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Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs

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Abstract

We assessed the effects of Saharan dust inputs of particulate matter (PM), total phosphorus (TP), total nitrogen, and water soluble organic carbon (WSOC) on bacterial abundance (BA) in two alpine lakes and two reservoirs in the Mediterranean region. We also experimentally assessed the effects of dust inputs on bacterial activity and community composition and explored the presence of airborne bacteria. We found synchronous BA dynamics at least in one of the study years for each corresponding pair of ecosystems, suggesting an external control. The link between BA dynamics and inputs of PM, WSOC, or TP occurred only in those ecosystems with severe P-limitation and low dissolved organic carbon. The response was most intense in the most P-limited ecosystem. Dust addition had a significant positive effect on bacterial growth and abundance, but not on richness, diversity, or composition of the indigenous bacterial assemblages. We also obtained experimental evidence that some airborne bacteria could develop in oligotrophic waters by observing the growth of gamma-proteobacteria, a group poorly represented in natural aquatic environments.

Every year, massive airborne plumes of Saharan dust are exported to both the Atlantic Ocean by the predominant westerly winds and to the Mediterranean region under particular meteorological conditions mostly occurring during spring and summer (Moulin et al. 1997). These dust inputs are a significant source of mineral nutrients (nitrogen, phosphorus, and iron) and organic carbon to both terrestrial (Okin et al. 2004) and aquatic ecosystems (Jickells et al. 2005; Morales-Baquero et al. 2006; Mladenov et al. 2008).

Dust-derived inputs of limiting nutrients can have biological effects in aquatic ecosystems. These effects have been studied mostly in seawaters with attempts to estimate the contribution of atmospheric inputs of phosphorus or iron to primary productivity (Herut et al. 2002; Ridame and Guieu 2002) or carbon cycling (Duarte et al. 2006; Pulido-Villena et al. 2008*a*). In contrast, studies in freshwater have mostly focused on chemical issues such as the effects of acidic deposition (both of nitrogen and sulphur compounds). Nitrogen deposition in the Northern hemisphere increased due to anthropogenic activity, and its effects and subsequent recovery have been intensively studied for decades (Stoddard et al. 1999; Baron et al. 2000). More recently, atmospheric inputs associated with dust deposition and global change have also been a prolific research area within freshwater systems due to the increase of atmospheric dust linked to desertification or human activity (Prospero and Lamb 2003; Neff et al. 2008). Dust deposition counteracts acidic deposition in diverse areas influenced by deserts (Xue and Schnoor 1994; Rogora et al. 2004) and, on the other hand, fertilizes inland waters. Although this fertilizing effect was first documented in the 1970s (Peters 1977), its biological responses are virtually unexplored (Morales-Baquero et al. 2006; Pulido-Villena et al. 2008*b*). In addition to the inputs of mineral and organic nutrients associated with dust events, viable bacteria are transported long distances with (so far) unknown implications for colonization of aquatic ecosystems and, consequently, for shaping biogeographical patterns of bacterial phyla (Kellogg and Griffin 2006).

Dust events have clear seasonal and climatic patterns in the Mediterranean region (Moulin et al. 1997) and are external forcings of aquatic communities (Pulido-Villena et al. 2008*b*). Synchrony (i.e., temporal coherence) of a given variable in different aquatic ecosystems within a geographical region is a suitable indication of this external control (Baines et al. 2000). Physical variables (e.g., temperature) are often synchronous, whereas chemical (e.g., nutrients) or biological (e.g., chlorophyll *a* [Chl *a*]) variables can be, to some extent, masked by other lake-specific drivers (Baines et al. 2000; Baron et al. 2000; Baron and Caine 2000). Aquatic bacteria, given their short generation time and their high susceptibility to temperature, are expected to be more responsive to external drivers than other biotic components. However, synchrony of bacterial dynamics, activity, or composition has been only recently explored (Chrzanowski and Grover 2005; Crump and Hobbie 2005; Kent et al. 2007).

We assessed whether dust-derived inputs can induce a direct response of bacterioplankton in four freshwater systems (two high mountain lakes and two reservoirs) located in the southwest Mediterranean region. We simultaneously monitored each pair of systems and atmospheric inputs of mineral and organic nutrients to

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search for significant correlations. The selected freshwater ecosystems present contrasting nutrient conditions, which allow us to explore the relative importance of lake-specific properties determining the bacterial response. Finally, we experimentally assessed the potential effects of dust inputs on bacterial activity and community structure.

Methods

Monitoring of bacteria abundance and atmospheric inputs—Alpine lakes in Sierra Nevada (Spain) show peculiar characteristics that make them particularly responsive to dust deposition. They are oligotrophic, with low concentration of dissolved organic carbon (Reche et al. 2001) and located near to the Sahara Desert. Previous research on these remote lakes has revealed a significant influence of Saharan dust inputs on lake biogeochemistry (Morales-Baquero et al. 2006; Pulido-Villena et al. 2006) and a fertilizing effect on phytoplankton and bacterioplankton (Morales-Baquero et al. 2006; Pulido-Villena et al. 2008*b*). Two contrasting alpine lakes (Caldera and Río Seco) were selected for this study (Table 1). Caldera is located on rocky terrain and has no inlets or outlets, that is, it does not receive nutrient inputs from runoff, and biota are extremely limited by phosphorus (N : P molar ratio = 196). Río Seco (N : P molar ratio = 57) is partially surrounded by meadows and has seasonal inlets that drain water from the catchment. Both lakes were sampled weekly during the ice-free period of 2001 and 2002. Mixed samples in equal proportion from 9, 7, 5, 3, and 1 m in Caldera and from 1.5 m in Río Seco were taken to determine bacterial abundance and Chl *a* concentration.

The two reservoirs also present contrasting characteristics, particularly with respect to nutrient limitation (Table 1). Quéntar is oligotrophic and extremely P-limited; it is located in the Sierra Nevada (N : P molar ratio = 381). By contrast, Béznar is eutrophic (N : P molar ratio = 79) and surrounded by agricultural land. More details on these two reservoirs can be found elsewhere (de Vicente et al. 2008). Quéntar reservoir was sampled weekly during 2004 and 2005 and Béznar reservoir was sampled exclusively during spring and summer of 2004 and 2005, corresponding to the stratification period. For both systems, samples were collected by pumping water from depths of 5, 10, and 15 m and mixing them in equal parts to produce a single representative sample for the epilimnetic zone.

Dry and wet atmospheric deposition samples were collected weekly using MTX® ARS 1010 automatic deposition samplers. Dry deposition (excluding visible insects) was collected by rinsing the bucket with 1000 mL of Milli-Q® ultrapure water, which was then used for chemical analyses. The volume of rain in the wet deposition bucket was recorded, and a 1000-mL aliquot was analyzed. If the rain volume was <1000 mL, it was brought up to that volume with Milli-Q® ultrapure water. One collector was installed in a high-altitude site in the Sierra Nevada Mountains (37°03′N, 3°23′W) at 2900 m above sea level (a.s.l.), near to the two alpine lakes and was sampled at the same day as the lakes. Data on nutrients delivered by atmospheric deposition to these alpine lakes have been

Table 1. Location, size, chemical, and biological characteristics (mean values and ranges) of the study ecosystems.

	Location	Size (km^2)	Catchment (km^2)	Total phosphorus ($\mu mol\ L^{-1}$)*,†	Total nitrogen ($\mu mol\ L^{-1}$)*,†	Dissolved organic carbon ($\mu mol\ L^{-1}$)†,‡	N : P molar ratio	Chlorophyll ($\mu g\ L^{-1}$)	Bacterial abundance (cell $mL^{-1} \times 10^6$)
Alpine lake Caldera	37°3′N, 3°19′W	0.021	0.235	0.14(0.04–0.33)	27.5(40.9–12.4)	60.1(23.4–145.7)	196	0.7(0.2–1.6)	1.08(0.49–2.32)
Alpine lake Río Seco	37°3′N, 3°20′W	0.004	0.099	0.52(0.17–2.78)	29.8(3.4–52.3)	142.6(62.1–283.5)	57	1.5(0.6–3.0)	1.87(1.29–2.77)
Reservoir Quéntar	37°12′N, 3°26′W	0.41	101.5	0.12(0.03–0.28)	40.0(12.9–94.3)	128.9(39.2–344.8)	381	2.4(0.9–6.9)	2.50(0.56–6.06)
Reservoir Béznar	36°54′N, 3°32′W	1.70	352	1.05(0.38–1.70)	82.9(27.1–305.7)	237.4(94–444.3)	79	40.4(22.6–73.6)	4.37(1.66–7.60)

* Data from Morales-Baquero et al. (2006).
† Data from O. Romero et al. (unpubl.).
‡ Data from Mladenov et al. (2008).

already published (Morales-Baquero et al. 2006; Pulido-Villena et al. 2008*b*). Two atmospheric samplers were placed near to the study reservoirs and sampled on the same day during 2004 and 2005. Atmospheric deposition samples were analyzed for particulate matter (PM), water soluble organic carbon (WSOC), total phosphorus (TP) and total nitrogen (TN). The high seasonal consistency among collectors placed at different altitudes (Morales-Baquero et al. 2006; Pulido-Villena et al. 2008*b*) or in locations separated by kilometers (O. Romera pers. comm.) highlights the relative importance of long-term dry deposition over local sources.

Detailed procedures for PM, TP, and TN analyses can be found elsewhere (Morales-Baquero et al. 2006). WSOC refers to the organic carbon from atmospheric deposition that is soluble in water and is measured as dissolved organic carbon (DOC). Samples for WSOC were analyzed by filtering collector samples through precombusted Whatman GF/F glass-fiber filters. The filtrate was collected in a combusted (>2 h at 500°C) flask, acidified with HCl (final potential hydrogen [pH] < 2) and stored at 4°C in the dark until analysis. WSOC concentration was measured by a high-temperature catalytic oxidation in a Shimadzu total organic carbon (TOC) analyzer (Model 5000 in 2001 and 2002 and Model TOC-V CSH in 2004 and 2005) equipped with a platinized-quartz catalyst for high-sensitivity analysis. Samples were purged for 20 min to eliminate remains of dissolved inorganic carbon (DIC). Three to five injections were analyzed for each sample and blank (Milli-Q water). Standardization of the instrument was done with potassium hydrogen phthalate (4-points calibration curve).

Samples for bacterial abundance (BA) were fixed in the field with neutralized 0.2-μm-filtered formaldehyde to a final concentration of 2%. BA was determined by epifluorescence microscopy using 4′6-diamidino-2-phenylindole fluorochrome stain. At least 450 cells in 30 random fields were counted per filter. Chl *a* was measured spectrophotometrically after pigment extraction with methanol.

Dust addition experiment—We performed one experiment in the laboratory by amending Quéntar reservoir water with dust deposition collected in the area from 14 June to 28 June 2005, during a Saharan dust event (Fig. 1). PM deposition during this event was 1.42 g m^{-2}. The experiment consisted of three treatments: control (no dust addition) and two dust-amended treatments (0.7-dust and 0.2-dust). Reservoir water was initially filtered through Whatman GF/F (pore size ~0.7 μm) filters for control and 0.7-dust treatments. This filtration removed grazers and phytoplankton. For the 0.2-dust treatment, GF/F-filtered water was passed through 0.2-μm polycarbonate filters to exclude bacteria. Dust was added to a final concentration of 0.22 mg L^{-1}, corresponding to the theoretical dilution of the incoming dust (1.42 g m^{-2}) into a 15-m depth epilimnion. All treatments were run in triplicate. Experimental bottles were incubated in the dark at 15°C and shaken every day. Samples for WSOC and soluble reactive phosphorus (SRP) were taken at the beginning of

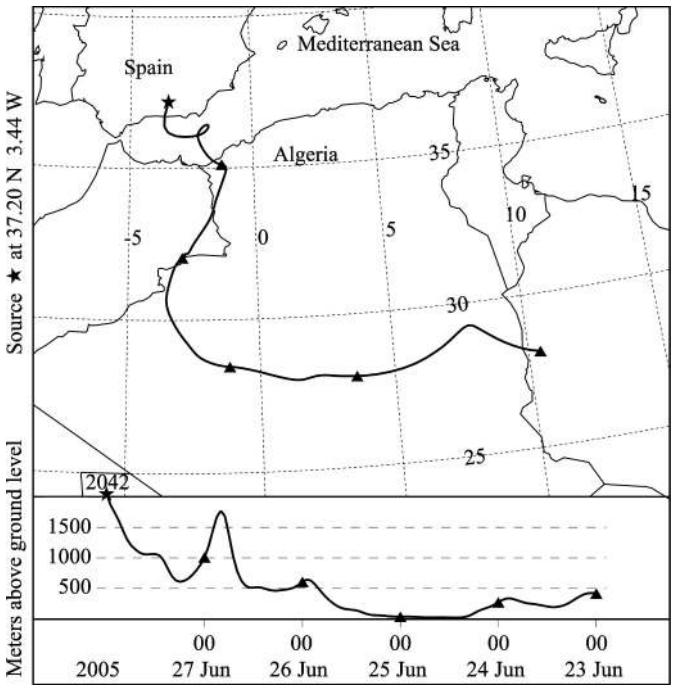

Fig. 1. Five-day air back-trajectories since 28 June 2005 arriving at Quéntar site where the atmospheric dust deposition was collected. The back-trajectory was obtained using the Hybrid Single-Particle Lagrangian Integrated Trajectories (HYSPLIT) trajectory model (Draxler and Rolph 2003; Rolph 2003) provided by the National Oceanic and. Atmospheric Administration Air Resources Laboratory (http://www.arl.noaa.gov/ready/).

incubation and samples for bacterial community composition (BCC) were taken at the beginning and at the end of incubation. Samples for bacterial abundance and production (BP) were taken sequentially (~24 h) during the incubation. WSOC and BA in the experimental bottles were analyzed using the same techniques described above. SRP was analyzed spectrophotometrically after Murphy and Riley (1962). BP was estimated from the rate of ^{3}H-leucine-protein synthesis following the microcentrifugation technique of Smith and Azam (1992). Briefly, 10 μL of L-[4,5-^{3}H] leucine (Specific Activity: 4.3 TBq mmol L^{-1}) was added to 1.5-mL samples, yielding a final concentration of 57.3 nmol L^{-1}.

To estimate changes in BCC during the experimental incubation, we performed denaturing gradient gel electrophoresis (DGGE) of the bacterial 16S rRNA gene and band-pattern analyses for the different treatments. Because ribosomal genes are represented by DGGE bands, our bacterial diversity data are not strictly species, but they are considered operational taxonomic units. We filtered the initial and final regrowth cultures through polycarbonate filters (0.2-μm pore; Nuclepore) to collect bacterial biomass. These filters were incubated with lysozyme, proteinase K and sodium dodecyl sulfate in lysis buffer (40 mmol L^{-1} ethylenediaminetetraacetic acid; 50 mol L^{-1} Tris [hydroxymethyl] aminomethane pH = 8.3; 0.75 mol L^{-1} sucrose). Deoxyribonucleic acid (DNA) was extracted with phenol-chloroform-isoamyl-alcohol (25 : 24 : 1, vol :

vol : vol) and with chloroform : isoamyl-alcohol (24 : 1, vol : vol) followed by isopropanol precipitation. Fragments of the 16S ribosomal DNA suitable for DGGE analysis were obtained by Polymerase Chain Reaction (PCR) using the universal bacterial primer set 341fGC-907r as previously described (Casamayor et al. 2002). DGGE was carried out with the D-Code system (Bio-Rad Laboratories) in a denaturing gradient 40% to 80% (100% denaturant agent is 7 mol L^{-1} urea and 40% deionized formamide) at 100 V and 60°C for 16 h. About 500 ng of PCR product was deposited in each well. After electrophoresis, the gels were stained for 30 min with SybrGold nucleic acid stain and visualized with ultraviolet (UV) irradiance. Digitized images were obtained with a UVIdoc gel documentation system (UVItec Limited) and saved as computer files. The intensity of each band was measured by integrating the area under the peak and was expressed as percentage of the total area in the lane. The number of bands and the intensity of each band were used to calculate the Shannon–Weaver diversity index (H′) in the control and 0.7-dust treatments:

$$H' = -\sum_{i=1}^{i=n} p_i \ln p_i \quad (1)$$

where n is the number of bands in each treatment and p_i the relative intensity of the ith band. The use of the number of visible bands and their relative intensity as surrogate for species richness and diversity is controversial (Lindström et al. 2007; Reche et al. 2007). However, they can be considered a viable metric for bacterial community structure as long as the definition is clear, used consistently, and the sampling effort among treatments is comparable.

DGGE bands at the same position in the different lanes of the gel were manually identified and a binary matrix (1/0) was made for all lanes, taking into account the presence or absence of individual bands. This matrix was used to build a dissimilarity matrix based on the Jaccard coefficient (Sj). Finally, a dendrogram showing the relationships among samples was obtained with the unweighted pair-group average linkage method. To identify the predominant amplified bacterial populations, prominent bands were excised from the gel, resuspended in 25 μL of MilliQ water and stored at 4°C overnight. An aliquot (2–5 μL) of supernatant was used for PCR reamplification with the original primer set, and the PCR product was purified and sequenced using external sequencing facilities (www.macrogen.com). Sequences were submitted to Basic Local Alignment Search Tool (BLASTN) search (www.ncbi.nlm.nih.gov/blast; Altschul et al. 1997) for preliminary identification. Nucleotide sequence accession numbers at European Molecular Biology Laboratory are the following: AM942752–AM942758.

Statistical analyses—Synchrony was evaluated by Pearson correlation coefficients between pairs of freshwater ecosystems (alpine lakes and reservoirs). To explore if bacterial response to atmospheric inputs was direct or phytoplankton-mediated, we performed regression analysis between BA vs. atmospheric inputs of PM, WSOC, TN, and TP and BA vs. Chl *a*, and we compared the significance of the obtained relationships.

For the experiment, the specific growth rates were obtained by fitting exponential or logistic models to the changes in bacterial abundance (μ) and in (^{3}H-leucine)-protein synthesis (b) throughout incubation. The best fits and the different parameters (μ, b, and the carrying capacity [k]) were obtained using the Nonlinear Estimations Module of the STATISTICA (version 7.0) software package. More details on this procedure can be found elsewhere (Pulido-Villena and Reche 2003).

To determine the significance of bacterial response to dust addition through incubation time, one-way repeated measures ANOVAs were performed. This analysis is useful for experiments where there may be a within-subject effect (incubation time) and a between-subjects effect (dust addition). This analysis tests three types of effects: do BA and BP change through incubation time?; are BA and BP different in control and the 0.7-dust treatment at every incubation time?; and does the difference between BA and BP in control vs. 0.7-dust treatment increase through incubation time?

Results

Monitoring of bacterial abundance and atmospheric inputs—Bacterial abundance in the alpine lakes oscillated around 1×10^6 cell mL^{-1} in Caldera and around 2×10^6 cell mL^{-1} in Río Seco (Fig. 2A; Table 1). We found significant BA synchronous dynamics in these two neighbor lakes only during the ice-free period of 2002 ($n = 11$, $r = 0.68$, $p = 0.021$). The concentration of Chl *a* in Caldera lake ranged from 0.2 μg L^{-1} to 1.6 μg L^{-1} in 2001 and from 0.6 μg L^{-1} to 1.0 μg L^{-1} in 2002. In Río Seco lake, Chl *a* varied from 0.6 μg L^{-1} to 1.6 μg L^{-1} in 2001 and from 1.3 μg L^{-1} to 3.0 μg L^{-1} in 2002. BA dynamics in Quéntar reservoir were different in the two study years (Fig. 2B). In 2004, BA was highly variable ranging from 0.5 $\times 10^6$ cell mL^{-1} to 6.1 $\times 10^6$ cell mL^{-1} with maxima during summer, whereas BA in 2005 was comparatively homogeneous with abundances that oscillated around 1.2 $\times 10^6$ cell mL^{-1}. BA in Béznar reservoir ranged from 2.5 $\times 10^6$ cell mL^{-1} to 7.6 $\times 10^6$ cell mL^{-1} in 2004, and from 2.3 $\times 10^6$ cell mL^{-1} to 8.5 $\times 10^6$ cell mL^{-1} in 2005. BA in these two neighbor reservoirs showed significant synchronous dynamics during 2004 ($n = 18$, $r = 0.58$, $p = 0.012$). Chl *a* in Quéntar reservoir ranged from 0.8 μg L^{-1} to 6.9 μg L^{-1} in 2004 and from 0.6 μg L^{-1} to 3.1 μg L^{-1} in 2005, whereas in Béznar reservoir it ranged from 15.5 μg L^{-1} to 73.6 μg L^{-1} in 2004 and from 9.2 μg L^{-1} to 100.3 μg L^{-1} in 2005.

PM and TN deposition mean values were higher at the high-altitude site than at the reservoir sites. By contrast, WSOC mean values were higher at the reservoir sites than at the high-altitude site. TP mean and range values were similar at all sites (Table 2).

We did not find significant correlations between BA and either Chl *a* or atmospheric inputs of PM, WSOC, TN, and TP in the two ecosystems with the lowest N : P ratios and the highest DOC concentrations (i.e., Río Seco and Béznar;

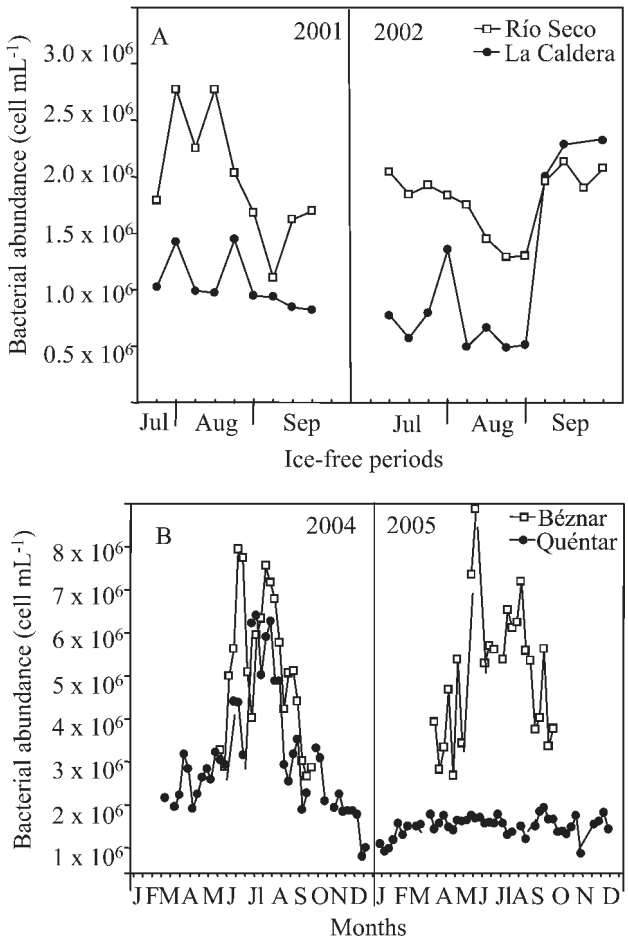

Fig. 2. Bacterial dynamics in the (A) two alpine lakes during the ice-free periods of 2001 and 2002, and (B) two reservoirs during 2004 and 2005.

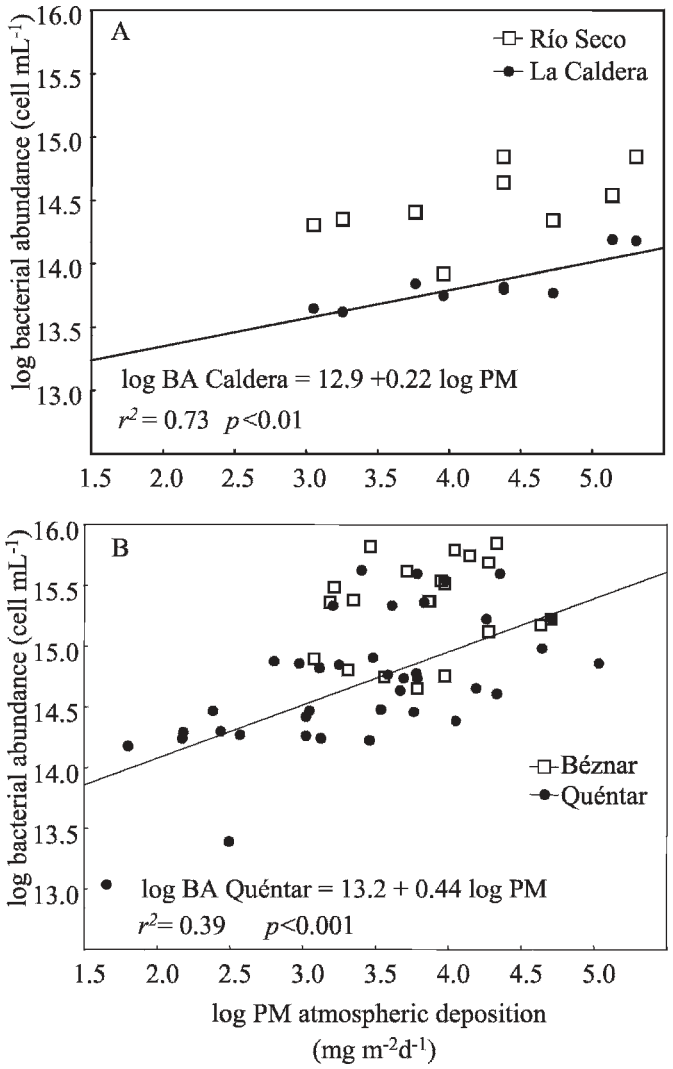

Fig. 3. Scatter plots of bacterial abundance (BA) and atmospheric deposition of particulate matter (PM) in the (A) two alpine lakes during ice-free period of 2001, and (B) the two reservoirs during 2004. Regression lines and equations are shown for the significant relationships in Caldera and in Quéntar.

Table 1). By contrast, in the alpine lake Caldera, BA was significantly and positively correlated to atmospheric inputs of PM (Fig. 3A), WSOC, TN, and TP during the ice-free period of 2001 in spite of no significant relationships of BA with Chl *a* (Table 3). The explained variance of BA was particularly high (>60%) in the relationship with atmospheric inputs of WSOC and TP. In the mountain reservoir Quéntar, BA was positively correlated to both Chl *a* and atmospheric inputs of PM (Fig. 3B), WSOC, and TP during 2004, but not with TN (Table 3). PM atmospheric deposition explained a higher variance of BA (39%) than Chl *a* (23%).

In summary, in the two ecosystems with severe P-limitation and the lowest DOC concentration (Caldera and

Table 2. Means and ranges (in brackets) of atmospheric inputs of particulate matter, water-soluble organic carbon, total nitrogen, and total phosphorus in three atmospheric deposition samplers located at a high-altitude site in Sierra Nevada Mountains and near Quéntar and Béznar reservoirs.

Atmospheric samplers	Sampling periods	Particulate matter (mg m^{-2} d^{-1})	Water-soluble organic carbon (μmol m^{-2} d^{-1})	Total nitrogen (μg m^{-2} d^{-1})	Total phosphorus (μg m^{-2} d^{-1})
High altitude	Jul–Oct 01	98.9(21.2–203.1)	279.3(75.7–1013.1)	1027(413–2114)	62.8(16.5–202.3)
(2900 m a.s.l.)	Jul–Sep 02	67.2(35.2–192.8)	269.9(36.6–868.7)	1986(445–8158)	43.9(12.9–78.2)
Quéntar	Feb–Dec 04	38.3(5.3–154.5)	263.7(6.4–798.9)	1036(26–6783)	68.2(1.9–248.8)
(1030 m a.s.l.)	Jan–Dec 05	41.5(3.6–201.4)	333.1(2.9–830)	863(75–2341)	49.1(2.9–169.3)
Béznar	May–Oct 04	52.1(21.8–111.3)	462.5(207.7–917.8)	700(110–2710)	68.0(20.8–247.8)
(485 m a.s.l.)	Mar–Oct 05	55.8(22.0–192.0)	433.7(109.6–1027.4)	728(88–2658)	98.0(11.5–412.8)

Table 3. Results of the regression analysis between Chl *a* concentration ($\mu g\ L^{-1}$) and atmospheric inputs of particulate matter ($mg\ m^{-2}\ d^{-1}$), water soluble organic carbon ($\mu mol\ m^{-2}\ d^{-1}$), total nitrogen ($\mu g\ m^{-2}\ d^{-1}$), and total phosphorus ($\mu g\ m^{-2}\ d^{-1}$) vs. bacterial abundance ($cell\ m^{-1}$) in the alpine lake Caldera and in the mountain reservoir Quéntar. Note all variables were natural-log-transformed.

Independent-variable log	Dependent-variable log bacterial abundance (BA)	*n*	*r*	*p*-value	Regression equation
Chl *a*	$Caldera_{2001}$	9	0.65	0.057	
	$Caldera_{2002}$	11	0.20	0.550	
	$Caldera_{all\ data}$	20	0.08	0.723	
	$Quéntar_{2004}$	28	0.48	0.008**	log BA=14.3+0.52 log Chl *a*
	$Quéntar_{2005}$	36	0.40	0.016*	log BA=14.2−0.40 log Chl *a*
	$Quéntar_{all\ data}$	64	0.35	0.004	log BA=13.9+0.49 log Chl *a*
Atmospheric particulate matter (PM)	$Caldera_{2001}$	9	0.85	0.004**	log BA=12.9+0.22 log PM
	$Caldera_{2002}$	10	0.58	0.075	
	$Caldera_{all\ data}$	19	0.13	0.60	
	$Quéntar_{2004}$	39	0.63	0.000***	log BA=13.2+0.44 log PM
	$Quéntar_{2005}$	42	0.29	0.065	
	$Quéntar_{all\ data}$	81	0.34	0.002**	log BA=13.5+0.23 log PM
Atmospheric water-soluble organic carbon (WSOC)	$Caldera_{2001}$	8	0.80	0.017*	log BA=13.0+0.17 log WSOC
	$Caldera_{2002}$	10	0.45	0.191	
	$Caldera_{all\ data}$	18	0.25	0.317	
	$Quéntar_{2004}$	33	0.51	0.003**	log BA=13.6+0.23 log WSOC
	$Quéntar_{2005}$	37	0.46	0.004**	log BA=13.4+0.09 log WSOC
	$Quéntar_{all\ data}$	70	0.21	0.08	
Atmospheric total nitrogen (TN)	$Caldera_{2001}$	9	0.74	0.023*	log BA=12.7+0.28 log TN
	$Caldera_{2002}$	10	0.42	0.227	
	$Caldera_{all\ data}$	19	0.26	0.276	
	$Quéntar_{2004}$	30	0.32	0.087	
	$Quéntar_{2005}$	36	0.26	0.125	
	$Quéntar_{all\ data}$	66	0.11	0.390	
Atmospheric total phosphorus (TP)	$Caldera_{2001}$	9	0.83	0.006**	log BA=13.8+0.20 log TP
	$Caldera_{2002}$	10	0.84	0.002**	log BA=13.9−0.84 log TP
	$Caldera_{all\ data}$	19	0.33	0.162	
	$Quéntar_{2004}$	33	0.47	0.006**	log BA=13.6−0.28 log TP
	$Quéntar_{2005}$	42	0.23	0.146	
	$Quéntar_{all\ data}$	75	0.36	0.001**	log BA=13.5−0.21 log TP

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Quéntar), BA appeared to be directly related to atmospheric inputs of nutrients (Fig. 3). In addition, the slope obtained for the relationship between BA and atmospheric PM inputs in Quéntar (0.44 ± 0.09), the most P-limited ecosystem, was considerably higher than the slope of the relationship in Caldera (0.22 ± 0.05).

Dust addition experiment—At the beginning of the incubations, the two dust-amended treatments (0.7-dust and 0.2-dust) had a higher concentration of organic substrate (WSOC = 56.1 ± 12.9 and 74.0 ± 1.6 $\mu mol\ L^{-1}$, respectively) and P (SRP = 0.93 ± 0.15 and 1.51 ± 0.33 $\mu g\ L^{-1}$, respectively) than the control treatment (WSOC = $36.2\ \mu mol\ L^{-1}$ and SRP = 0.34 ± 0.16 $\mu g\ L^{-1}$). Bacterial abundance growth rates (μ) were similar in the 0.7-dust treatment and in the control, but with a higher carrying capacity (k) in the 0.7-dust treatment (Fig. 4A). However, growth rate (b) was markedly higher than the control and fitted to an exponential function in the 0.7-dust treatment (Fig. 4B). We found a significant and positive effect of dust addition on both bacterial abundance and production accentuated over incubation time (Table 4). An exponential increase in both bacterial abundance (Fig. 4C) and production (Fig. 4D) in the 0.2-dust treatment was particularly accentuated after 2 d of incubation.

The 0.2-dust treatment did not produce amplification products at the initial time. We observed good agreement among replicates in the control (lanes C, D, and E) and 0.7-dust (lanes I, J, and K) treatments at the final time (Fig. 5). The replicates for 0.2-dust treatment were comparatively more variable, but well-contrasted from the other treatments at final time (lanes F, G, and H). The band richness (S) in the different lanes ranged between 22 and 12. The 0.2-dust samples showed consistently lower band richness than control and 0.7-dust treatments. No significant changes in bacterial diversity (H′) were observed between control and 0.7-dust treatments.

Analysis of banding pattern based on absence or presence clearly discriminated three clusters: (1) initial, (2) final time for control and 0.7-dust treatments with exactly the same qualitative fingerprint in both treatments, and (3) final time for the 0.2-dust treatment (Fig. 6). Therefore, dust addition did not provoke a substantial change on the composition of indigenous bacterial assemblages. In contrast, dust addition to lake water <0.2 μm (0.2-dust treatment) led to a different final assemblage likely

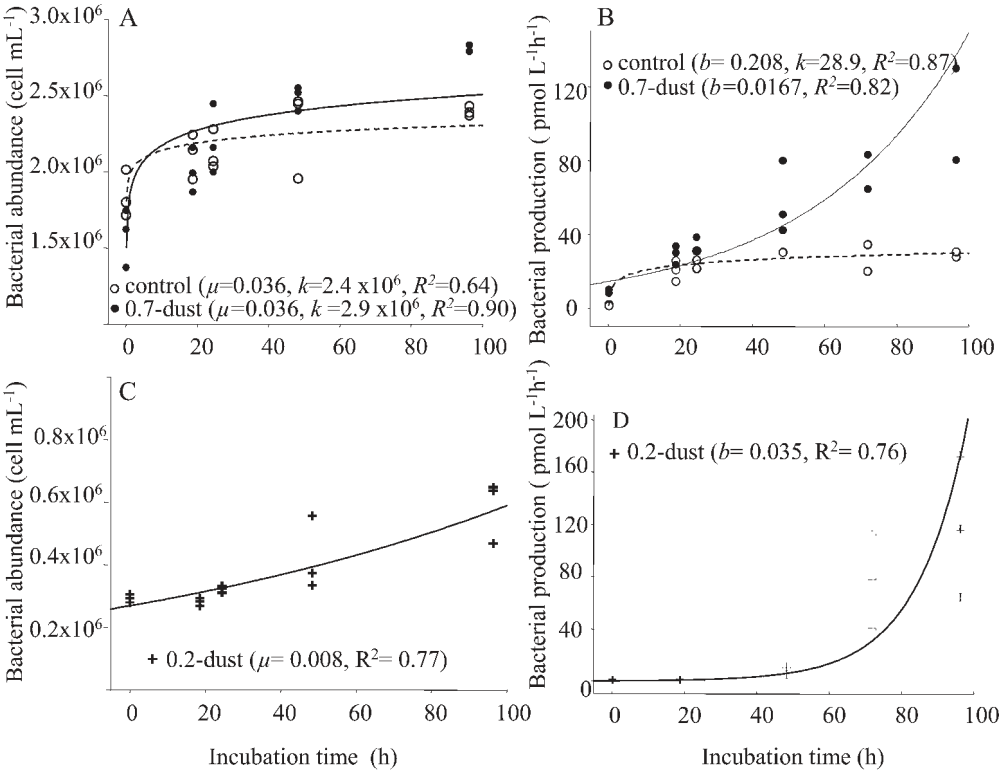

Fig. 4. Changes over time in bacterial abundance (A, C) and production (B, D) in the different treatments: control (open circle), 0.7-dust (filled circle), and 0.2-dust (cross). Exponential or logistic functions are shown (solid and dotted lines). Note different scale for 0.2-dust treatment.

associated to both viability of airborne bacteria present in the dust and growth of autochthonous bacteria present still in the 0.2-μm filtered water (*see* Discussion). Thus, some of the bacterial populations with ability to grow in 0.2 filtered water from Quéntar mixed with dust, were already present in the system (e.g., band 7), whereas some others (e.g., bands 3, 6) were under detection limits in the original community (i.e., 0.5–1% of total targeted bacteria; Casamayor et al. 2000) or not present at all.

Bands from different lanes stopping at the same position in the DGGE gel had the same 16S rRNA sequence (Table 5). Seven different phylotypes were identified belonging to alpha- beta- and gamma-proteobacteria and Bacteroidetes. For all the cases, the sequences had closely related relatives in GenBank database (similarity in partial 16S rRNA gene sequence >99%). The original bacterial community was dominated by alpha- and beta-proteobacteria and Bacteroidetes whereas in the 0.2-dust treatment Gammaproteobacteria closely related to *Acinetobacter johnsonii* predominated over the others in the final assemblage.

Table 4. Results of one-way repeated-measures ANOVA of the experiment of dust addition for bacterial abundance and production.

	Treatment	Df	*F*	*p*-value
Bacterial abundance	0.7-dust	1	6.48	0.0636
	Time	5	14.05	0.0000
	0.7-dust×time	5	3.13	0.0301
Bacterial production	0.7-dust	1	11.87	0.0749
	Time	5	18.76	0.0001
	0.7-dust×time	5	7.21	0.0042

Discussion

Field evidence of effect of dust inputs on bacterial dynamics—We found synchronous BA dynamics at least in one of the study years for each corresponding pair of ecosystems (Fig. 2). BA synchrony in freshwater ecosystems has been previously reported but with a lower correlation coefficient (0.48; Chrzanowski and Grover 2005) than those obtained in this study for the alpine lakes (0.68) and the reservoirs (0.58). All these coefficients are still notably lower than that found for BP in two temperate rivers (0.98; Crump and Hobbie 2005). Usually, BP is more dynamic than BA both across and within systems (Gasol and Duarte 2000) suggesting a higher sensitivity of production to external changes. Synchrony of biological variables has been comparatively less explored than chemical or physical variables because food-webs interactions and stochastic population dynamics can overwhelm the external signal, leading to noisier results. Atmospheric inputs as a climatic forcing generating synchrony has been previously reported for chemical variables particularly in mountain areas (Baron and Caine 2000; Morales-Baquero

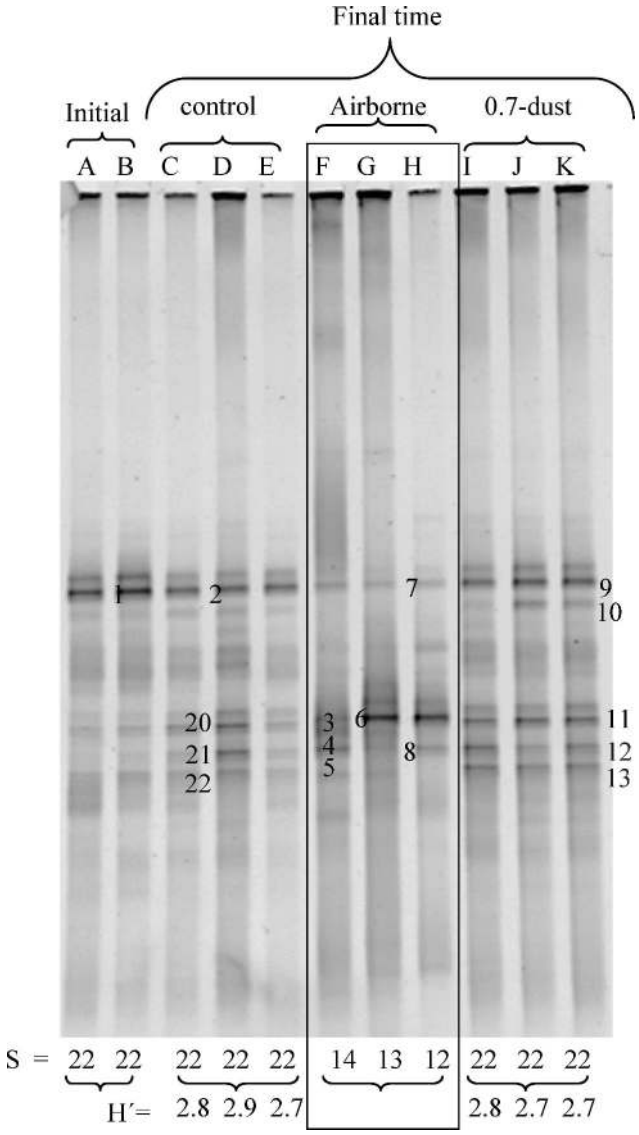

Fig. 5. Denaturing Gradient Gel Electrophoresis (DGGE) for bacterial 16S rRNA genes in the assemblages at the initial and final times in the different treatments. Labeled bands were identified after excision and sequencing (Table 5). S is band richness and H′ is the diversity index for each lane.

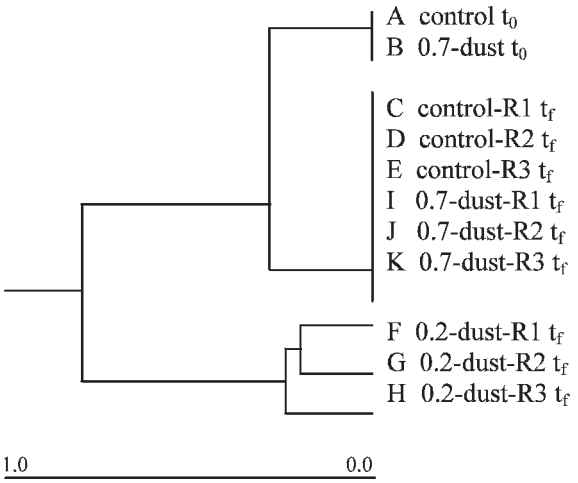

Fig. 6. Dissimilarity dendrogram for bacterial assemblage fingerprints using Jaccard values obtained from the binary data (absence or presence) of the DGGE gel in Fig. 5. A to K are the different DGGE lanes shown in Fig. 5. t_0 and t_f are initial and final times of the regrowth cultures, respectively. R1, R2, and R3 are the three replicates.

et al. 2006; Pulido-Villena et al. 2006) but, to our knowledge, this study represents the first evidence for bacterial variables.

Despite synchronous BA dynamics in the alpine lakes and reservoirs, no manifest link between BA dynamics and atmospheric inputs of nutrients were established in any of these ecosystems. Significant relationships between BA and atmospheric inputs of PM, WSOC, or TP were observed exclusively in the ecosystems with severe P-limitation and low availability of organic substrates (i.e., Caldera and Quéntar; Fig. 3; Table 3) and the response was comparatively more intense (higher slope) in Quéntar reservoir, the most P-limited ecosystem. The ecosystem specificity of the response to atmospheric inputs underlines that external forcing includes an array of climatic drivers such as temperature, solar radiation, and evaporation, not just atmospheric inputs of nutrients, which may have synergetic or counteracting influences on bacterial variables. Thus, bacterial-specific responses to atmospheric inputs of nutrients depend on the corresponding trophic status and organic carbon availability of the study system. These lake-specific responses have been also observed for chemical variables (Nydick et al. 2003; Morales-Baquero et al. 2006).

Bacterial response to atmospheric inputs of mineral and organic nutrients has been previously reported. Seitzinger and Sander (1999) and Pulido-Villena et al. (2008*a*,*b*) found a significant stimulus of bacteria to the organic nitrogen delivered by rainfall and to the SRP in dry deposition, respectively. In contrast, other studies did not find noticeable response of bacterioplankton to rainwater or water-soluble aerosol (Klein et al. 1997; Duarte et al. 2006). In Caldera lake and Quéntar reservoir, the two ecosystems where a positive response of bacterioplankton to atmospheric inputs was apparent, the relationship with the highest explained variance was obtained with PM inputs as the independent variable (Table 3). Atmospheric inputs of PM can be considered a surrogate of both P and organic C inputs as well as other mineral nutrients such as iron (Jickells et al. 2005). It is well-documented that P availability has a considerable influence on bacterial processing of organic matter, enhancing assimilation of carbon to generate new biomass increasing bacterial growth efficiency (Reche et al. 1998; Smith and Prairie 2004).

The stimulation of BA by atmospheric inputs appeared to be direct rather than being an indirect consequence of phytoplankton activity. The reliance of bacterioplankton on phytoplankton through excretion of dissolved organic matter or after decomposition of dead cells has been extensively explored and reported for decades (Gasol and Duarte 2000). In fact, Kent et al. (2007) showed synchrony

Table 5. Closest relatives after BLAST search of partial 16S rRNA gene sequences for the selected denaturing gradient gel electrophoresis (DGGE) bands shown in Fig. 5.

Phylogeny	DGGE band	Closest relative (acc no.)	Identities	Similarity	Acc no.
Alpha-proteobacteria	1,2,7,9	Uncultured bacterium (AM888002)	505/506	>99%	AM942752
	8,12,21	*Brevundimonas* sp. (EU333887)	512/513	>99%	AM942753
Beta-proteobacteria	13,22	Uncultured bacterium (EU263743)	539/542	>99%	AM942754
	4	Uncultured bacterium (EF034834)	526/527	>99%	AM942755
Gamma-proteobacteria	3,6	*Acinetobacter* sp. (EU182832)	534/534	100%	AM942756
Bacteroidetes	10	Uncultured bacterium (EF520592)	503/507	>99%	AM942757
	20,11	Uncultured bacterium (AJ697706)	518/518	100%	AM942758

at the community level among phytoplankton and bacteria. In our study, by contrast, the stronger relationship between BA and PM atmospheric inputs in comparison to the relationship between BA and Chl *a*, and even the absence of the latter relationship in the alpine lake Caldera (Table 3) suggests a direct response of bacterioplankton to mineral and/or organic nutrients delivered by atmospheric deposition.

Dust addition experiment—The experimental results corroborated the direct response of BA and BP to dust inputs (Fig. 4 and Table 4). Increasing mineral (SRP) and organic (WSOC) nutrients associated with Saharan dust increased the BA carrying capacity (*k*) and induced an exponential growth of BP (Fig. 4). The relatively more powerful response of BP in comparison to BA to atmospheric dust deposition was also reported by Herut et al. (2005), suggesting that BP may be more reactive to changes. No significant changes in band richness (S), diversity (H′; Fig. 5) or composition (Fig. 6) were observed as a consequence of dust addition. Dust addition (final concentration = 0.22 mg L^{-1}) was at the lower end of concentrations reported in the literature (from 0.2 mg L^{-1} to 9 mg L^{-1}) in similar experiments (Herut et al. 2005; Duarte et al. 2006; Pulido-Villena et al. 2008*a*,*b*). Therefore, our results obtained are very conservative; changes in the frequency (pulses) or loading (quantity) of dust additions could give different responses.

The few available studies analyzing the effects of atmospheric inputs on bacteria have typically considered the effect of nutrients on autochthonous bacterioplankton. However, the effect of airborne bacteria inputs on bacterioplankton community structure has recently started to be explored (Hervás and Casamayor 2009), despite the increasing evidence of the long-range transport of viable airborne bacteria (Kellogg et al. 2004; Hua et al. 2007). We observed (Fig. 4C,D) that some airborne bacteria could develop in the oligotrophic waters of Quéntar reservoir. There was significant bacterial growth after 48 h of incubation in the 0.2-dust treatment (coincident with a lag-time for endospores to activate) and an unexpected predominance of Gammaproteobacteria, a bacterial group poorly represented in natural aquatic environments, but readily cultured (Agogué et al. 2005). We cannot rule out a potential growth of ultramicrobacteria present in the lake that could have passed through the 0.2-μm filter (Hahn 2004), or even resistance forms of other autochthonous bacteria. Nevertheless, DGGE fingerprints showed that band richness (S) of potential airborne bacteria growing in water from Quéntar reservoir, was significantly lower (S = 12–14) than autochthonous bacteria (S = 22; Fig. 5). This fact points to at least two major constraints to cosmopolitism: (1) a different viability of long-range dispersed bacteria (Kellogg and Griffin 2006), and (2) a variable capacity to grow in lake waters. These two processes can generate historical factors leading to spatial patterns of microbial distribution (Reche et al. 2005; Martiny et al. 2006). Some of the presumed airborne bacteria dwell already in the reservoir (e.g., bands 1, 7) corroborating that long-range transport of bacteria could be a successful vehicle of dispersion. On the other hand, relatively important presumed-airborne bacteria with capacity to grow in reservoir waters (e.g., band 6) appear to have a low presence in reservoir waters (Fig. 5) suggesting a competitive exclusion of airborne bacteria in natural conditions. The comparison of the control (exclusively indigenous bacteria) and the 0.7-dust (indigenous and airborne bacteria) treatments at the final time suggests that dust deposition likely stimulated mostly the indigenous bacteria (e.g., bands 11, 12, and 13). The time required for endospores to germinate could be the critical factor determining the result of competitive exclusion. The absence of germination of some airborne bacteria could fuel the bacterial seed bank in a particular aquatic ecosystem which can contribute to ''occasional or rare'' species (Pedrós-Alió 2006). All these processes have crucial implications for bacterial community composition in natural waters.

Currently, climatic change is promoting an increase in the global content of aerosols mostly derived from arid soils (Prospero and Lamb 2003). The persistent droughts in the Sahara, Sahel, and Lake Chad basin are increasing substantially the frequency and intensity of dust events with potential consequences for aquatic microbiota that have been scantily explored. Therefore, the study of viability, and the competitive capacity of indigenous vs. airborne bacteria associated with dust aerosols will be of great interest to predict the potential changes of bacterial community composition and activity in aquatic ecosystems under future scenarios of fertilization by mineral and organic nutrients and invasions of airborne bacteria.

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