low addition level (DL) and a 10 times higher value (0.5 g L^{-1}) as our high addition level (DH). P treatments were enriched with inorganic PO_4^{3-} at a concentration of 0.5 μM, again as a single initial addition. This was estimated to be the amount of P that we would add with the dust, and would act as a control of the effect of a P addition without the other components of the dust.

Turbulence was generated with a vertically oscillating grid device (Peters *et al.*, 2002). A turbulent kinetic energy dissipation rate of 10^{-2} cm^2 s^{-3} was applied, a value within the range of turbulence intensities in coastal areas (Kiørboe and Saiz, 1995). Containers corresponding to turbulence (T) treatments were subjected to turbulent conditions for 3 days, similar to the duration of events in the Blanes area (Guadayol and Peters, 2006), and remained still until the end of the experiment, whereas still (S) treatments were kept still throughout.

The experimental temperature was 17°C corresponding to the *in situ* water temperature. Light conditions inside the containers were 225 μmol photons m^{-2} s^{-1}. A 16:8 h light:dark cycle, characteristic of the time of the year, was applied. The experiment started within 4 h of water collection.

Chlorophyll *a* and nutrients

Samples (100 mL) for estimating chlorophyll *a* (Chl *a*) concentration were filtered through 25 mm diameter Whatman GF/F filters, which were immediately frozen until extraction. Filters were then placed in 90% acetone at 4°C for 24 h and the fluorescence of the extract measured using a Turner Designs fluorometer. Total phosphorus (particulate + dissolved organic and inorganic phosphorus) was determined in duplicates after an acid persulphate oxidation followed by a spectrophotometric analysis using the molybdenum blue method. For analysis of DOC concentration, filtered (GF/F) samples (10 mL) were collected in precombusted (450°C, 12 h) glass ampoules and acidified with H_3PO_4 to pH < 2. The ampoules were heat-sealed and stored in the dark at 4°C until analysis. DOC was measured with a Shimadzu TOC-V organic carbon analyser. Initial chlorophyll concentration was 2.45 mg m^{-3}, initial total N was 1.73 μM and initial Si concentration was 0.13 μM. Other values are given in Table I.

In order to evaluate the increase of fluorescent dissolved organic matter (FDOM) due to dust addition, we added 0.05 g to a litre of sea water, previously filtered through a combusted GF/F filter. After mixing, we took

Table I: Concentrations of total phosphorus (μM) and of DOC (μM) at the start of the experiment (t0), after addition of dust (SD) and inorganic phosphorus (P), and after 18 h (t18h) or 3 days (t3d) of incubation

	Target concentrations		Measured concentrations		
			Total phosphorus (μM)		DOC (μM)
	SD (g L^{-1})	P (μM)	t0	t3d	t18h
Source seawater			0.095 ± 0.007		59.0 ± 0.7
CT	0	0		0.115 ± 0.021	
CS	0	0		0.120 ± 0.014	
DHT	0.5	0		3.34	264.8 ± 2.6
DHS	0.5	0		3.37	
DLT	0.05	0		0.425 ± 0.021	73.2 ± 2.0
DLS	0.05	0		0.445 ± 0.007	
PT	0	0.5		0.42	
PS	0	0.5		0.47	

C, control; DL, low dust concentration; DH, high dust concentration; P, phosphorus addition; T, turbulence treatment; S, still treatment. When possible, average ± standard deviation of two replicated tanks.

samples for DOC and FDOM analyses. FDOM samples were analysed immediately after collection. Single measurements of the aliquots were performed with an LS 55 Perkin Elmer Luminescence spectrometer, equipped with a xenon discharge lamp, equivalent to 20 kW for 8 μs duration. The detector was a red-sensitive R928 photomultiplier, and a photodiode worked as a reference detector. Measurements were performed after filtering the water sample through a combusted GF/F filter, at a constant room temperature of 20°C in a 1 cm quartz fluorescence cell. The Ex/Em wavelengths used for single measurements were those established by Coble (Coble, 1996): Ex/Em: 340/440 nm peak as an indicator of terrestrial-like substances and at 320/410 nm peak as an indicator of marine humic-like substances. Single fluorescence measurements were calibrated against a quinine sulphate dihydrate (QS) standard made up in 0.05 M sulphuric acid. Single fluorescence measurements are expressed in quinine sulphate units (QSU), μg eq QS L^{-1}.

Organism abundance

Bacterial abundance and the percentage of high nucleic acid (HNA) cells were measured by flow cytometry (Gasol and del Giorgio, 2000). Samples were run in a Becton–Dickinson FACSCalibur cytometer after staining with 10× SybrGreen I (Molecular Probes), and bacteria were detected by their signature in a plot of side scatter (SCC) versus FL1 (green fluorescence). Regions were established on the SSC versus green fluorescence plot in order to discriminate cells with HNA content from cells with low nucleic acid content. Cell abundance was determined for each subgroup. Bacterial biomass was calculated from abundance assuming a carbon content of 12 fg C $cell^{-1}$ (Fukuda *et al.*, 1998).

Live samples were analysed in a FACSCalibur flow cytometer (Becton–Dickinson) in unstained aliquots for the enumeration of picophytoplankton populations. Milli-Q water was used as a sheath fluid and 1-μm yellow–green latex Polysciences beads were used as an internal fluorescence standard. The samples were run at the highest possible speed (around 60 μL min^{-1}) for 5 min, and the data were acquired in log mode. Abundances were calculated by the ratiometric method from the known amount of added beads, calibrated daily against TrueCount (Becton and Dickinson) beads. *Synechococcus* were detected by their signature in a plot of orange fluorescence (FL2) versus red fluorescence (FL3), picoeukaryotes (Peuk) had higher FL3 signals and no FL2 signals. No *Prochlorococcus* were present at the time of sampling.

Single-cell bacterial physiology

We used the activity probe 5-cyano-2,3-ditolyl tetrazolium chloride (CTC, Polysciences) to enumerate highly actively respiring prokaryotes, as an indication of the number of very active cells. Two 0.5 mL sample aliquots were spiked with 5 mM final concentration of CTC from a freshly prepared stock solution (50 mM in Milli-Q water) and incubated for 90 min in the dark, at *in situ* temperature. The samples were immediately run through the FACSCalibur flow cytometer. An additional 0.5 mL aliquot was fixed with paraformaldehyde at time 0 and spiked with 5 mM final concentration of CTC for a background control of CTC fluorescence on dead samples. CTC-formazan is excited by wavelengths between 460 and 530 nm and has bright red fluorescence. CTC+ particles were those that showed red fluorescence (>630 nm, FL3 in our instrument), higher than the background fluorescence level (Gasol and Arístegui, 2007). A dual plot of 90° light scatter and red fluorescence was used to separate CTC+ cells from background noise. The FL2 versus FL3 plot was used to differentiate the populations of photosynthetic microbes from the CTC+ particles.

Bacterial production

Bacterial heterotrophic production was estimated using the ^{3}H-leucine incorporation method (Kirchman *et al.*, 1985). Triplicate or quadruplicate aliquots of 1.2 mL were taken for each sample and one or two trichloroacetic acid (TCA)-killed controls. The Leu tracer was used at 40 nM final concentration in incubations of ~2 h. The incorporation was stopped with the addition of 120 μL of cold TCA 50% to the samples and, after mixing, they were kept frozen at −20°C until processing, which was carried out by the centrifugation method of Smith and Azam (Smith and Azam, 1992). We used the standard conversion factors of 3.1 kg C mol Leu^{-1}.

Community metabolism

For PP, two light and one dark acid-cleaned polycarbonate bottles were filled with 160 mL water samples from each sample, inoculated with $\sim 3.7 \times 10^{5}$ Bq (10 μCi) of ^{14}C-bicarbonate (VKI, Denmark). Incubations were done in the same chamber where the main samples were incubated, and with the same light levels, and lasted for ~2 h at *in situ* temperature. Samples were

then filtered onto Whatman GF/F filters (25 mm diameter). Filters were placed in 6 mL vials and fumed overnight with HCl 35%. Finally, 4.5 mL of ReadySafe liquid scintillation cocktail was added before determination of disintegrations per minute (dpm) in the laboratory by means of a Beckman LS6500 liquid scintillation counter. Dark bottle dpm were subtracted for correction of non-photosynthetic ^{14}C fixation. No isotope discrimination factor was used. Conversion to carbon units was performed assuming an ambient inorganic C concentration of 25 000 mg C m^{-3}. Conversion to daily values was done considering a 16:8 h dark:light cycle, and the assumption of constant production during the light hours.

For CR, we followed the changes in dissolved oxygen during dark incubations of unfiltered water. Eight BOD bottles were carefully filled, and three replicate bottles were immediately fixed with Winkler reagents to determine the initial oxygen concentration. Four replicate bottles were incubated in the darkness at *in situ* temperature (as in Vázquez-Domínguez *et al.*, 2007) and fixed with Winkler reagents after ~24 h. Dissolved oxygen measurements were made with an automatic titrator (DL50 Graphix, Mettler Toledo) based on potentiometric endpoint detection (Outdot *et al.*, 1988). The rate of respiration was determined by regressing the oxygen concentration against the time at which the samples were withdrawn. This estimation assumes that the disappearance of oxygen was linear (Model II regression) and the slope of the regression is equal to the respiration rate. We assumed a respiratory quotient of 0.88 (see further details in Alonso-Sáez *et al.*, 2008).

Collection of community DNA

Microbial biomass was collected by sequentially filtering around 5 L of seawater through a 3 μm pore size polycarbonate filter (Millipore, 46 mm) and a 0.2 μm polycarbonate filter (Millipore). Microbial biomass was treated with lysozyme, proteinase K and sodium dodecyl sulphate, and the nucleic acids were extracted with phenol and concentrated in a Centricon-100 (Millipore). Nucleic acids were extracted by a standard protocol using phenol/chloroform (see details in Schauer *et al.*, 2003).

Fingerprinting analysis

Denaturing gradient gel electrophoresis (DGGE) and gel analysis were performed essentially as described previously (Schauer *et al.*, 2003; Sánchez *et al.*, 2007). Briefly, 16S rRNA gene fragments (around 550 bp in length) were amplified by PCR, using the universal primer 907rm and the bacterial-specific primer 358f containing a GC-clamp. The PCR products were loaded on a 6% polyacrylamide gel with a DNA-denaturant gradient ranging from 40% to 80%. The gel was run at 100 V for 16 h at 60°C in 1× TAE running buffer. DGGE gel images were analysed using the Diversity Database software (BIO-RAD). A matrix was constructed for all lanes taking into account the relative contribution of each band (in percentage) to the total intensity of the lane. On the basis of this matrix, we obtained a dendrogram by the UPGMA clustering method (Euclidean distances, Statistica 6.0). DGGE bands were excised, reamplified and verified by a second DGGE. Bands were sequenced using primer 358f without the GC clamp, with the BigDye terminator cycle-sequencing kit (Perkin Elmer Corporation) and an ABI PRISM model 377 (v3.3) automated sequencer. Dendrograms were used to compare microbial assemblage structures.

Statistical analyses

ANOVAs and *post hoc* tests to compare treatments were run with software JMP 7.0.

RESULTS

General experimental considerations

Our DL additions resulted in an increase in P concentration of ca. 0.3 μM above the 0.1 μM present in the water at the time of sampling (Table I). In the DH treatment, the increase was 10 times higher, up to 3.3 μM. Similarly, the addition of DL concentrations enriched the background DOC concentration by ca. 14 μM (from 59 to 73 μM), whereas the DH treatment assumed an enrichment of slightly more than 10-fold, up to ca. 260 μM (Table I). Maximum DOC recorded at Blanes Bay is ca. 170 μM (Pinhassi *et al.*, 2006; Alonso-Sáez *et al.*, 2008) and, thus, the DH treatment was well above the common values in the Bay. Although the addition of DOM in the DL treatment assumed an increase of 24.0 ± 3.3%, the terrestrial and marine humic-like fractions of DOC (as measured by FDOM) increased by 65.6 ± 2.7% and 49.4 ± 1.9% respectively. Thus, the water became enriched in humic compounds that are characterized by low lability.

Microorganism abundance

The experiment started with an initial Chl *a* value close to 3 mg m^{-3}, which is one of the highest values that have been measured in the Bay (Alonso-Sáez *et al.*, 2008), and this indicates that we had sampled a bloom.

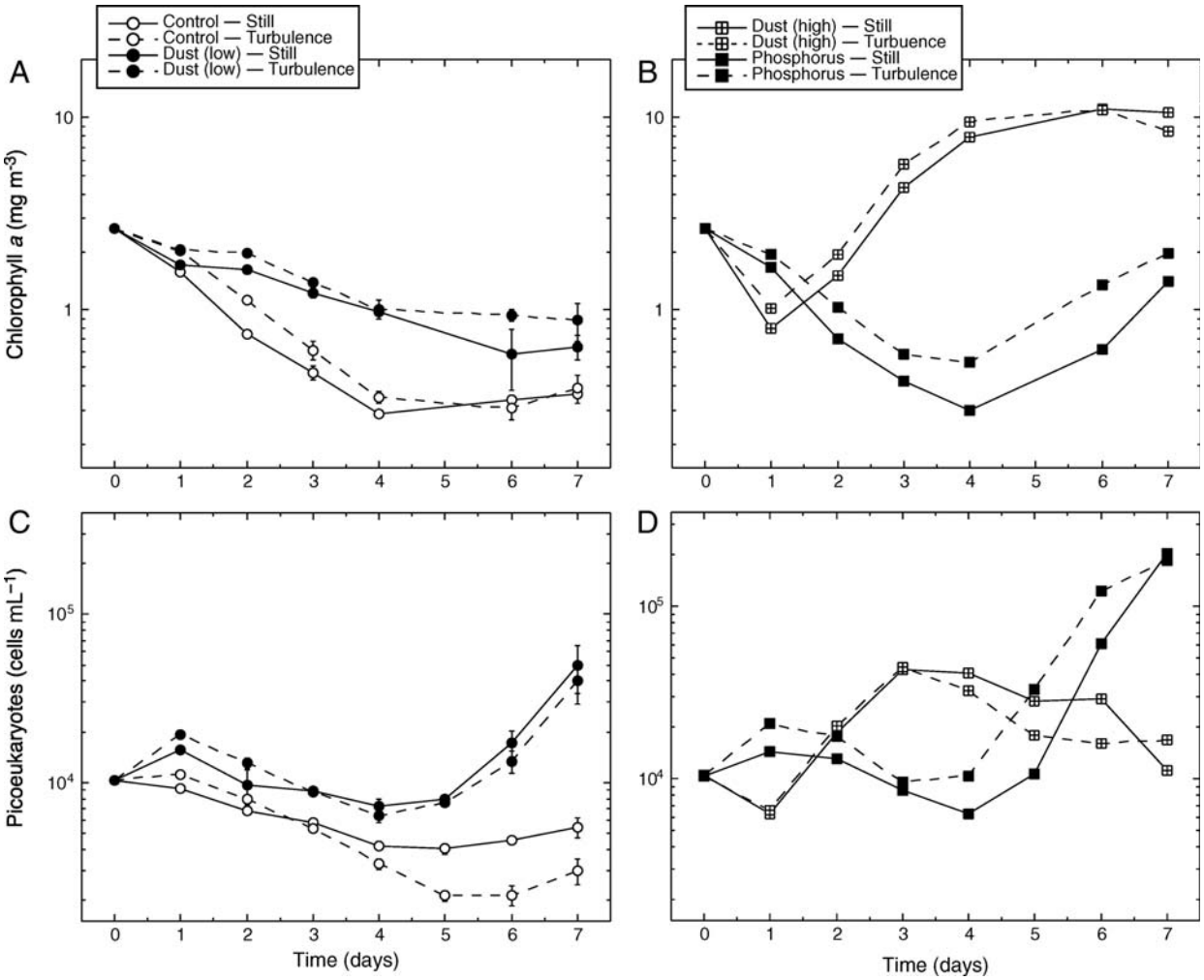

Fig. 1. (**A**) Changes in Chl *a* concentrations (mg m^{-3}) in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**B**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions. (**C**) Picoeukaryote abundances (cell mL^{-1}) in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**D**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions.

The bloom decayed throughout the experiment (Fig. 1A), but did less so in the DL treatment. The P treatment reached values intermediate between those of the C and DL treatments, with a small bloom developing towards the end of the experiment. In the DH treatment, a bloom developed reaching values of up to 10 mg m^{-3} (Fig. 1B). Treatment was a significant variable explaining the data (Table II), particularly between treatments DH and C. Average Chl *a* concentrations was 1.5 times in the DL when compared with the control. *Synechococcus*, which were initially at 3×10^4 cells mL^{-1}, disappeared from all treatments at similar rates (data not shown), while Peuk responded differently to each treatment: they maintained abundances and even increased slightly at the end of the experiment in the DL treatment (Fig. 1C), while they decreased in the control. They reached a peak of 4×10^4 cells mL^{-1} in the DH treatment in Days 3 and 4, and a peak of 2×10^5 mL^{-1} in the P treatments towards the end of the experiment (Fig. 1D). Average Peuk abundance was 2.7 times in the DL treatment when compared with the C treatment (Table II). Comparison of the evolution of Chl *a* and Peuk abundance suggests that while the Chl *a* peak at the end of the experiment can, in part, be assigned to the Peuk development, the one occurring in the DH treatment needs to be assigned to cells larger than Peuk. Indeed, the group responsible for this Chl *a* peak in the DH treatment was nanoflagellates and diatoms, mostly of genus *Nitzschia* (E. Romero *et al.*, in preparation).

Table II: Results of a one-way ANOVA with the time-integrated (for production) or time-averaged (for stocks) values for each treatment

	Chl *a* total	BA	BP	Peuk	%HNA	P/B
Global ANOVA *P*	<0.001	<0.001	0.005	0.002	0.002	NS
Treatment						
CS	D	D	D	C D	C	A
CT	C D	D	C D	D	B C	A
DLS	B C	C	A	B	A	A
DLT	B C	C	A B	B C	A	A
DHS	A	B	A B	B	A	A
DHT	A	A	A B C	B	A	A
PS	C D	C D	B C D	A	A B	A
PT	B C	C D	A B C	A	A	A
Post hoc tests						
P difference DL/C	0.001	0.004	0.002	0.007	<0.001	NS
Factor (×)	1.5	1.8	2.3	2.7	1.4	—
P difference DL/P	NS	NS	NS	0.01	NS	NS

Treatments with the same letters are not significantly different from each other. The *post hoc* tests give the probability of DL treatments being different from the C treatments, and of the P treatments being different from the DL treatments. The "factor" is the ratio between the time-integrated value of treatment DL to that of treatment C. BA, total bacterial abundance; BP, bacterial production; Peuk, picoeukaryote abundance; P/B, biomass-specific bacterial production.

While heterotrophic bacteria decreased in the control tanks until Day 3, and then increased towards the end of the experiment (Fig. 2A), they showed an initial peak in the DL treatment on Day 1, which was suppressed on Day 2. The response in the DH treatment was much higher (>10× in just 1 day, Fig. 2B), and the high bacterial abundances lasted for 3 days after returning to abundances similar than those in the control treatment on Day 4. In both, P and DH treatments, bacteria increased steadily from Day 4 to the end of the experiment. The final bacterial yield in the P and DH treatments was slightly higher ($4-8 \times 10^6$ cells mL^{-1}) than in the C or DL treatment ($3-5 \times 10^6$ cells mL^{-1}). Addition of dust (DL) increased bacterial abundance by a factor of 1.8 on average compared with the control (Table II).

The differences between treatments were stronger in the physiological structure of the bacterial assemblage. The %HNA cells increased immediately in all treatments, particularly in the DL, in contrast to the C (Fig. 2C and D). Some effects of turbulence were obvious in this parameter, with %HNA being higher in the T replicates of the C, P and DL treatments. The number of actively respiring bacteria (CTC+) followed a pattern quite similar to that of the %HNA cells, with a clear difference between the DL and the C treatments (Table III), a much larger response in the DH, and a relatively higher response in the turbulent replicates of the C, P and DL treatments. Although the differences in %HNA between the C and DL treatments lasted for the whole incubation, those in the number or proportion of CTC+ cells were mostly apparent during the first 4 days of incubation. Table III is split into two parts (Days 1–3 and 4–7) to reflect better these differences. In any case, the difference in the number of CTC+ cells was significant between the C and DL treatments for both periods, and it was also significant for the %CTC+ cells in the first period (Table III).

Bacterial activity

Bacterial production was stimulated by dust addition in DL by a factor 2.3 compared with the control (Table II). The effect of the addition of P was more pronounced where turbulence was applied during the incubation (Fig. 3B). In fact, a positive enhancement in the turbulent treatments was evident in the P and C treatments (Fig. 3). Bacterial growth rates were higher in the DL treatment than in the C treatment at Days 2 and 3, but were similar later on (Fig. 3C). Days 2 and 3 correspond with a decrease in bacterial abundances, probably indicating concomitant effects of grazing by flagellates. The addition of a high amount of dust (DH) did not promote bacterial production above the values generated by the addition of lower amounts of dust (DL). Since we added 40 nM of leucine tracer in all treatments, it is possible that incorporation in the DH treatment was actually below saturation in these samples that had, at some point, ca. 10^7 cells mL^{-1}. The relatively low growth rates measured for the DH treatments at the start of the experiment (Fig. 3D) are not compatible with the large increase in abundances measured (Fig. 2B) and support this possibility.

Community metabolism

We measured PP twice during the experiment and CR once. Dust addition increased both, PP and CR (Table IV), the DL addition by factors ranging from 1.6 to 3.6. The DH addition produced much larger increases, with factors of 6–15. In all cases, the increases in metabolism caused by the dust were stronger for PP than for CR, thus turning the communities towards net autotrophy from an already heterotrophic initial community. At the end of the experiment, there was a clear difference between the DL and the P treatments: PP was higher in the P treatment than in the DL treatment, whereas CR was similar in both treatments. The net result of the addition of DL was, at the end of the experiment, a more heterotrophic system, whereas the net result of the DH and P additions were balanced or net autotrophic communities.

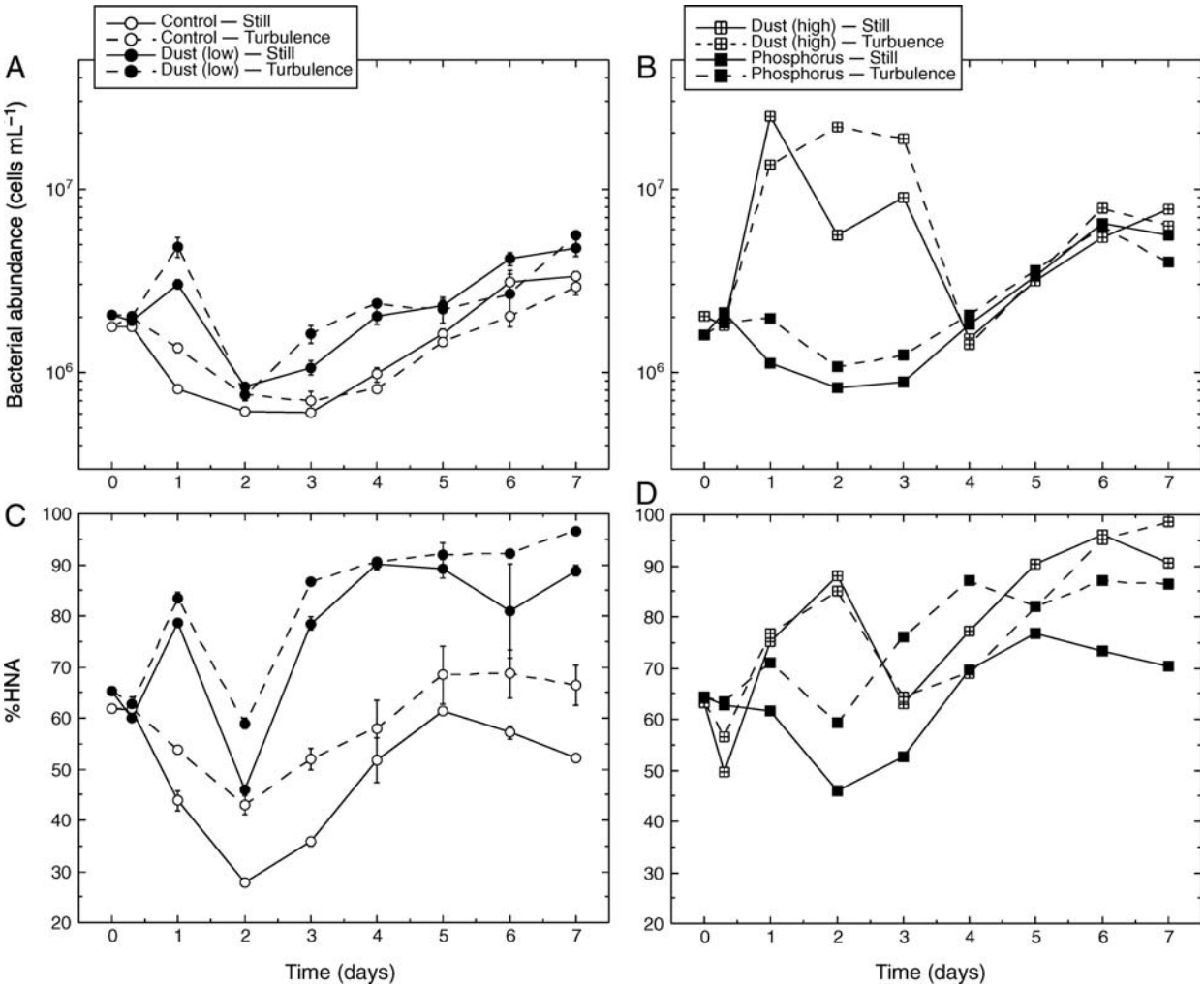

Fig. 2. (**A**) Bacterial abundance (cell mL^{-1}) in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**B**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions. (**C**) Single-cell bacterial physiological characteristics measured as the %HNA in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**D**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions.

Bacterial community structure

We used DGGE to compare fingerprints of the bacterial assemblage composition (Fig. 4). Ward's clustering method was used in order to explore the similarities between the samples based on bacterial assemblage structure, and to compare the effect of the dust addition to those of phosphorus and control treatments. The P and DL communities clustered together in the tanks and were distant from the C or DH communities (Fig. 4B). The control tanks were similar to the initial sample. There were some effects of turbulence, more obvious at the end of the experiment (Time 7) than at Time 3. We retrieved 13 bands that were dominant in different treatments (Table V). All the sequences that could be retrieved from the DGGE gel were found to have similarities in GenBank to previously described organisms, with values >97% (Table V). Just one phylotype was dominant in all the treatments (SD2), four appeared only in phosphorus treatments, three only where dust had been added and three in both P and dust treatments.

DISCUSSION

The fertilizing nature of airborne dust

The amount of dust that we added in our main experimental treatment, 0.05 g L^{-1}, was slightly higher than the doses used experimentally in a couple of recent

Table III: Average concentrations of CTC+ cells, %CTC+ (over total bacteria) for Days 1–3 and 4–7

	CTC+ abund.		%CTC+	
	Days 1–3 ($n = 3$)	Days 4–7 ($n = 4$)	Days 1–3 ($n = 3$)	Days 4–7 ($n = 4$)
CS	$0.82 \times 10^5 \pm 0.20$	$1.91\ 10^5 \pm 0.28$	10.4 ± 2.9	7.7 ± 3.0
CT	$0.94 \times 10^5 \pm 0.05$	$0.74\ 10^5 \pm 0.12$	10.9 ± 2.3	5.2 ± 0.7
DHS	$49.30 \times 10^5 \pm 14.73$	$2.46\ 10^5 \pm 0.05$	45.7 ± 22.4	6.3 ± 0.9
DHT	$51.22 \times 10^5 \pm 14.60$	$3.55\ 10^5 \pm 2.29$	29.3 ± 8.2	26.1 ± 17.1
DLS	$3.85 \times 10^5 \pm 0.81$	$3.52\ 10^5 \pm 0.85$	26.8 ± 8.5	6.9 ± 1.1
DLT	$6.80 \times 10^5 \pm 1.82$	$2.17\ 10^5 \pm 0.25$	30.6 ± 6.2	8.5 ± 2.0
PS	$0.91 \times 10^5 \pm 0.19$	$1.49\ 10^5 \pm 0.48$	9.7 ± 2.1	4.0 ± 0.4
PT	$2.24 \times 10^5 \pm 0.21$	$2.76\ 10^5 \pm 1.07$	16.2 ± 1.4	8.4 ± 2.4
Prob. diff. DL/C	<0.001	<0.001	<0.001	NS
Factor (×)	6.02	2.15	2.69	1.20
Prob. diff. DL/P				
S	NS	NS	NS	NS
T	<0.001	NS	NS	NS

Average values ± SE of all replicates and times. In the lower part, results of the Student's *t* comparisons between treatments C and LD, or between treatments P and LD. In this last case, separating still from turbulence treatments.

studies (Herut *et al.*, 2005; Pulido-Villena *et al.*, 2008), but was within the limits of what can be expected to occur in the ocean. Most Saharan rains carry between 5 and 8000 mg of dust L^{-1} (Ridame and Guieu, 2002). In the experimental tanks, we measured a release of ca. 3 μmol PO_4^{3-} g^{-1} of dust (Table I), which is lower than the range reported in Ridame and Guieu (Ridame and Guieu, 2002), between 0.97 and 3.2 μmol PO_4^{3-} L^{-1} for 0.05 g L^{-1} of dust. This is an amount of P that increased the initial P concentration by a modest factor of 1.3 (Table I) and thus, addition of a smaller dust amount would most probably not have affected the microbial communities of the coastal waters we used for the experiment. This might contrast with the experiments of Herut *et al.* (Herut *et al.*, 2005) and Pulido-Villena *et al.* (Pulido-Villena *et al.*, 2008), which used oceanic waters, initially more oligotrophic than the waters we used. It is worth remarking that while the dust we used for the additions originated in a "Sahara dust" rain event, the effects of our experimental additions might not be exactly comparable to the results obtained in experiments in which dust collected in the Sahara is added to seawater. Rain events in coastal areas bring not only Sahara dust but also other types of particles, particularly in windy events, which might be enriched in nutrients other than P. While this makes the experiments less constrained and more difficult to compare, it does make them more realistically close to what occurs in coastal marine areas.

In spite of the fact that the P addition associated with the dust was relatively modest, it favoured a slower decrease in Chl *a* and an increase in picoeukaryote development (an average factor of 2.7×) and in PP (an average factor of 1.5× at Day 3 and 2.7× at Day 7, Table IV). That dust enhances PP had been previously demonstrated in several experimental studies (Blain *et al.*, 2004; Mills *et al.*, 2004; Herut *et al.*, 2005), and the yield we observed (on average 0.5 μg Chl *a* per 0.05 g dust, i.e. 10 μg g^{-1}) is about the same yield observed by Herut *et al.* (Herut *et al.*, 2005) in the Eastern Mediterranean, but smaller than the observations of Blain *et al.* (Blain *et al.*, 2004) and Mills *et al.* (Mills *et al.*, 2004) in the North Atlantic. Enhancement of chlorophyll in the DH treatment was on the order of 10 times the enhancement generated in the DL treatment (Fig. 1), which is consistent with the 10-fold difference in the addition. Since SD contains nutrients other than P (Jickells, 1995), the hypothesis that other limiting nutrients were the responsible for the enhancement of primary producers cannot be disregarded and, in fact, the addition of P alone resulted in a smaller enhancement than the DL addition (Fig. 1), although on average the difference was not statistically significant (Table II).

Bacteria were also stimulated by the dust addition: abundance (an average factor of 1.8×), production (a factor of 2.3×) and the number of actively respiring cells (factors of 2–6×), and also the single-cell indications of cellular activity, such as the %HNA (a factor of 1.4×) and the %CTC+ cells (a factor of 2×). However, bacterial growth rates were not statistically enhanced by the DL addition. The stimulation of bacterial heterotrophic activity also resulted in a stimulation of CR, by a factor of 1.6–2.4 (Table IV). Bacterial abundance and production were, thus, enhanced by factors higher than the enhancement of Chl *a*. P enrichment in the DL treatment was of a factor of 1.3× and DOC enrichment was also of a similar factor (1.24×). However, the background DOC concentration at the

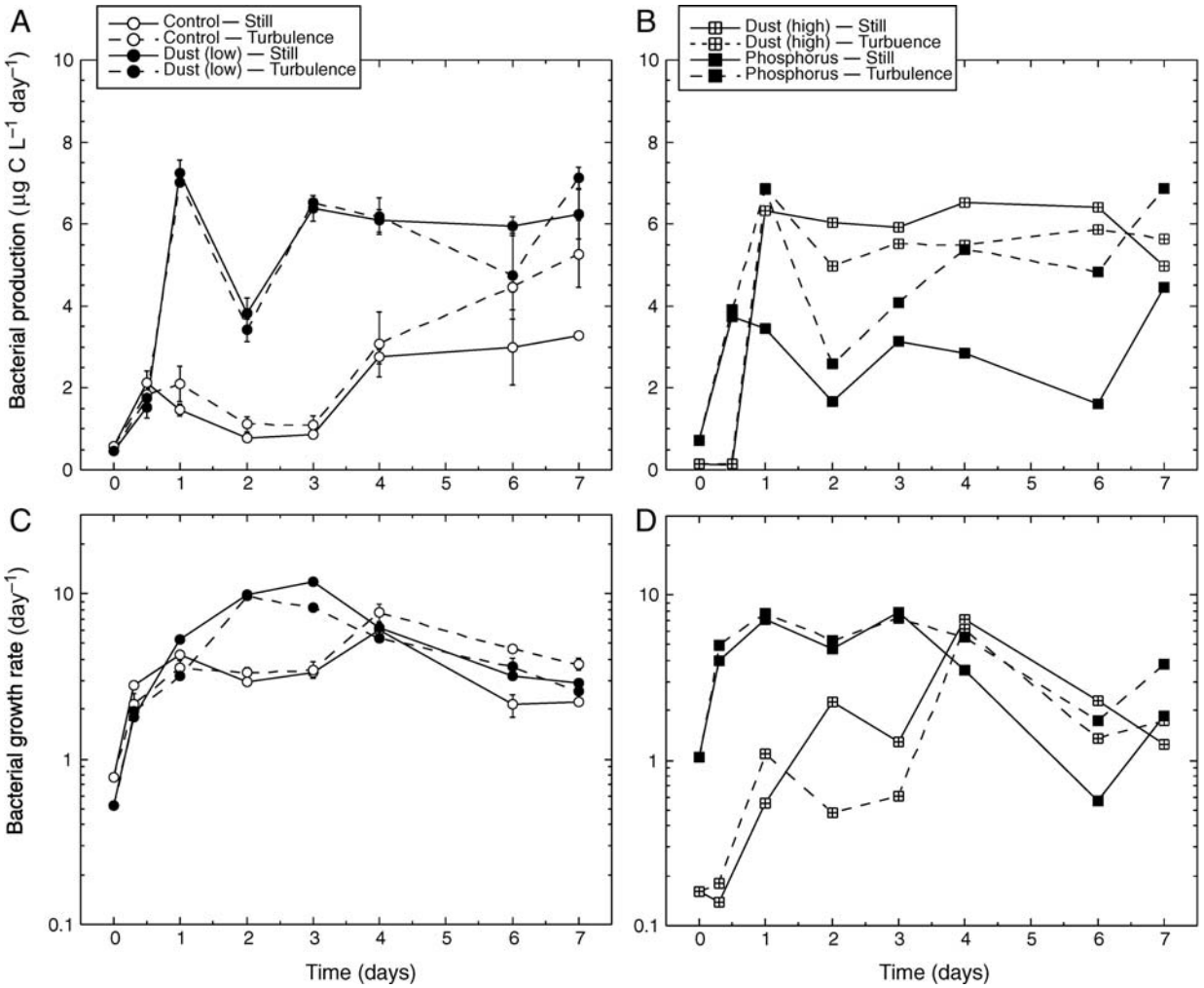

Fig. 3. (**A**) Bacterial production (μg C L^{-1}) in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**B**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions. (**C**) Bacterial-specific growth rate (day^{-1}) in the control tank (white circles) and the tanks having received dust (DL, black circles) under still and turbulent conditions, and (**D**) in the tank having received phosphorus (white squares) and the one with very high dust concentration (black squares), under still and turbulent conditions.

start of the experiment (59 μM, Table I) is typically formed by mostly non-reactive C (Hansell and Carlson, 1998). The 14 μM added with the DL treatment could represent a "de facto" larger enrichment factor, if this added C were of a degradable nature. The only approach we could use to get some information about the lability nature of the added DOC was the study of the characteristics of the FDOM. The terrestrial and marine humic-like fractions of DOC increased relatively more than the added DOC, an indication that the water became enriched in humic compounds of relatively low biolability. The 14 μM enrichment was probably not all of a usable nature.

A response of the heterotrophic community to the SD addition had already been observed by Bonnet *et al.* (Bonnet *et al.*, 2005) and Herut *et al.* (Herut *et al.*, 2005), even though they did not measure the added DOC. In these studies, as in our DL treatment, the stimulation of bacterial production was not followed by a proportional stimulation of bacterial abundance, something that they assigned to heterotrophic nanoflagellates (HNF) development cropping the stimulated production. HNF reached a peak at Day 3 in our experiment (E. Romero *et al.*, in preparation) and likely cropped most of the new production in the first days of the experiment, but not after Day 4 (Fig. 2), maybe because bacteria then developed grazing-defence strategies, or because grazers on flagellates developed in the microcosms. The burst of bacterial growth caused by the nutrient dose allowed bacteria to escape HNF control in the DH treatments

Table IV: Values of community metabolism in the different treatments

	Primary production (mg C m^{-3} day^{-1})	Community respiration (mg C m^{-3} day^{-1})	CR/PP (mg C m^{-3} day^{-1})
Initial	80.1	111.8	1.40
CS			
Day 3	37.0 ± 5.2		
Day 7	19.8 ± 8.6	27.1 ± 3.4	1.36 ± 0.7
CT			
Day 3	38.3 ± 6.4		
Day 7	16.5 ± 1.1	81.6 ± 3.4	4.93 ± 0.2
DLS			
Day 3	58.1 ± 3.6		
Day 7	36.0 ± 17.7	65.8 ± 1.7	1.82 ± 1.4
DLT			
Day 3	51.9 ± 2.5		
Day 7	59.2 ± 26.1	130.1 ± 0.9	2.19 ± 1.2
Factor DL/C			
Day 7—S	1.82	2.42	
Day 7—T	3.56	1.59	
DHS			
Day 3	251.5		
Day 7	627.1	438.5	0.70
DHT			
Day 3	402.2		
Day 7	346.3	472.8	1.37
PS			
Day 3	24.7		
Day 7	99.1	73.9	0.74
PT			
Day 3	34.0		
Day 7	142.3	86.9	0.61
Factor P/DL			
Day 7—S	2.75	1.12	
Day 7—T	2.42	0.67	

When errors are present, they correspond to the standard deviation of two replicated microcosms. Production is converted to daily units using the 16:8 h light:dark cycle.

(Fig. 2B). Viral abundance peaked on Day 2 (Weinbauer *et al.*, in preparation), when bacterial abundance was already low, and thus viral lysis could have also contributed to the losses of bacteria.

The bacterial production that we obtained per unit of dust added was similar to that measured by Herut *et al.* (Herut *et al.*, 2005): these authors obtained ca. 75 pmol leucine h^{-1} per mg dust, and we obtained 70 pmol h^{-1} per mg dust. The bacterial abundance increase we measured (2×10^9 cells per 0.05 g dust, in the DL treatment and 2×10^{10} cells per 0.5 g in the DH treatment) was consistent between treatments, but lower than the 1×10^9 cells per 2 mg measured by Pulido-Villena *et al.* (Pulido-Villena *et al.*, 2008). In the DH treatment, however, leucine incorporation rates should had been 10 times those we measured in the DL treatment and were similar, something that we believe was caused by a too low amount of leucine tracer added in relation to the enrichment we generated.

Biogeochemical effects of dust: autotrophy versus heterotrophy

Dust enhanced CR in our experiment, as Saharan dust (SD) did in the experiments and natural observations of Pulido-Villena *et al.* (Pulido-Villena *et al.* 2008). These authors measured a 2× enhancement of bacterial respiration, while we measured a factor of 1.6−2.4× in whole CR (Table IV) with a higher dust dose. Our results suggest that this enhancement had to do with the inorganic nutrient fertilization caused by the dust, but also with the DOC that was introduced with it. Depositions rich in SD contain carbon in amounts that might easily reach 1% dw (Eglinton *et al.*, 2002). This carbon originated in vegetation fires accumulated and stored in soil (Eglinton *et al.*, 2002) and is, thus, likely not to be very labile. Our results suggest some use of this carbon by the resident (see below) bacterial community. It is also possible that the DOC used by the bacteria was not present in the SD but in the particles accompanying the dust during the rain event.

We could only measure respiration at the end of the experiment (Table IV), when respiration in the S treatment was not significantly different between the P and DL treatments, but was much higher in the DL treatment under turbulence. A similar result could be observed in the number of actively respiring cells (CTC+, Table II), which were, under turbulence, much higher in the DL treatment than in the P treatment. In some studies, the number of CTC+ bacterial cells has been seen to vary coherently with bacterial respiration (Smith, 1998) and increases in the number of CTC+ cells are usually associated with bursts in growth of bacteria (del Giorgio and Gasol, 2008).

The metabolic balance of the microbial community was altered by the dust addition in a way different from the alteration caused by P addition. P shifted the community towards autotrophy (final CR/PP values ca. 0.6). In the DL treatment, the values were net heterotrophic (1.82 ± 1.4 and 2.19 ± 1.2). The control treatment stayed close to 1.36 in the S treatment but became very heterotrophic under turbulence (see later). Interestingly, the high addition of dust (DH, 0.5 g L^{-1}) shifted the community towards net autotrophy, whereas the DL treatment shifted it towards heterotrophy. In the DH treatment, a large diatom bloom developed with Chl *a* values of ca. 10 mg m^{-3}. In contrast, the 1.5× increase in Chl *a* measured in the DL treatment consisted in pico- and nanoeukaryotic cells. Apparently, at least from what can be extrapolated from our few data, a low addition of dust can shift the system to heterotrophy, but a larger input might trigger the development of diatom blooms that would turn the system net

autotrophic. The type of algae using the added P might explain this difference, as well as the possibility that some nutrient thresholds exist that shift the "winners" from picoeukaryotes to diatoms. Silica, which is known to be present in Sahara dust but with low availability (Bonnet *et al*., 2005), might be used by the autotrophic plankton when added at some concentrations. Indeed, we observed that the dust that we used contained silica, that this was released into the water, and that it was presumably used up by the microbial community in the DH treatment (E. Romero *et al*., in preparation). Some systems, such as the Eastern Mediterranean sites studied by Herut *et al*. (2005), have such an important nutrient limitation that addition of P alone does not generate a large phytoplankton growth because the systems becomes immediately N-limited. This was not likely to be the case at our site on the Western coast where there was enough N for microbial growth.

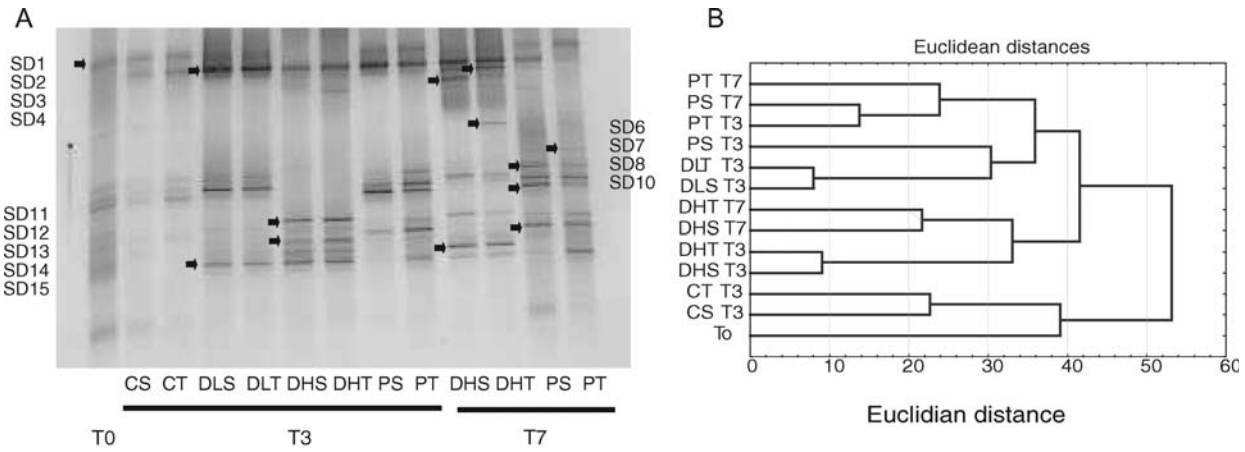

Fig. 4. (**A**) DGGE fingerprint of the bacterial 16S rRNA gene fragments from samples of different treatments and at different times of incubation. (**B**) Dendrogram classification (Ward's method, Euclidean distances according to the band pattern) of DNA samples.

Table V: Phylogenetic affiliation of 16S rRNA gene sequences from excised DGGE bands obtained in the different treatments

Band ID#	P	D	T	Access. #	Closest relative	Access. #	% similarity	Phylogenetic group
SD2	X	X	—	FJ406502	Uncultured *Tenacibaculum* sp.	AY573521	100	Bacteroidetes
					Polaribacter dokdonensis strain MED152	AY573521	98	
SD3	—	X	X	FJ406502	Uncultured *Tenacibaculum* sp.	AY573521	99	Bacteroidetes
					Tenacibaculum sp.	AB274770	98	
SD4	—	X	—	FJ406503	Uncultured *Tenacibaculum* sp.	AY573521	99	Bacteroidetes
					Polaribacter dokdonensis strain MED152	DQ481463	98	
SD6	—	X	X	FJ406504	Uncultured *Cytophaga* sp.	AJ487533	98	Bacteroidetes
SD7	X	—	X	FJ406505	Uncultured Bacteroidetes	AB266010	96	Bacteroidetes
					Lewinella nigricans	AB301615	92	
SD8	X	—	—	FJ406506	Uncultured Bacteroidetes	AB266010	99	Bacteroidetes
					Lewinella nigricans	AB301615	90	
SD10	X	—	—	FJ406507	Uncultured alphaproteobacterium	EF506911	99	Alphaproteobacteria
					Marinosulfonomonas methylotropha	AY771769	97	
SD11	X	X	—	FJ406508	Uncultured alphaproteobacterium	AY922243	97	Alphaproteobacteria
					Roseobacter gallaeciensis	AJ867255	96	
SD12	X	—	—	FJ406509	Uncultured alphaproteobacterium	DQ446171	98	Alphaproteobacteria
					Thalassobius sp.	EF587951	97	
SD13	—	X	—	FJ406510	*Roseobacter* sp.	AY258088	97	Alphaproteobacteria
					Antarctobacter heliothermus	Y11552	97	
SD14	—	X	—	FJ406511	Rhodobacteraceae bacterium	AJ810845	99	Alphaproteobacteria
					Ruegeria atlantica	AF124521	99	
SD15	X	X	—	FJ406512	*Ruegeria algocolus*	X78315	98	Alphaproteobacteria

For each phylotype, the closest relatives in GenBank are shown, together with their accession numbers and sequence similarity. We also indicate whether the phylotype was detected in the P treatments, in the D treatments, or whether the band tended to be more common in the turbulence treatments.

In this sense, it is interesting to refer to the models of the microbial food web developed by Thingstad *et al.* (Thingstad *et al.*, 2007). The models idealize competition for P between bacteria, flagellates and diatoms. Addition of DOC shifts the uptake towards the bacteria (and, thus, the system turns net heterotrophic), whereas addition of silicate above a threshold shifts it towards the diatoms (and the system would turn net autotrophic). We hypothesize that our DL treatment would be a situation of the first type, whereas the DH treatment would be of the second type. The metabolic response of the plankton community to dust additions would be concentration-dependent.

Pulido-Villena *et al.* (2008) presented evidence that on top of the fertilizing nature of atmospheric dust events that could increase PP and C drawbacks to the ocean, the stimulation of heterotrophic bacteria could actually reduce the amount of C sequestration caused by these events. These authors calculated that the dust-induced bacterial growth could mineralize up to 70% of the bioavailable DOC annually exported to the deep Mediterranean. Our results concur with those of these authors in assigning a very relevant role to bacteria in the use of the nutrients accompanying the dust pulse, but suggest that the real metabolic effects are concentration-dependent, and might depend on the bioavailability of the P, Si and organic C attached to the dust, something that is currently very poorly constrained.

Although our experiment was not designed to be a proper test of the effect of turbulence as an external factor modifying the response of the microbial community, we observed a systematic, but weak, positive effect of turbulence on Chl *a* development (Fig. 1A and B). Experimental turbulence has been seen to enhance chlorophyll development in several laboratory studies (Arin *et al.*, 2002; Pinhassi *et al.*, 2004), and in non-enriched and slightly enriched waters of Blanes Bay particularly in spring (Guadayol *et al.*, 2009). Turbulence had more effect on the sample receiving P (Fig. 1B) and less effect in the control sample, something that was also seen in Arin *et al.* (2002). While Chl *a* showed a positive enhancement by turbulence, picoeukaryote development (Fig. 1C and D, and Table II) and bacteria (Table II) were less affected. Shifts in food web structure and flagellate feeding rates caused by turbulence could explain these differences (Peters *et al.*, 1998, 2002). The most relevant effects on bacterial activity were again seen in the treatment that had received inorganic nutrients alone (P), which showed a large difference in leucine incorporation (Fig. 2) and in the number of CTC+ cells (Table III). These effects resulted in relatively similar levels of autotrophic and heterotrophic metabolism at Day 7 in the P treatment (Table IV), but in very strong differences in the control treatment, which respired more under turbulence than under still conditions (*t*-test, $P < 0.001$). The DL treatment also respired more under turbulence than under still conditions (*t*-test, $P < 0.001$), while the balance CR/PP was significantly higher for the control under turbulence than for the still control (*t*-test, $P < 0.001$). The difference in the metabolic balance was not significant between still and turbulence for the DL treatment.

It has to be taken into account that turbulence was applied only from Days 1 to 3 and, thus, the difference reported in Table IV occurred 4 days after turbulence had stopped. In accordance with our results, Alcaraz *et al.* (Alcaraz *et al.*, 2002) found a higher microbial CR and heterotrophic metabolic balance under turbulent conditions. In contrast, Petersen *et al.* (Petersen *et al.*, 1998) found no effects of turbulence on CR.

Turbulence had little effect on bacterial community structure (Fig. 4), something that is compatible with the explanation of Pinhassi *et al.* (Pinhassi *et al.*, 2004) that claimed that changes in community structure were linked to the changes in photosynthetic community structure caused by the turbulence and not to the physical stress by itself.

The potential for affecting bacterial community structure

We tested whether bacterial community structure was affected by the different treatments. In particular, we were interested to see whether the DL and P treatments were significantly different. It has been speculated that airborne bacteria can travel associated with SD particles (Griffin *et al.*, 2001; Kellogg and Griffin, 2006; Griffin, 2007; Hervàs and Casamayor, 2009; Hervàs *et al.*, 2009). If that were to be a relevant source of allochthonous bacteria that would develop in the ocean, we should see it in our analyses. Of course, if it were only a small contribution by inactive bacteria, we would not see it with DGGE, as this technique has a low resolution (picks up only <30 dominant phylotypes; Muyzer *et al.*, 1997).

The main differences observed in community structure were those between the DH treatment and the DL and P treatments (Fig. 4), indicating that the evolution with time of the bacterial community caused by the low addition of dust was very similar to that caused by P alone. Even the high addition of dust did not seem to affect the number of bands that were detected (about 12–15 per sample), in a pattern similar to which others have reported (Reche *et al.*, 2009).

Mainly alphaproteobacteria and bacteroidetes could be retrieved from the DGGE and identified. We did not

retrieve any gammaproteobacteria, but this might be related to the fact that gammaproteobacteria, particularly *Alteromonas*, tend to develop at the beginning of these type of mesocosm experiments (Schäfer *et al.*, 2001; Allers *et al.*, 2007) and tend to be substituted by alphaproteobacteria at the end of the experiments, particularly by Rhodobacteraceae of types similar to the SD11 and SD13 bands seen in our experiment.

Several of the Bacteroidetes that we retrieved were similar to *Tenacibaculum* and *Polaribacter* (Table V). This group contains the genome-sequenced *Polaribacter dokdonensis* strain M152 (very similar to band SD2) which has been seen to contain a substantial number of genes for attachment to surfaces or particles, gliding motility and polymer degradation (in agreement with the currently assumed life strategy of marine Bacteroidetes), but also the proteorhodopsin gene, which together with a remarkable suite of genes to sense and respond to light, may provide a survival advantage in the nutrient-poor sun-lit ocean surface when in search of fresh particles to colonize (González *et al.*, 2008). Organisms of this group have been retrieved in culture, in DGGE bands from enrichment experiments, but are also appearing *in situ* in Blanes Bay (I. Lekunberri *et al.*, submitted for publication), something that make them part of the most interesting marine Bacteroidetes, as they grow in culture but at times dominate *in situ*. Some *Tenacibaculum*-like organisms (SD3 and SD4) appeared in SD additions, but the sequence closer to M152 (SD2) appeared in almost all experimental treatments.

Bacteroidetes of the *Lewinella* type (bands SD7 and SD8), relatives of *Chitinophaga*, are not commonly detected in the area (I. Lekunberri *et al.*, submitted for publication). They appeared only in the P treatment. Within the alphaproteobacteria identified, bacteria of the *Ruegeria/Silicibacter* cluster appeared in the D and P treatments at the end of the experiment (SD14 and SD15). These are common organisms in Blanes Bay, easy to isolate in plates, and common in mesocosm experiments (I. Lekunberri *et al.*, submitted for publication). The other alphaproteobacteria identified (SD10 to SD13) also commonly develop in mesocosm experiments (Allers *et al.*, 2007). In that study, different types of sequences related to Rhodobacteraceae dominated the different treatments in nutrient-enriched mesocosms and most of the treatments resulted in the development of Peuk. This might be the reason for the similarity of these sequences to those of Allers *et al.* and for the difference between the DL and DH treatments (Fig. 4). As pointed out by Pinhassi *et al.* (Pinhassi *et al.*, 2004), the type of algae developing in the system determined the bacterial community structure.

Bands 3, 4, 6 and 13–15 were associated in some way with the dust addition, but most of them appeared also as faint bands in some P treatments (e.g. band SD15). Band SD2, which was very similar to band SD4, appeared in all treatments. It is, thus, unlikely that any of the bands identified can be considered as specific of the dust addition treatments, and the affiliation of the bands also do not support this specificity. Bacterial community structure in the DL treatment was likely to be mainly generated by the P pulse, and the associated development of algae. The situation was probably slightly different in the DH treatment.

CONCLUSION

A small airborne dust addition collected during a Sahara dust rain on the Mediterranean coast affected bacterial abundance, activity and diversity in our mesocosm experiments with coastal waters. It also shifted the metabolic balance of the community to heterotrophy, something that we assign to the DOC addition simultaneous to the P addition from the dust. However, a higher addition of dust caused a shift towards autotrophy. Bacterial community structure was affected similarly by dust and by P alone. The results reinforce the potential for dust inputs to affect bacterial diversity and function, but clearly indicate the need for better knowledge of the composition of the inputs, as the results encountered were, in part, dependent on the concentration of added nutrients.

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