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High similarity in bacterial bioaerosol compositions between the free troposphere and atmospheric depositions collected at high-elevation mountains

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ABSTRACT

The long-range transport of bioaerosols takes place in the free troposphere and has lately gained a renewed interest in both environmental and health-related disciplines. Sampling free troposphere bioaerosols has been, however, historically challenging and requires of expensive and complex facilities. We analysed different bacterial bioaerosols studies carried out by sequencing of the 16S rRNA gene, available from the literature. The dataset was compared with bacterial bioaerosols present in rain and dry deposition passively collected at high-elevation sites in Sierra Nevada along a set of sampling periods lasting 3 years. Up to 65% of OTUs and 82% of the bacterial genera were shared between wet and dry bioaerosols. Interestingly, only Oxalobacteraceae were notably more abundant in wet deposition, with *Noviherbaspirillum* and *Massilia* as dominant genera. We demonstrated that the bacterial composition of bioaerosols collected by passive natural deposition at high-elevated mountains were closer to the bacterial microbiome from the free troposphere. Interestingly, the meta-analysis showed a different bacterial composition and community structure in bacterial bioaerosols collected at low-elevated areas, over the open ocean, or during desert dust events. Since the boundary layer can be easily reached in high mountain areas, and the local landscape is surrounded by rocks and meadows, alpine stations are potentially optimal research sites with reduced influence of surface aerosols, minimizing local contaminations. Consequently, sampling alpine bioaerosols could be a good proxy for bioaerosols monitoring, long-range dispersal studies, and the dynamic characterization of the free troposphere microbiome.

1. Introduction

Global-scale bioaerosols dispersal is an ubiquitous phenomenon (Griffin et al., 2017). Bioaerosols originated from different terrestrial and marine sources can reach the free troposphere and experience long-range intercontinental transport. Depending on wind conditions and particles size, bioaerosols can be injected above the boundary layer at typical heights from 500 m to 2 km before being washed out by wet (rain and snow) or dry deposition (Burrows et al., 2009). Due to their microscopic size, bioaerosols can remain airborne and viable for long periods (Barberán et al., 2014; Hervàs et al., 2009) and have the potential to travel over continental or transoceanic scales fuelled by trade winds or dust storms (Hua et al., 2007; Prospero et al., 2005). During the airborne journey, bacteria are typically co-transported embedded within organic detritus and/or soil particles that provide UV shielding and a certain level of humidity that may increase their viability and dispersal ranges (Griffin et al., 2017). Hence, aerosol transport may represent a successful mechanism for worldwide dispersal of viable microorganisms and genes (Hervàs et al., 2009; Zhu et al., 2017). Airborne particles may therefore act as very effective vectors for intercontinental disease transmission and spreading of allergens. Moreover, their influence in atmospheric and climate processes, as condensation nuclei for cloud droplets, ice crystals, and precipitation is well recognized (Delort et al., 2010; Fröhlich-Nowoisky et al., 2016; Joly et al., 2013).

Sampling bioaerosols from the free troposphere has been historically challenging, and there is a need of systematic bioaerosols studies across time and space in the high atmosphere (Smith, 2013a; Caliz et al., 2018) like it is usually carried out with other common air pollutants (Bianchi et al., 2016; Murray et al., 2009). A number of potential options for large spatio-temporal studies of free troposphere bioaerosols has been recently reviewed, including aircrafts sampling,

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scientific balloons, rockets, and remote sensing satellites (Smith, 2013b). These methodologies are, however, expensive, need of complex logistics, and usually yield limited microbial biomass that require of extensive controls and careful sterilization procedures. Conversely, high altitude research stations have been used to assess tropospheric background concentrations and long-range transport of pollutants for decades (for instance, the Swiss station at Jungfraujoch and Mount Washington in New Hampshire, USA) (Bianchi et al., 2016; Murray et al., 2009). Sampling bioaerosols at high-elevation mountains (Barberán et al., 2014; Bowers et al., 2012, 2009; Caliz et al., 2018) and studying the composition of their natural atmospheric depositions (Amato et al., 2017; Hervàs et al., 2009; Peter et al., 2014) may also offer realistic possibilities for temporal dynamics and long-range studies of bioaerosols mimicking the medium-to-upper troposphere microbiome (DeLeon-Rodriguez et al., 2013). However, the vertical distribution of airborne microbial communities in different atmospheric layers have been only locally studied (Maki et al., 2015), and there is a lack of studies to determine whether or not collecting wet and dry atmospheric depositions could be representative of the free troposphere bioaerosols.

In the present study, we analysed the bacterial bioaerosols deposited under wet (rain) and dry conditions, which were passively collected at a high-elevation mountain (Sierra Nevada, SE Spain, 2900 m above sea level) along a set of sampling consecutive periods (over summer to mid-autumn) lasting 3 years by high-throughput sequencing of 16S rRNA gene amplicons. Next, we carried out a meta-analysis combining the Sierra Nevada dataset with data from different studies in the literature, covering a wide range of environmental conditions, elevations, and bioaerosol origins. We hypothesized a close relationship between bacterial bioaerosols collected on the top of high mountains and the free troposphere bioaerosols. We compared data from bacterial bioaerosols obtained in variety of situations: (i) above the boundary layer by aircraft sampling (DeLeon-Rodriguez et al., 2013; Zweifel et al., 2012); and under the influence of dust episodes (Maki et al., 2017; Yamaguchi et al., 2012); (ii) from the ground surface at high altitude research stations by means of air dynamic samplers (Bowers et al., 2012), and passively collecting wet (Barberán et al., 2014) or bulk (Peter et al., 2014) depositions; (iii) from the ground surface at low altitude by means of air dynamic samplers (Bertolini et al., 2013; Bowers et al., 2013; Jeon et al., 2011; Mazar et al., 2016; Rosselli et al., 2015) or collecting wet depositions (Itani and Smith, 2016); and (iv) close to the ocean surface by means of air dynamic samplers (Mayol et al., 2017).

2. Materials and methods

2.1. Sampling settings

Atmospheric depositions were collected above tree line in Sierra Nevada (Southeast Spain, 37°03'N, 3°23'W) far away from vegetation, trees, or agricultural activities, using a passive MTXH ARS 1010 automatic sampler (MTX, Bologna, Italy) installed at 2896 m above sea level on a metallic structure with 1.1 m legs on rocky soil at the Astrophysical Observatory of Sierra Nevada (See Figure S1 —supplementary information) surrounded by rocks and meadows. This passive collector discriminated between dry and wet atmospheric deposition using a humidity sensor that activated an aluminium lid to cover/uncover two containers (dry and wet, respectively). In this area, the annual mean for the boundary layer was located at 1.7 ± 0.5 km altitude during the studied period —as determined by LIDAR data— (Granados-Muñoz et al., 2012), showing that the samples were predominantly collected above the boundary layer. Moreover, temporal evolution of LIDAR signals corresponding to isolated atmospheric events previously analysed in this area (Mladenov et al., 2010) supported this fact. This region is under the influence of the global dust belt, and has frequent intrusions of Saharan dust (Mladenov et al., 2011). The origin of air masses reaching the Sierra Nevada Mountains was determined using the transport and dispersion Hybrid Single-Particle Lagrangian Integrated (HYSPLIT) model (Draxler and Rolph, 2014). HYSPLIT backward trajectories were obtained using archived data from the Global Data Assimilation System with a 120 h run time at 2896 m asl. In addition, to verify the origin of the air masses, we also generated maps of time-averaged dust-column mass density (kg m^{-2}) for the study periods using the second Modern-Era Retrospective analysis for Research and Applications (MERRA-2) model. More details on these two procedures can be found in Reche et al. (2018).

Dry and wet (rain) deposition samples were collected along three summers to mid-autumn periods, fortnightly during 2006 and 2007, and weekly during 2008 (Reche at al. 2018). The sample set consisted of n = 46 samples (28 from the dry container and 18 from the wet). Dry deposition was obtained rinsing the dry bucket with 1000 mL of Milli-Q ultrapure, 0.2 μm-filtered, and UV-sterilized water. In the wet deposition bucket, the volume of rain was recorded and up to 1000 mL aliquot was processed when available. Blank controls in the used Milli-Q ultrapure water were below detection level after bacterial counts with flow cytometry (Reche et al., 2018). For particulate material determinations, water samples with suspended materials were filtered using a 0.2 μm policarbonate filter (47 mm diameter). Filtration was stopped when 0.2 μm filter was saturated. Filters containing microbial biomass were stored at −20 °C before DNA extraction. The total iron content determinations in the collected aerosols were carried out following standard protocols as described earlier (Mladenov et al., 2010).

2.2. DNA extraction and sequencing

DNA was extracted using the UltraClean Plant DNA Kit (Mobio Laboratories, Inc.) according to manufacturer's instructions. DNA concentrations were measured using QubitTM fluorometer (Invitrogen). The purified DNA extracts were stored at −80 °C until use. Bacterial bioaerosols were analysed by PCR amplified 16S rRNA gene tag sequencing with the primer pair 357F/926R (357F-CCTACGGGAGGCAG CAG, 926R-CCGTCAATTCMTTTRAGT) matching the V3−V5 hypervariable regions (Sim et al., 2012) by 454 FLX following the Research and Testing Laboratory protocols (Lubbock, TX, http://www.researchandtesting.com). Quality checking and reads denoising were processed using the UPARSE pipeline (Edgar, 2013), version usearch8.1.1861_i86osx32. Sequences were trimmed in length, 250 pb, which substantially reduce the error rate. Approximately 99,2% of the original reads passed the filter (145627 final reads). The sequences were then clustered into operational taxonomic units (OTUs) using a cut-off of 0.03% and, further analysed with UCHIME to discard chimeric reads by both de novo and reference-based chimera filtering step against "Gold" reference database available for ChimeraSlayer. Unique sequences (i.e. singletons) were removed. Overall, 132957 sequencing reads were assigned to OTUs, which were then parsed to create an OTU table. An average sequence depth of 2461 sequences per sample was reached. In order to minimize biased effects for differences in sampling effort, the original OTU table was average rarefied after 100 random subsamplings (Caliz et al., 2015) and set to a depth of 900 sequences per sample. Taxonomic assignment was carried out with SINA – Aligner and classifier (version 1.2.11) using SILVA_128 reference database (Pruesse et al., 2012; Quast et al., 2013) with the de-replicated version at 99% sequence identity. Chloroplasts, mitochondria, and a few untargeted species were also discarded from the analysis. As a result, the final OTU table contained 1469 bacterial OTUs, and 46 samples. The whole gene sequence dataset was uploaded to the NCBI Sequence Read Archive facility and is available through BioProject record ID PRJNA509787. Metadata summarizing air masses origins —based on the analyses of backward trajectories, as reported by (Reche et al., 2018)— are available in the BioProject record.

2.3. Statistical analyses

Statistical analyses were run in the R environment (http://www.r-project.org/). Community ecology related parameters were calculated using the vegan package (Oksanen et al., 2017) and figures were drawn with ggplot2 (Wickham, 2009). Community similarities were represented by non-metric multidimensional scaling (*metaMDS* function) using Bray-Curtis dissimilarities after Hellinger standardization (Legendre and Gallagher, 2001). Analyses of similarities (ANOSIM) were performed based on 1000 permutations. The ANOSIM R statistic is based on the difference of mean ranks between groups and within groups and ranges from 0 (no separation) to 1 (complete separation) (Clarke and Warwick, 1994). With the aim to rule out heteroscedasticity among groups the permutest.betadisper function was check before proceeding with ANOSIM. This measure was also useful to assess the beta-diversity. We used Mantel tests with the Spearman method to determine the correlation between bacterial community similarity patterns and numeric variables. Shannon index and expected species richness were calculated with functions *diversity* and *rarefy* (vegan package), respectively. Similarly, Berger-Parker Dominance index was calculated by using the diverse package (Guevara et al., 2016). Venn diagrams were carried out with VennDiagram package (Chen, 2016). Ward's hierarchical agglomerative method based on Bray-Curtis distances was used for cluster analysis (*hclust* function).

2.4. Meta-analysis

We carried out a meta-analysis combining published data from different genetic studies of bacterial bioaerosols (see Table 1 for details) including data from the Sierra Nevada dataset. Taxonomic compositions of bioaerosols were compared for a range of sampling sites, methods, elevation, origins and occurrence of dust events. For each bioaerosol study, we computed a summarized taxonomic profile. We used hierarchical clustering analysis to group all bacterial bioaerosol studies according to taxonomic similarity.

3. Results

Bacterial bioaerosols from dry and wet depositions collected in Sierra Nevada were barely separated as indicated by a rather low, though significant, ANOSIM statistic (R: 0.17; $p < 0.01$). A high degree of community overlapping is assumed for $R < 0.25$ (Ramette, 2007). The NMDS ordination analysis based on Bray-Curtis dissimilarities showed high similarity as well (Fig. 1 – left panel). No differences in beta diversity were observed between dry and wet depositions (*Betadisper* test, p-value > 0.05) (Fig. 1– right panel). In addition, the amount of deposited particulate materials and Fe —a marker signature indicating desert soil origin— did not support samples dissimilarities (Mantel r – Spearman: 0.13; Significance: 0.07) (Figure S2). This result could be explained by the mostly mixed origin of the aerosols samples in our dataset. Both wet and dry bioaerosols showed high values of Good's coverage of the bacterial communities (> 0.9) (Fig. 2), and no significant differences were observed in Shannon index and Berger-Parker Dominance (T-test, p-value > 0.05). Species richness and Faith's phylogenetic diversity were significantly higher in dry bioaerosols (Fig. 2), even though the degree of taxa overlapping was substantially high between dry and wet depositions (Figure S3). Up to 65% of OTUs and 82% of the bacterial genera were shared between wet and dry bioaerosols, and the dominant general taxa (i.e., relative abundances > 1%) were mainly the same (Figure S4, upper panel) and positively correlated (Spearman rho: 0.54; p-value < 0.01) at the family level. Overall, a combined dispersion plot for the mean relative abundances showed largely convergent bioaerosols compositions at the taxonomic level of family (Figure S4, lower panel). Interestingly, only Oxalobacteraceae were notably more abundant in wet deposition, with *Noviherbaspirillum* and *Massilia* as dominant genera.

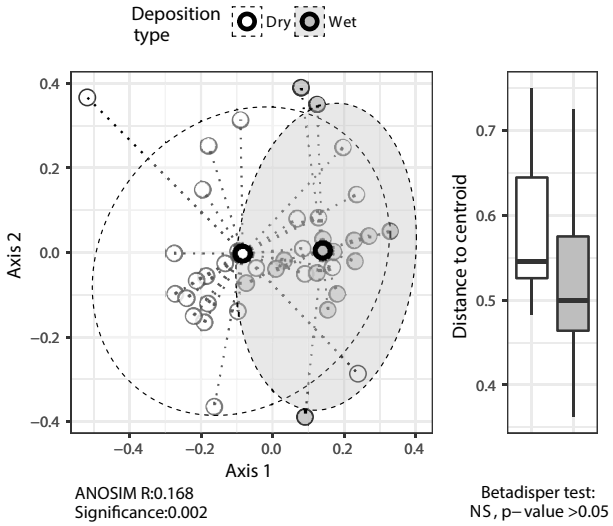

Fig. 1. Non-metric multidimensional scaling (NMDS) ordination analysis of bacterial community similarities based on Bray-Curtis dissimilarities (left panel). Dots colour according to the type of deposition. Dotted lines join each sample with its corresponding group centroid. The ellipsoid areas are indicative of distribution (and dispersion) of each group on the ordination space (at 0.95 confidence level). The right panel shows boxplots of beta-diversity as distance to centroid.

We carried out a meta-analysis according to taxonomic similarities combining the Sierra Nevada dataset with data from different bioaerosols studies in a variety of situations and sampling protocols including those from the free troposphere and the Pyrenees (Table 1). Hierarchical clustering analysis divided the dataset into three main groups, named 1, 2a and 2b in Fig. 3. Cluster 1 included most of the studies within the boundary layer from low-elevation areas and desert dust plumes collected from the free troposphere by aircrafts, owning a substantial influence of ground materials. These samples showed a strong dominance of Firmicutes sequences (relative abundance 31.6 ± 13.0 in cluster 1 *vs* 3.6 ± 3.5 and 9.4 ± 5.9 in clusters 2a and 2b, respectively). Conversely, cluster 2a included bioaerosols mostly collected above the boundary layer, both by aircrafts during desert dust free episodes and by passive deposition at high-elevated mountains. Cluster 2b contained a heterogeneous variety of sampling protocols and presence of dust, mainly representing local terrestrial inputs sampled by air dynamic methods both at high altitudes and in suburban localities, as well as marine aerosols. Bacterial communities from clusters 2a and 2b were more similar among them, with higher proportion of Proteobacteria sequences than cluster 1 and always dominated by α-, β- and γ-Proteobacteria. α-Proteobacteria clearly dominated cluster-2a (45.8 ± 11.3), while cluster 2b showed a higher mean relative abundance of β- (32.0 ± 22.0) and γ-Proteobacteria (17.7 ± 13.8).

The different studies grouped in cluster 2a were also highly coincident in bacterial composition and structure at the family taxonomic level (Fig. 4). The most abundant families such as Sphingomonadaceae/Methylobacteriaceae (α-Proteobacteria) and Oxalobacteraceae/Comamonadaceae (β-Proteobacteria) were recurrently detected in all studies. Pseudomonadaceae (γ-Proteobacteria) were also abundant in several studies and almost evenly detected. ANOSIM statistic further confirmed the high degree of overlapping in bacterial composition between aircrafts sampling and passive deposition at high-elevated mountains (*R*: 0.15; significance: 0.2).

4. Discussion

Tropospheric communities are expected to be less complex than many of the biological habitats on Earth (DeLeon-Rodriguez et al.,

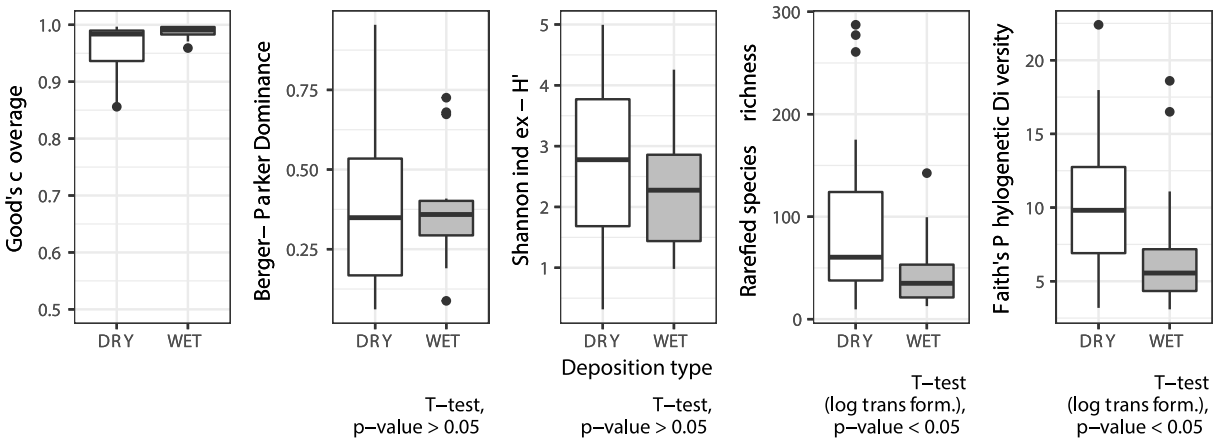

Fig. 2. Box-plots comparing the values of good's coverage and a set of different alpha-diversity parameters for dry and wet deposition.

Table 1
Studies included in the meta-analysis. Details on geographic and sampling sites, elevation, sampling technique and occurrence of dust events are shown. The right-most column shows position in the cluster analysis shown in Fig. 4. *ABL* above boundary layer, *BL* boundary layer, n/*a* non-applicable, *NS* non-specified. * sampling site ABL just during night-time.

Reference	Geographic site	Atmospheric layer	Sampling point	Sampler	Deposition	Dust Episode	Cluster Fig. 4
Rosselli et al. (2015)	Sardinia-Italy (EU)	BL	Ground surface - low altitude	Air dynamic	n/a	No	1
Yamaguchi et al. (2012)	Japan (Asia)	ABL	Air	Air dynamic - Aircraft	n/a	Yes	1
Rosselli et al. (2015)	Sardinia-Italy (EU)	BL	Ground surface - low altitude	Air dynamic	n/a	Yes	1
Jeon et al. (2011)	Seoul (Asia)	BL	Ground surface - low altitude	Air dynamic	n/a	Yes	1
Maki et al. (2017)	Japan (Asia)	ABL	Air	Air dynamic - Aircraft	n/a	Yes	1
Mazar et al. (2016)	Israel (Asia)	BL	Ground surface - low altitude	Air dynamic	n/a	No	1
Bowers et al. (2013)	Colorado (USA)	BL	Ground surface - low altitude	Air dynamic	n/a	NS	1
Mazar et al. (2016)	Israel (Asia)	BL	Ground surface - low altitude	Air dynamic	n/a	Yes	1
Bertolini et al. (2013)	Italy (EU)	BL	Ground surface - low altitude	Air dynamic	n/a	NS	1
This study	Spain (EU)	ABL	Ground surface - high altitude	Deposition	Wet	n/a	2a
Barberán et al. (2014)	Spain (EU)	ABL	Ground surface - high altitude	Deposition	Wet	NS	2a
DeLeon-Rodriguez et al. (2013)	USA	ABL	Air	Air dynamic - Aircraft	n/a	NS	2a
Zweifel et al. (2012)	Sweden (EU)	ABL	Air	Air dynamic - Aircraft	n/a	NS	2a
This study	Spain (EU)	ABL	Ground surface - high altitude	Deposition	Dry	n/a	2a
Maki et al. (2017)	Japan (Asia)	ABL	Air	Air dynamic - Aircraft	n/a	No	2a
Itani and Smith (2016)	Beirut (Asia)	BL	Ground surface - low altitude	Deposition	Wet	Yes	2b
Peter et al. (2014)	Austria (EU)	nd	Ground surface - high altitude	Deposition	Bulk	No	2b
Bowers et al. (2012)	Colorado (USA)	BL*	Ground surface - high altitude	Air dynamic	n/a	NS	2b
Mayol et al. (2017)	Ocean (Global)	BL	Ocean surface	Air dynamic	n/a	NS	2b
Peter et al. (2014)	Austria (EU)	nd	Ground surface - high altitude	Deposition	Bulk	Yes	2b
Jeon et al. (2011)	Seoul (Asia)	BL	Ground surface - low altitude	Air dynamic	n/a	No	2b

2013), and the meta-analysis of microbial communities from a communal Earth' samples catalogue (Thompson et al., 2017) has shown lower alpha-diversity in bioaerosols than in soils, sediments, plants rhizosphere and freshwaters. Wet depositions collected at a high-elevated mountain in the Pyrenees also showed not very diverse bacterial communities in comparison with source Saharan desert soils, and alpine lakes neuston at the air-water interface (Barberán et al., 2014). In our study, the ecological diversity and specific richness were quite low for most of the collected bacterial bioaerosols. Shannon index (H') values < 2 for all the cases indicate both low richness and high dominance of a small number of taxa. This fact contrasts with the high diversity of bioaerosols collected by air dynamics near the ground (Bowers et al., 2012; Brodie et al., 2007) where most probably contamination of the bioaerosol fraction with local ground surface microbes increases diversity. Indeed, it has been shown that bacterial bioaerosols diversity increases due to the presence of desert dust inputs both in the free troposphere and near to the ground (Maki et al., 2017; Mazar et al., 2016).

As a relevant finding, we demonstrated that the bacterial composition of bioaerosols collected by passive natural deposition at high-elevated mountains were closer to bacterial communities from the free troposphere. Consequently, sampling alpine bioaerosols could be a good proxy for bioaerosols monitoring and long-range dispersal studies. In fact, it has been recently shown a closer relationship between bioaerosols collected by air sampling at the top of mountains and at the medium-to-upper troposphere than with those collected closer to oceanic or soil sources (DeLeon-Rodriguez et al., 2013). As shown in our meta-analysis, these alpine airborne communities share major orders, families and genera with the free troposphere. More in detail, shared taxa were Sphingomonadaceae (*Sphingomonas*), Methylobacteriaceae (*Methylobacterium*), Oxalobacteraceae (*Massilia*, *Herbaspirillum*, *Noviherbaspirillum*), Pseudomonadaceae (*Pseudomonas*) and also other Rhizobiales. Besides, some of these taxa include members related to known bacteria able to use C1-C4 compounds or to resist UV and desiccation, which have been recurrently detected in a long-term monitoring of aerosols in a mountain-top study in the Pyrenees (Caliz et al., 2018), evidencing their ability to potentially survive and remain alive in the atmosphere. Then, our data is comparable to the results obtained by means of sampling on an aircraft with a dynamic air setting (DeLeon-Rodriguez et al., 2013; Maki et al., 2017; Zweifel et al., 2012), and the homogeneity of airborne communities could be attributed, in part, to the different biological response of bacteria to cope with the atmospheric environmental conditions. It is an obvious concern that research aiming to address long-range airborne microbial dispersal should

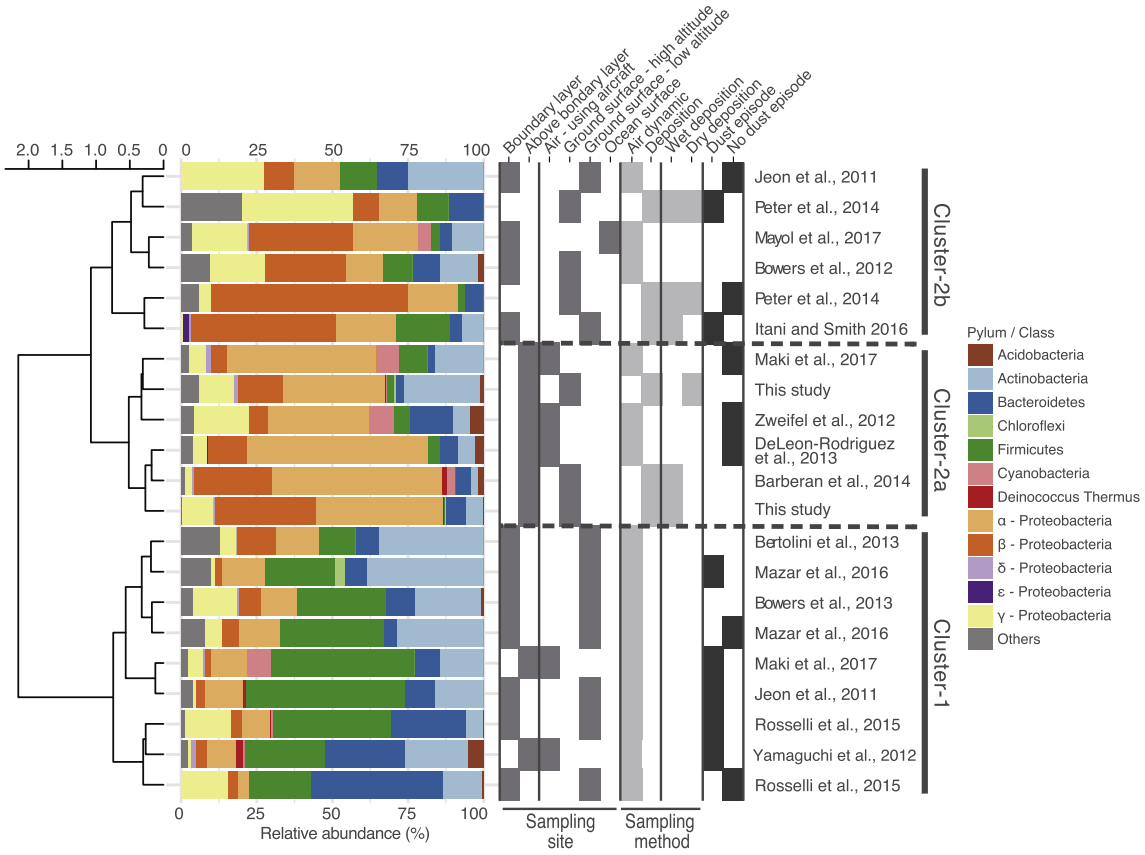

Fig. 3. Hierarchical clustering analysis (Ward's hierarchical agglomerative clustering method) based on Bray-Curtis distances of bacterial bioaerosols reported from different studies. Bar plots show the relative abundance of dominant phyla or classes. Information on sampling sites, sampling techniques, and presence of desert dust is shown. See additional details in Table 1.

carefully avoid ground-surface contamination, and collect samples from the free troposphere (Maki et al., 2017; Smith, 2013b). Since the boundary layer can be easily reached in high mountain areas, and the local landscape is surrounded by rocks and meadows, alpine stations are potentially optimal research sites with reduced influence of surface aerosols, minimizing local contaminations. Subsequently, high mountain sites appear as suitable sampling network infrastructures to address studies on the composition and fate of long-range transported bioaerosols. The links of bacterial origins with trajectories is certainly an issue of major interest that has been recently addressed in a high mountain station in the Pyrenees, using a large number of samples within a 7 yrs long-term study (Caliz et al., 2018).

Interestingly, the meta-analysis showed a different bacterial composition and community structure in bacterial bioaerosols collected at low-elevated areas, or during desert dust events, with dominance of Firmicutes, Actinobacteria and Bacteroidetes. These phyla are commonly found in soils (Fierer, 2017), and prevailed in both the bioaerosols associated with Asian dust events and soils from different Asian deserts (Jeon et al., 2011; Maki et al., 2017). These observations indicate that ground surface microbes can greatly influence the bioaerosols collected at low-elevated sites, or during dust intrusion episodes (see listed works in cluster 1, Fig. 3). In agreement, bioaerosols collected over the open ocean showed lower proportion of these phyla and greater signal of terrestrial microbes are detected in marine bioaerosols collected closer to landmasses than in the open ocean (Mayol et al., 2017).

In addition, sampling of passively deposited aerosols results advantageous in comparison with air dynamic sampling on ground, as it would collect the microbiome present in the entire air column above the boundary layer. Yet, we cannot obviate some potential biases and contaminations. For instance, ground surface microbes deposited into the collectors from surrounding areas by turbulent flows could represent a source of contamination. However, the location of the collector above tree line and on rocky naked soil, and far away from vegetation, trees, or agricultural activities strongly minimized this potential source of contamination. MilliQ water blanks were used to control for potential contaminations in dry depositions, that was negligible. For wet deposition, we did not expect substantial enrichment of bacteria inside the collector according to previous tests and test carried out in samples of the same dataset (Reche et al., 2018). Thus, wet and dry deposition rates for bacteria were not significantly different (Kruskal–Wallis test KW-H = 2.88; p = 0.089, Reche et al., 2018), supporting no substantial growth in the wet collector. Nevertheless, the use of bacterial growth inhibitors could be an appropriate strategy to minimize potential biases during monitoring and handling of bioaerosols, together with the synchrony in atmospheric deposition among distant sites (Reche et al., 2018). Passive sampling of natural depositions allows to minimize maintenance logistics and powering of sampling instrumentation, as there is no need for pumps in continuous running —either for filtration settings or liquid impingers.

5. Conclusions

Overall, we observed that naturally deposited aerosols in areas with little influence of ground surface contamination, such as the top of high-mountains, strongly resembled the bioaerosols of the free troposphere. Thereby, this approach would be useful on reaching a descriptive view of microbes that move thousands of kilometres away from source regions throughout the troposphere. Under certain circumstances, wet collectors can recover higher bacterial loads as compared with dry ones, for instance, during Saharan dust intrusions (Reche et al., 2018). Then, long term monitoring studies employing

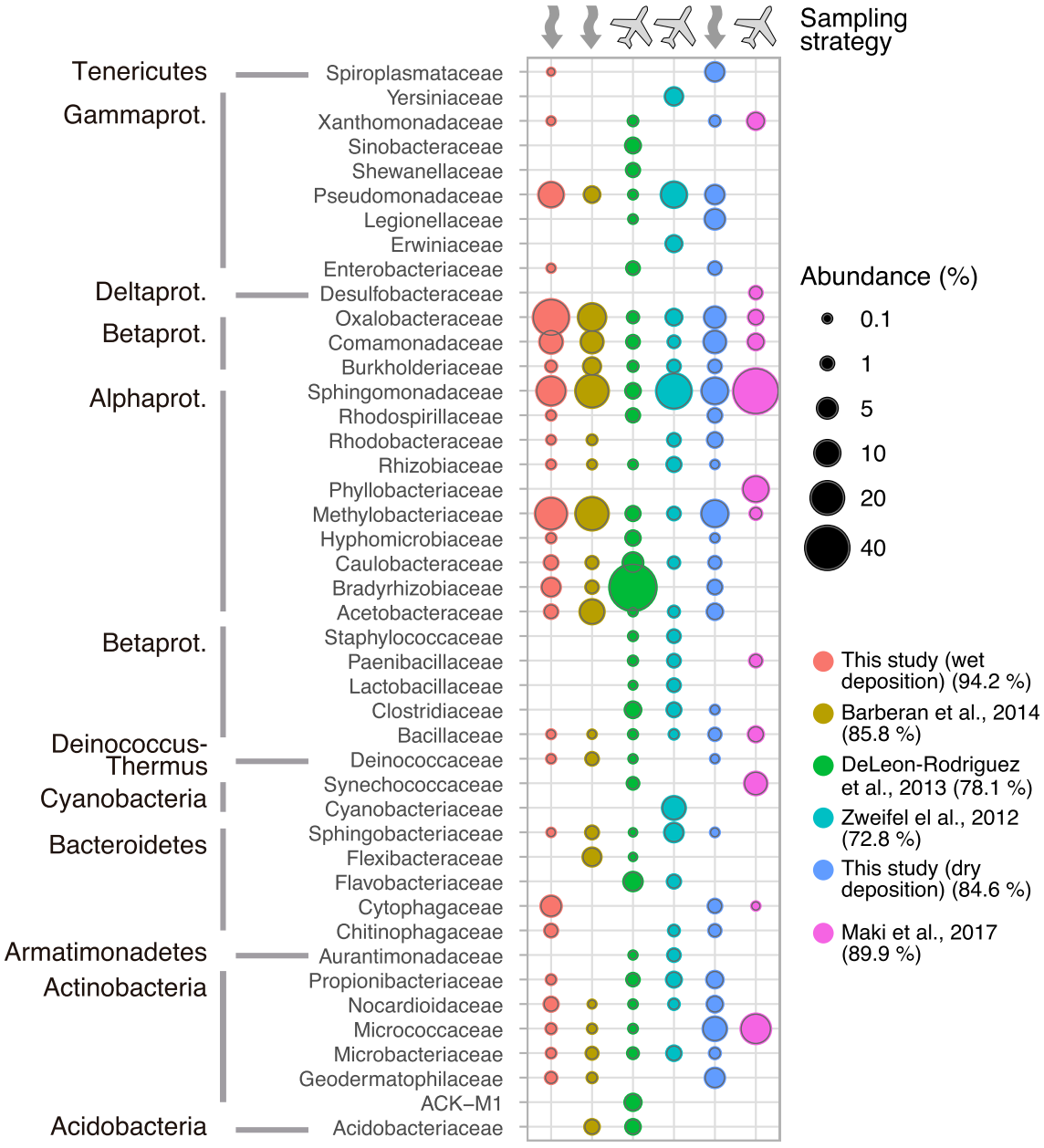

Fig. 4. Composition and abundance of taxonomic bacterial families in bioaerosols from studies in cluster 2a of Fig. 3. Only families with relative abundances > 0.1% are shown. Total abundance percentage analysed shown within brackets in the colour legend. Labels for sampling strategy (upper part): *arrow,* deposition collection on ground surface at high altitude sites; *airplane,* aircrafts equipped with air dynamic samplers (see also Table 1).

molecular methods could be more easily affordable by means of collecting bioaerosols washed from the atmosphere. This would allow circumventing the problem of limited biomass, which could hamper bacterial aerosols studies. The proposal of monitoring aeroplankton is noteworthy at the light of statements in need of monitoring microbes just like usually done with other types of common air pollutants (Smith, 2013a; Caliz et al., 2018). Then, cost-effective, and long-term sustainable monitoring programs are needed for this aim. Monitoring naturally deposited bioaerosols at accessible high-elevated areas would be a good enough method for a realistic and general prospection of the free troposphere microbiome.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atmosenv.2019.01.038.

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