Structure, inter-annual recurrence, and global-scale connectivity of airborne microbial communities

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HIGHLIGHTS

- We analyzed the composition of microbial communities in airborne dust deposition collected in a high altitude area.
- Additional analysis of lake surface waters and Mauritanian soils indicate very different microbial composition in the three habitats.
- Communities in aerosol deposition varied in time with a strong seasonal component of interannual similarity.
- Communities immediately following dust deposition were closer to Saharan soils than those found when dust inputs receded.
- A high microbial biodiversity was found in the aerosol deposition

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ABSTRACT

Dust coming from the large deserts on Earth, such as the Sahara, can travel long distances and be dispersed over thousands of square kilometers. Remote dust deposition rates are increasing as a consequence of global change and may represent a mechanism for intercontinental microbial dispersal. Remote oligotrophic alpine lakes are particularly sensitive to dust inputs and can serve as sentinels of airborne microbial transport and the ecological consequences of accelerated intercontinental microbial migration. In this study, we applied high-throughput sequencing techniques (16S rRNA amplicon pyrosequencing) to characterize the microbial communities of atmospheric deposition collected in the Central Pyrenees (NE Spain) along three years. Additionally, bacteria from soils in Mauritania and from the air-water interface of high altitude Pyrenean lakes were also examined. Communities in aerosol deposition varied in time with a strong seasonal component of interannual similarity. Communities from the same season tended to resemble more each other than those from different seasons. Samples from disparate dates, in turn, slightly tended to have more dissimilar microbial assemblages (i.e., temporal distance decay), overall suggesting that atmospheric deposition may influence sink habitats in a temporally predictable manner. The three habitats examined (soil, deposition, and air-water interface) harbored distinct microbial communities, although airborne samples collected in the Pyrenees during Saharan dust outbreaks were closer to Mauritian soil samples than those collected during no Saharan dust episodes. The three habitats shared c.a. 1.4% of the total number of microbial sequences in the dataset. Such successful immigrants were spread in different bacterial classes. Overall, this study suggests that local and regional features may generate global trends in the dynamics and distribution of airborne microbial assemblages, and that the diversity of viable cells in the high atmosphere is likely higher than previously expected.

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1. Introduction

The Saharan desert in Africa is the largest source of aerosolized soil dust on Earth (50 to 75% of the global dust production), contributing as much as one billion metric tons of dust per year to the atmosphere (Kellogg and Griffin, 2006). The global atmospheric mobilization of dust has likely increased in recent decades due to persistent drought in the Sahara-Sahel region over the past 40 years, the onset of commercial agriculture, and an increase in land-use practices (such as livestock grazing) that have desiccated large aquatic regions such as the Chad lake, decreasing vegetation cover and increasing the frequency and intensity of dust storms (Mulitza et al., 2010; Hulme, 2001). Dust storms can transport microscopic particles thousands of kilometers away from the source, a phenomenon that was noted more than 160 years ago by Charles Darwin (Darwin, 1846). Airborne dust may particularly

mobilize microscopic organisms (Bovallius et al., 1978; Prospero et al., 2005; Yamaguchi et al., 2012). It is well known that bacteria and fungi are commonly found associated with dust (even at altitudes of 20,000 m; Griffin, 2004) and many of these microbes can survive prolonged transport in the atmosphere and may even be metabolically active while aloft (Sattler et al., 2001; Amato et al., 2005). The topic of microbial dispersal via dust events has generated general interest due to concerns about health effects of allergens and the possible long-distance transport of pathogens (Kellogg and Griffin, 2006; Hervàs et al., 2009). In addition, ecologists are interested in understanding the role of these transoceanic and transcontinental dust events in injecting large pulses of viable microorganisms into the atmosphere, thereby expanding the geographical range of some organisms and possibly altering microbial community composition in sink environments by facilitating long-distance dispersal events.

Microorganisms enter the atmosphere as aerosol particles, can remain in the atmosphere for many days and can be transported by wind over long distances before being washed out by precipitation (rain and snow) or dry deposition (Burrows et al., 2009a). The general assumption of the atmosphere as a mere conduit for the dispersal of microorganisms rather than a dynamic habitat itself has been recently questioned (Womack et al., 2010). Although the impact of microbial atmospheric deposition on sink environments is a poorly studied issue of unpredicted consequences, very few efforts have been addressed to estimate the composition of extraneous microorganisms, their temporal dynamics, and the significance of immigration for community assembly (Hervàs et al., 2009; Jones et al., 2008). To gain knowledge on the ecology of these processes field studies should ideally combine in addition to state-of-the-art molecular methods (i) long-term temporal tracking of atmospheric depositions, (ii) careful selection of pristine and distant sink environments connected to the source, and (iii) these environments should experience frequent and intense atmospheric deposition episodes.

In this study, we applied high-throughput sequencing techniques (16S rRNA amplicon pyrosequencing) to characterize the microbial communities of the atmospheric deposition on the Central Pyrenees (NE Spain) along a period of three years. In this area, higher frequency of African dust outbreaks is observed during late spring and summer. High mountain lakes are remote and often considered pristine ecosystems largely unaffected by local anthropogenic factors due to their inaccessibility. For this reason, it has been proposed that high mountain lakes can serve as sentinels of global change (Catalan et al., 2006). The first natural collector/interceptor of these atmospheric depositions is the neuston, the biological community inhabiting the hydrophobic surface microlayer located within the first millimeter of the air-water interface (Hervàs and Casamayor, 2009). Thus, ideally the neuston should capture the remote effects that long-distance dust deposition may have on the composition of microbial communities. Our objectives were (i) to describe the microbial structure and dynamics of atmospheric deposition in the long-term, (ii) to assess the temporal pattern structuring the deposition-associated microbial communities along a inter-annual survey, and (iii) to explore the potential biological connectivity lithosphere (bacteria of soils in Mauritania)-atmosphere (airborne bacteria)-hydrosphere (neuston of high altitude lakes) by global-scale transport of microorganisms.

2. Materials and methods

2.1. Study site and sampling

The atmospheric deposition was obtained from an automatic dry/wet passive collector MTX ARS 1010 equipped with two 667 cm2 area polyethylene containers and a hygroscopic sensor cell, placed at c.a. 1,800 m altitude on a rocky landscape within the protected area of Aigüestortes National Park (Pyrenees, NE Spain). Samples were collected approximately twice per month during three years (from 15th May

2007 to 26th May 2010; Table 1 and Fig. 1). Particles deposited in the wet container (i.e. those washed from the atmosphere by rain or snow precipitation) were collected onto precombusted (450 °C, 4 hours) Whatman GF/F filters and then, were dried in a laboratory heater for 4 hours and kept in the dark (Hervàs et al., 2009). The wet container remained covered and isolated from the atmosphere but during the rain/snow precipitation intervals when the hygroscopic sensor remained activated. Thus, we were mostly targeting viable airborne microbes with the potential to develop in highly diluted waters (e.g., rain, oligotrophic lakes) and, therefore, the initial relative proportions of the different airborne populations may have been changed. The timing of Saharan dust events was determined by TOMS (Total Ozone Mapping Spectrometer) which provides a measure of the atmospheric loading of UV-absorbing aerosols (i.e., mineral dust and soot from anthropogenic and natural combustion sources; Herman et al., 1997). Saharan dust intrusion data were obtained from www.calima.ws. The temporal trend of Saharan dust intrusion to the Pyrenees region (NE Spain) showed the highest frequency during late spring and summer (from May to September) for the period 2004-2012 (Fig. 1).

Different samples from Mauritanian sandy soils located within the Sahel region, 40 km SE of Boûmdeid, from 4 different locations in the Karakoro river basin (c.a. 3,000 km distant from the Pyrenees), were pooled together for each location to characterize the composition and heterogeneity of soil samples in the area. This area is subjected to frequent dust storms and is potential source of dust plumes (Kellogg and Griffin, 2006). The soil was treated to obtain soil particles of size < 0.63 µm as reported in Hervàs et al. (2009).

Finally, the lakes sampled belong to the Limnological Observatory of the Pyrenees (LOOP; Spanish Pyrenees; 42°33 N, 00°53 E) within the protected area of the Aigüestortes National Park (Table 2). The air-water surface microlayer was collected from the upper c.a. 400 µm of the surface film with a nylon screen sampler as previously reported (Auguet and Casamayor, 2008). The lakes were sampled during 14-24 July 2008 within a 10-days interval, to minimize temporal variability, and covered a Saharan dust intrusion episode of several days. Water samples were pre-filtered in situ through a 40-µm pore-size net to retain large zooplankton and algae, and 300-500 mL were subsequently filtered on 0.2 µm pore-size polycarbonate filters. The filters were stored in lysis buffer (40 mM EDTA, 50 mM Tris pH 8.3, 0.75 M sucrose) and enzymatically digested as reported in Ferrera et al., 2004.

2.2. Molecular methods and sequence processing

DNA was extracted using the Mobio PowerSoil DNA Isolation Kit (Mobio Laboratories). Preparation of extracted DNA for pyrosequencing followed the protocol described in detail in Fierer et al., 2008a, 2008b. In brief, the variable V4 and V5 regions of the 16S rRNA gene (c.a. 250 nucleotides) were amplified with the primers F515 (5 -GTGCCAGC MGCCGCGGTAA-3) and R806 (5 -GGACTACVSGGGTATCTAAT-3). The 515 F primer included the Roche 454-B pyrosequencing adapter and a GT linker, while 806R included the Roche 454-A sequencing adapter, a 12-bp barcode (unique to each sample), and a GG linker. The region amplified by this primer set is well suited for accurate phylogenetic placement of bacterial sequences (Liu et al., 2007) and should amplify nearly all bacteria and archaea with a few biases against particular groups (Bates et al., 2011). The resulting barcoded PCR product was normalized

Table 1
Summary of the atmospheric deposition samples analyzed in this study. Mean and standard deviation are indicated for the number of sequences and number of OTUs at 97% identity.

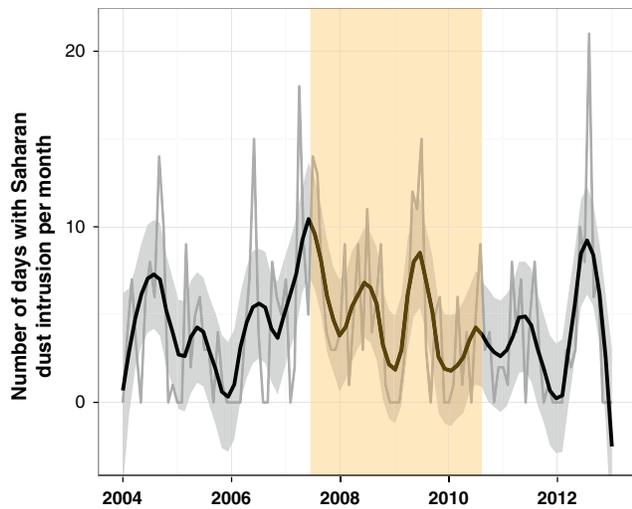| Year	Number of samples	Number of sequences	Number of OTUs (97%)
2007	10	4,080 ± 1,435	328 ± 126
2008	14	3,256 ± 1,281	371 ± 209
2009	15	4,194 ± 1,103	334 ± 135
2010	10	3,324 ± 680	361 ± 206

Fig. 1. Number of days with Saharan dust intrusion per month in Northeast Spain from 2004 to 2012. Temporal trend is shown by a local polynomial regression (loess; in black) with shaded 95% confidence interval. Sampling period (from May 2007 to May 2010) is indicated in yellow.

in equimolar amounts and sequenced on a Roche GS-FLX 454 automated pyrosequencer at the Environmental Genomics Core Facility (Engencore) at the University of South Carolina.

Raw 16S rRNA gene sequence data generated from pyrosequencing were processed in QIIME (Caporaso et al., 2010). Briefly, sequences were quality trimmed (>200 bp in length, quality score >25, exact match to barcode and primer, and containing no ambiguous characters) and clustered into operational taxonomic units (OTUs) with uclust (Edgar, 2010) using both a 97% identity threshold (standard 'species-level' OTU cutoff; Konstantinidis and Tiedje, 2007). We obtained a total of approximately 260,000-curated sequences from the 68 samples (4 soil, 15 air-water interface, and 49 atmospheric deposition samples) with each sample having more than 1,000 sequences. At the 97% identity level, the final OTU table contained c.a. 10,500 distinct OTUs. Taxonomic assignment was carried out with the RDP Bayesian Classifier (Wang et al., 2007), and manually curated by BLAST searches against the GenBank non-redundant nucleotide database (nt).

2.3. Statistical analyses

Community similarity was represented by non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity distance metric after Hellinger standardization (Legendre and Gallagher, 2001). Analysis

of similarities (ANOSIM; Clarke, 1993) and permutational multivariate ANOVA (PERMANOVA; McArdle and Anderson, 2001), both based on 1,000 permutations, were used to test for significant differences between habitat categories. We used standard and partial Mantel tests to determine the correlation between community similarity (Bray-Curtis dissimilarity distance) and time. All statistical analyses were carried out in the R environment (www.r-project.org) using the vegan package (Oksanen et al., 2007).

3. Results

We initially explored changes in airborne microbial community structure in time analyzing the 49 atmospheric deposition samples collected from May 2007 to May 2010. Interestingly, we observed a consistent trend in the temporal decay pattern of the microbial community similarity (Fig. 2, panel A). Overall, deposition samples from disparate dates tended to have more dissimilar communities than samples closer temporally (Fig. 2A linear trend in orange; Mantel test $r_M = 0.21$, p-value < 0.001). However, this general trend was affected by a strong seasonal pattern (i.e. deposition samples from the same season tend to resemble more each other without regard of the year; Fig. 2A local polynomial trend in green). The possible effect of Saharan dust outbreaks on shaping microbial atmospheric deposition communities was tested after relating the values of community similarity and the difference in days between the last Saharan intrusion episode and the day the sample was collected from the container. The smaller the difference the closer the similarity among evolved airborne communities may be expected. However, we did not observe any clear pattern in the dataset (Fig. 2B, Mantel test $r_M = 0.09$, p-value = 0.085) suggesting a quite heterogeneous microbial composition for the three-years dataset of Saharan dust episodes. None of these relationships changed after controlling for possible inter-matrix correlations by partial Mantel tests ($r_M = 0.21$, p-value < 0.001; $r_M = 0.10$, p-value = 0.055, respectively).

Next, we additionally explored microbial community composition in two other habitats potentially connected by atmospheric transport: the source of Saharan dust plumes (i.e., soil samples from the Saharan desert), and the aquatic environment where most probably the atmospheric load is first deposited on NE Spain (i.e., the air-water interface of ultraoligotrophic high mountain lakes in the Pyrenees). The three habitats examined (i.e., soil, deposition, and air-water interface) harbored distinct microbial communities (ANOSIM R = 0.93, p-value < 0.001; PERMANOVA $R^2 = 0.18$, p-value < 0.001) and were clearly separated in an ordination analysis (Fig. 3, panel A). Microbial richness tended to be higher in those deposition samples that were closer in ordination space to Saharan desert soils (Fig. 3A). The soil samples were more diverse (Fig. 3B) with up to five predominant taxa with relative abundances >10% (*Alpha*- and *Betaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes*) whereas only two

Table 2
Geographic coordinates, altitude, morphological characteristics, and number of sequences and OTUs at 97% identity for the Pyrenean lakes studied.

| Lake	Latitude (°)	Longitude (°)	Altitude (m)	Area (ha)	Depth (m)	Number of sequences	Number of OTUs (97%)
Pica Palomera	42.79	0.87	2,308	4.9	10	6,238	407
Certascan	42.70	1.30	2,335	56.9	130	3,579	318
Aixeus	42.61	1.37	2,370	3.4	15.5	4,662	390
Pois	42.65	0.71	2,055	4.4	13	5,056	452
Redon	42.64	0.78	2,240	24	73	2,380	373
Llong Lliat	42.81	0.87	2,140	27.1	32	4,348	536
Granotes	42.57	0.97	2,330	0.7	5	5,247	299
Llebreta	42.55	0.89	1,620	8	11.5	3,135	573
Roi	42.57	0.80	2,310	3.5	10	2,864	390
Bassa Oles	42.72	0.77	1,600	1.3	1	4,818	636
Romedo	42.70	1.32	2,110	11.9	40	4,325	424
Podo	42.60	0.93	2,450	4.6	20	2,577	395
Gerber	42.63	0.99	2,170	14.9	63	4,225	545
Filià	42.45	0.95	2,140	1.4	5.5	5,071	429
Plan	42.62	0.93	2,188	5	11	4,337	520
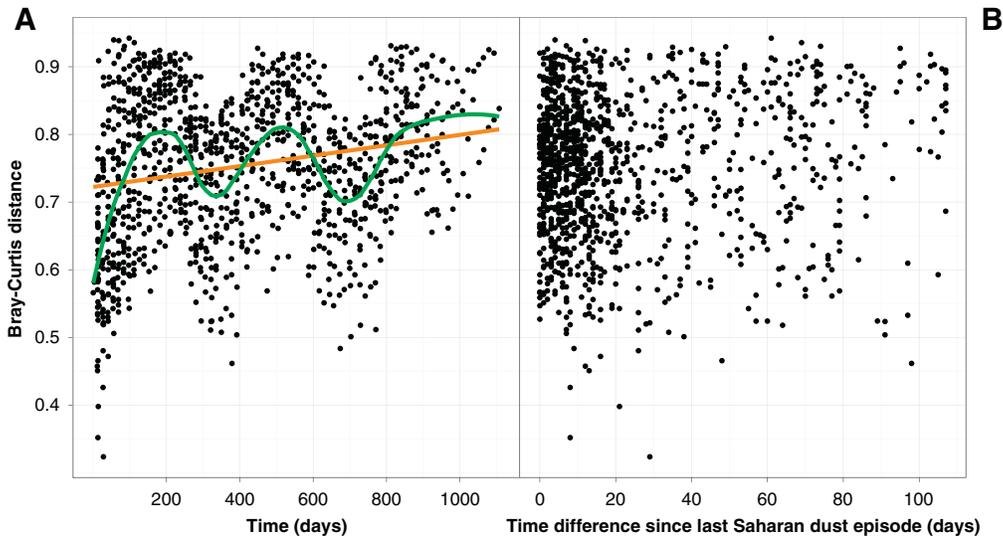
Fig. 2. Microbial community dissimilarity distance of atmospheric deposition against time (A, significant trend, p < 0.001) and the difference in days between the last Saharan intrusion episode and the day the sample was collected from the container (B, no significant trend). Inter-annual trend is shown by a fitted linear regression (in orange) and seasonal trend by a local polynomial regression (loess; in green).
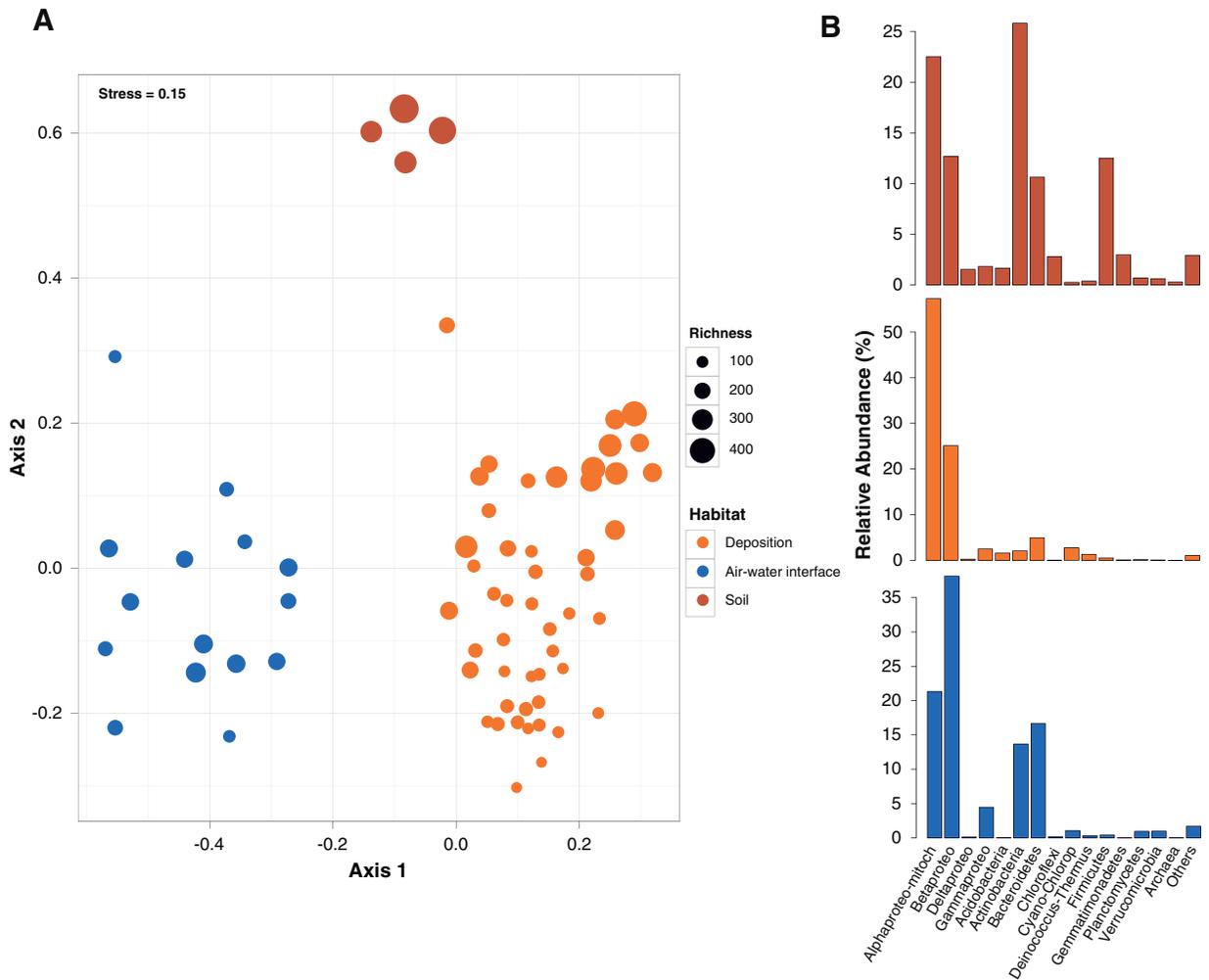

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot of the three different habitats sampled (i.e. desert soil, atmospheric deposition and lake neuston) for the 97% identity OTU table (A). Size corresponds to richness (number of OTUs) calculated after 1,000 randomizations to correct for different sample size. Relative abundance of major phylogenetic groups in the different habitats (B).

(*Alpha-* and *Betaproteobacteria*) or four (*Alpha-* and *Betaproteobacteria*, *Actinobacteria*, and *Bacteroidetes*) taxa were found as predominant groups in atmospheric deposition and lake neuston communities, respectively. *Archaea* were minor components in all the cases.

To further explore the intercontinental connection soil-atmosphere-lake water, we analyzed the OTU overlap among habitats (Fig. 4). Overall, atmospheric deposition samples shared 766 OTUs with soil and 732 OTUs with lake neuston, whereas Neuston and soil samples only shared 309 OTUs. The three habitats potentially connected by atmospheric transport shared a total amount of 138 OTUs (c.a. 1.4% of the total number of OTUs). Barplots in Fig. 4B show the taxonomic composition of the different compartments: OTUs unique to atmospheric deposition samples, OTUs found in desert soils and deposition samples, OTUs shared by deposition and lake neuston samples, and OTUs found in all three habitats. Overall, the distribution of different microbial groups was very similar in all compartments. Interestingly, potential soil-atmosphere migrants presented a higher proportion of OTUs from *Alphaproteobacteria-Mitochondria* and *Bacteroidetes*, and lower proportion of *Actinobacteria*, while *Bacteroidetes* were less prevalent in potential deposition-neuston colonizers. OTUs shared by the three habitats (potential successful migrants and colonizers) showed lower percentage of *Betaproteobacteria* and *Acidobacteria*, and higher percentage of *Firmicutes* and *Cyanobacteria-Chloroplast* than OTUs found only in atmospheric deposition samples. *Firmicutes* OTUs were more prevalent in shared habitats (i.e., potential migrants, colonizers or both) than in OTUs unique to atmospheric deposition (Fig. 4B). A more informative depiction of the OTUs shared by the three different habitats with the relative abundance and taxonomic hierarchy is shown in Fig. 5. These OTUs would potentially represent successful migrants and colonizers.

Along the survey period of the airborne microbes, different air masses and dust of local and remote origin can be mixed in the signal explored. To check for microbial community differences in local vs. remote deposition effect, we split the airborne samples in two contrasted groups (Fig. 6): those samples collected less than 2 days after a reported Saharan intrusion event (maximal influence of remote sources expected) and those coming from periods longer than 40 days without Saharan dust outbreaks (minimal or no African influence). Interestingly, we noticed significant differences between the groups (ANOSIM R = 0.46, p-value < 0.001; PERMANOVA R^2 = 0.16, p-value = 0.003), with

microbial communities present in < 2 days samples tending to be more similar to soil samples than > 40 days samples (*t*-test p-value = 0.002), suggesting that we captured the African signal in our dataset. No significant differences were observed in regard to lake neuston samples (*t*-test p-value = 0.077).

4. Discussion

A few studies have addressed the structure of airborne bacterial communities by culture-independent techniques (e.g., Hervàs et al., 2009; Jones et al., 2008). It has been proposed that microbial colonization via atmospheric deposition may only represent stochastic noise independent of robust internal dynamics of sink aquatic environments because it has been estimated that deposition over a day only represents 0.0001-0.1% of the bacterial planktonic pool in a lake, and other processes such as particle sinking flux and grazing rates remove equal or greater numbers of cells than estimated to be deposited (Jones et al., 2008). Such estimations are likely in the low range since the study by Jones et al. was carried out in northern Wisconsin, an area far away from any kind of (Saharan or Asian) desert dust influence and with lakes with bigger volume than those for the remote, small alpine lakes of this study. However, in both cases the influence on the total sink diversity pool (i.e. the rare biosphere; Pedrós-Alió, 2006) may be paramount due to the extraneous metabolic potential, introducing new functional traits and expanding genetic potential, especially in pristine environments such as high-altitude lakes.

In our dataset, we detected a clear temporal signal of microbial communities from atmospheric deposition reaching the Pyrenees region. Communities from the same season tended to resemble more each other than communities from different seasons, and samples from disparate dates slightly tended to have more dissimilar microbial assemblages (i.e., temporal distance decay). The observed temporal pattern suggests that although the magnitude of extraneous deposition may not be relevant for sink environments in terms of magnitude, atmospheric deposition may influence sink habitats in a temporally predictable manner. Inter-annual recurrence in microbial communities has also been observed in other environments (e.g. Fuhrman et al., 2006; Galand et al., 2010) and apparently it would be the case here, reinforcing the idea that the atmosphere has environmental characteristics
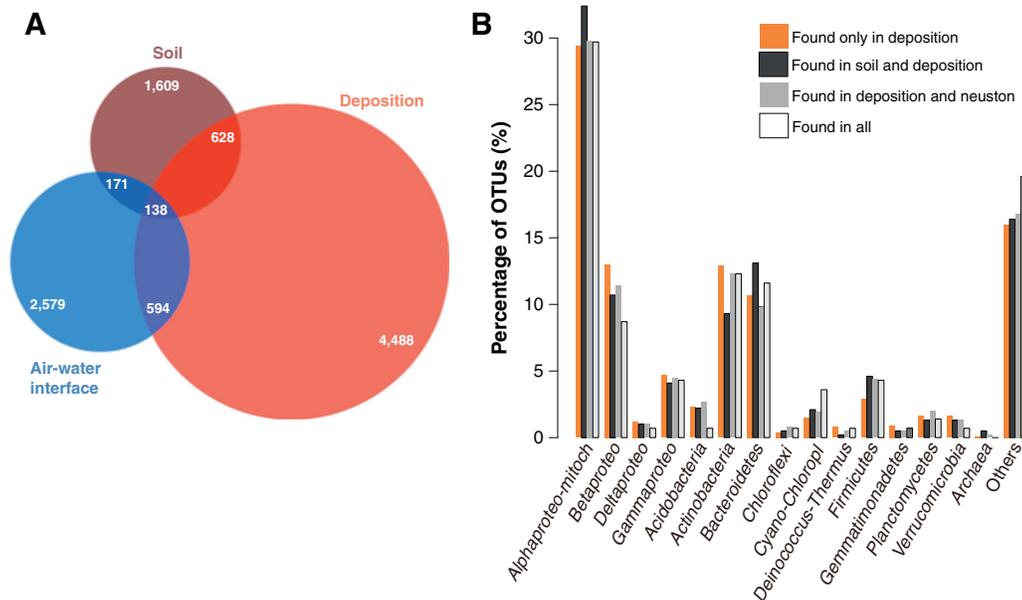

Fig. 4. Venn diagram showing 97% identity OTUs overlap among habitats (A). Taxonomic composition between the different compartments: (i) only found in atmospheric deposition samples, (ii) potential soil-atmosphere migrants (shared by soil and deposition habitats), (iii) potential deposition-neuston colonizers (shared by deposition and lake habitats), and (iv) potential successful migrants and colonizers (shared by the 3 different habitats, see more details in Fig. 5) (B).
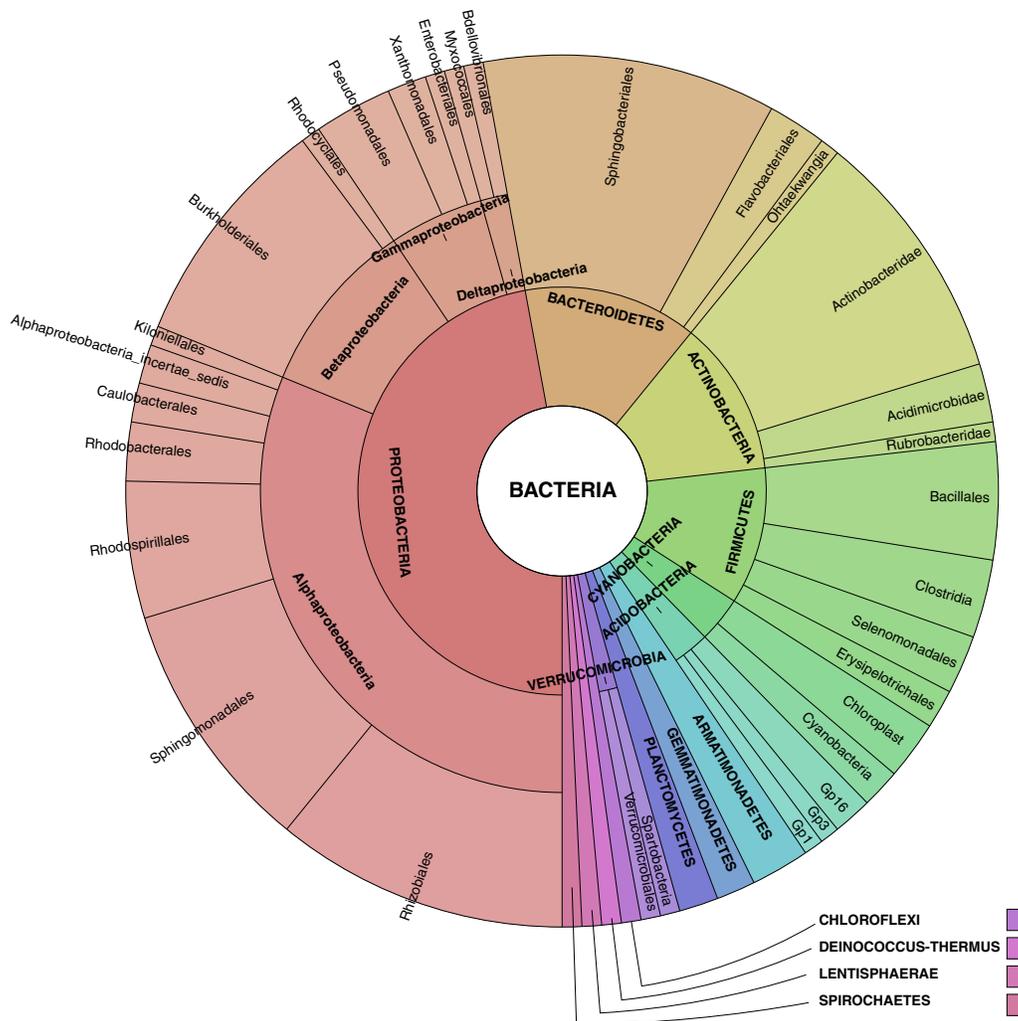
Fig. 5. Abundance and taxonomic hierarchy of potential successful migrants and colonizers OTUs (shared by the 3 different habitats; see Fig. 4) displayed using Krona radial space-filling (Ondov et al., 2011). Taxonomy nodes are shown as nested sectors arranged from the top level of the hierarchy at the center and progressing outward.

consistent with other habitats where biogeochemical cycling occurs (Womack et al., 2010).

Although the microbial presence in atmospheric aerosols (particularly dust) has been recognized since the 19th century (Darwin, 1846), it has not attracted much multidisciplinary research effort till the last decade specially due to methodological limitations for describing microbial community composition and estimating the magnitude of the atmospheric loads, and because of its recently recognized links with climate change. Generation of atmospheric aerosols and remote dust deposition is a global process, enhanced by perturbations linked to the global change (Moulin and Chiapello, 2006; Prospero and Lamb, 2003; Mulitza et al., 2010). Airborne dust may act both as fertilizer agent and as carrier vehicle for airborne microorganisms. It has been largely demonstrated that nutrient inputs from dust depositions increase bacterial abundance and production in the upper layers of nutrient-limited waters (Herut et al., 2002; Lekunberri et al., 2010; Reche et al., 2009; Morales-Baquero et al., 2006; Mladenov et al., 2011). Furthermore, it is now recognized that dust plumes carry a large set of viable airborne microorganisms with the potential to colonize new environments if conditions turn favorable (Herut et al., 2002; Hervàs et al., 2009). Despite the fact that intercontinental atmospheric dust transport probably may play a key role for global microbial dispersal, still little effort has been made to characterize potential routes of immigration for microorganisms.

The atmosphere is not just an intercontinental microbial corridor. Dust particles may provide shadow protection, resources, and a way to capture atmospheric humidity, helping microorganisms to survive the strong desiccation and UV doses during this long journey (Tong and Lighthart, 1998; Yamaguchi et al., 2012). Our results suggest that the atmospheric deposition can be considered a truly microbial habitat with idiosyncratic dynamics and a particular community composition, different from potential source and sink habitats. Some deposition-associated microorganisms probably were directly mobilized from the Saharan soils by winds or could have also been injected from local sources (including seawater or vegetation) by advection and vertical mixing in convective clouds. We noticed that the temporal pattern of microbial communities in atmospheric deposition was independent of the temporal pattern of Saharan dust outbreaks. Despite this general observation, deposition sampled after a very recent dust intrusion tended to resemble more the Saharan desert soil than deposition sampled after a long period without Saharan dust intrusion. Additionally, atmospheric deposition samples closer in ordination space to Saharan soil samples tended to be richer (i.e., higher number of microbial OTUs). We cannot however rule out some potential sources of "noise" in our dataset such as selective growth of different phyla during the period between two samplings (ca. two weeks) modifying the relative abundances, and the fact that neuston
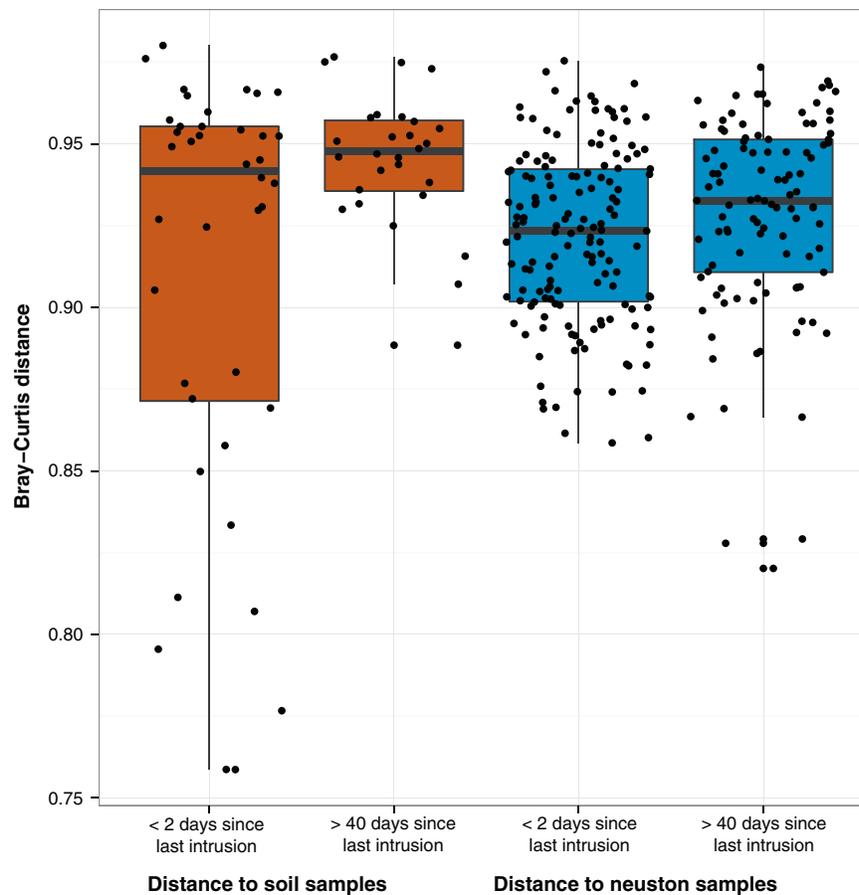
Fig. 6. Microbial community distance in contrasting atmospheric deposition samples from two extreme cases: less than 2 days since last Saharan intrusion event (n = 10) and more than 40 days since last intrusion (n = 7), against desert soil samples (n = 4) and lake neuston samples (n = 15).

samples also likely included the microbial assemblages associated to dry deposition.

The factors shaping microbial community structure in atmospheric deposition are manifold. Recent studies have stressed how the composition of airborne microbial communities is related to land-use type and season (Bowers et al., 2011a, 2011b), and probably these local and regional features generate global patterns in the dynamics and distribution of airborne microbial assemblages. In fact, a survey carried out in remote Himalayan lakes located at c.a. 5,000 m a.s.l., showed most of the bacterial populations detected as cosmopolitan, closely related to ribosomal sequences recovered from distant alpine and glacier regions (Sommaruga and Casamayor, 2009). In addition, a previous study highlighted the short-term temporal variability of airborne bacterial composition with many of the specific taxonomic groups retrieved being common to other cold and highly oligotrophic environments (Fierer et al., 2008a, 2008b). It has been proposed that airborne microbial community members may act as ice-nucleation particles (Bowers et al., 2009) and it remains to be tested whether or not the taxonomic composition of the airborne assemblages may influence cloud dynamics.

Remote lakes are excellent sentinels because they are usually unaffected by direct human influence, yet they receive inputs of atmospheric pollutants, dust, and other aerosols both inorganic and organic. Dust is responsible for the most important difference in precipitation chemistry and, consequently, in the acid-base balance of mountain lakes, and also leads to a significant increase in sulfate, nitrate, ammonium, and calcium deposition (Psenner, 1999; Pulido-Villena et al., 2006). Furthermore, dissolved organic matter (DOM) from atmospheric deposition may act as an energy source for bacterial growth (Mladenov et al., 2011). Specifically, the deposition maximum from the Sahara to Europe

occurs at 2,000-3,000 m altitude (Psenner, 1999) where the lakes sampled in this study were located. Correlations between bacterial abundance, elevation and water residence time suggest that at high elevations, fertilization by dust may be important because of minimal catchment vegetation influences, and relatively greater exposure to atmospheric deposition (Mladenov et al., 2011).

Although the potential extent of microbial dispersal is expected to be huge (Bovallius et al., 1978), successful colonization actually involves arrival and establishment. Asexual microorganisms tend to be excellent colonizers because of their dispersal capabilities and because a single individual can form a new local population (Brown, 1995). Dormancy (i.e. a reversible state of low metabolic activity that may represent up to 50% of total cell counts in natural communities) is a common strategy that allows microbial species to contend to temporal variability of environmental conditions by reducing the risk of local extinction through the recruitment from the seed bank, and by avoiding mortality and thus, increasing the probability of successful colonization (Lennon and Jones, 2011). In this study, we tentatively pointed to broad taxonomic groups with the faculty to thrive in the three potentially connected habitats. These potential migrants (desert soil to atmosphere) and successful colonizers (atmosphere to lake neuston) OTUs belonged mainly to *Alphaproteobacteria* (*Sphingomonadales* and *Rhizobiales*), *Actinobacteria* (*Actinobacteridae*), *Bacteroidetes* (*Sphingobacteriales*) and *Betaproteobacteria* (*Burkholderiales*). Although spore-forming bacteria (such as *Actinobacteria* and *Firmicutes*) were identified, it appears that the presence of viable cell forms in the atmosphere goes far beyond the traditionally considered well-adapted sporulated forms. Recently, some *Gammaproteobacteria* closely related to *Acinetobacter* have been proposed as indicators of airborne transport from the Sahara (Hervàs

et al., 2009). These cells, although are a minor component under in situ conditions, succesfully developed in cultures obtained from Saharan sands and airborne samples collected in the Pyrenees (Hervàs et al., 2009). *Acinetobacter*-like bacteria contain opportunistic human pathogens; and they are ubiquitous in soil and water, easily transported by air, and resistant to dry conditions (Bergogne-Berenzin and Towner, 1996). Although it is reasonable to assume that atmospherically transported pathogens may remain viable over long distances, no study has demonstrated the movement of a bacterial pathogen and linked it to the occurrence of disease (Griffin, 2007).

Overall, our study described the microbial community composition of atmospheric deposition reaching the Pyrenees in NE Spain during a period of three years, where we observed a conspicuous temporal pattern not directly affected by Saharan dust intrusion. We cannot definitely solve the issue of the significance of microbial colonization via atmospheric deposition, but our results indicate that besides its probable low-level and random variability, the detected temporal signal may point to regular and potentially predictable influences on sink communities. Further modeling (Burrows et al., 2009b) and experimental (Hervàs et al., 2009) approaches may enhance our understanding of the biological connectivity lithosphere-atmosphere-hydrosphere by global-scale transport of airborne microorganisms.

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