UNIVERSITY OF SASSARI

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D.U.S.T.
Desert Upon Sardinian Territory

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Abstract

Intercontinental dust transport represents one of the mechanisms of microbial dispersion, contributing to biodiversity of ecosystems and impacting on human health when of pathogenic nature. Studies on airborne dust microbiology have just begun reporting variable densities of microorganisms depending on seasons and environmental conditions, in particular a low number of culturable microrganisms, only 1%.

In this study, a combined culture-dependent and independent DNA-based analyses via NGS of 16S genes was applied for the first time to investigate the airborne dust microbiology in air sampled during dust events at two opposite coastal sites of italian Island Sardinia, which represents the crossroad of wind circulation from Africa towards Europe.

The results of culturable method showed that microrganisms possessing adaptive strategies to persist in the atmosphere during dust transportation survived, as Gram positive Bacillus spp and filamentous fungi.

By using NGS analysis, we observed distinct differences in bacterial community composition. A major conserved core microbiome was evidenced but increases in species richness and presence of specific taxa were observed in relation to each wind regime and seasons. Taxa which can feature strains with clinical implications were also detected. Data suggested the existence of a main pattern facilitating airborne bacteria dispersion and local scale migration after the dry seasons, which offers a refined interpretive understanding of overall environmental microbiology dynamics.

Keywords: airborne dust-transported microrganisms, culture-independent studies, microbial diversity, metagenomics, 16S rRNA sequencing

Introduction

Scientists have known for a long time that microorganisms could be transported by wind.[1] Bacterial transport is favored by specific meteorological situations and by microbial features: metabolically active bacteria are abundant in the upper atmosphere, particularly downwind of arid regions, where winds can mobilize large amounts of topsoil and dust.[2] Satellite data and forecasting models foretell geographical dispersion of African dust, revealing that the main sources are represented by the Sahara and Sahel regions, located in the north-western part of Africa continent. The finer dust particles can be carried to intercontinental distances. It is known, that there is a seasonal variability in dust transport and dust concentration can vary greatly from year to year. The atmosphere is just the conveyor belt for the transfer of microbes from one location to the other. As far as I know, microbes are not able to fly by themselves and thus they are not able to remain in the atmosphere and use this compartment as a habitat for growth/evolution. The mean synoptic situation that favours the transport of dust towards Italy is characterised by a deep low-pressure system over the Saharan region and a strong high-pressure system centred over Libya and Tunisia that extends across the Mediterranean sea. Strong heating on desert regions produces convective winds which elevate a great quantity of dust in the atmosphere.[1] Thanks to the deep gradient between the low and high systems, the suspended dust is then carried by a south-westerly flow towards the Mediterranean and Europe in fan-like plumes that differ every time. The primary source regions, which include the Sahara and the Sahel regions of North Africa and the Gobi and Takla Makan regions of Asia, are capable of dispersing 2-3 billion metric tons of soil dust are estimated to be transported in the Earth's atmosphere each year. Global transport of desert dust is believed to play an

important role in many geochemical, climatological, and environmental processes[3]. This dust carries minerals and nutrients, but it has also been shown to carry pollutants and viable microorganisms capable of harming human, animal, plant, and ecosystem health. Notwithstanding a considerable amount of research by scientists has addressed atmospheric pathways and aerosol chemistry, very few studies to determine the numbers and types of microorganisms transported within these desert dust clouds and the roles that they may play in human health have been conducted. Bioaerosols of microorganisms have gained increasing interest because of their potential to spread pathogens over long distances [4 5].

After 2000, the United States Geological Survey (USGS) in St. Petersburg, Florida, began the USGS Global Dust Program (initially funded by NASA) to investigate whether live microorganisms would be consistently transported in dust masses (initially in Saharan dust)[6]. Scientists at USGS using DNA sequencing of the ribosomal gene, were able to isolate and identify over 200 viable bacteria and fungi in samples from St. John in the U.S. Virgin Islands in 2000 during dust air-driven particle transport events[7]. Many of the viable microorganisms identified in Saharan dust are known from clinical records to cause respiratory diseases (allergic reactions, asthma, and pulmonary infections), cardiovascular diseases, or skin infections[8,9]. Other microbes in airborne dust are known to be pathogenic to humans, including those causing plague, anthrax, tuberculosis, or towards livestock, relatively to foot and mouth disease or to plants, causing sugarcane rust, and other pathologies of cotton, peach, rice and beans. Moreover, the association between microorganisms and air pollutants like PM2.5 and PM10 (particulate matter with diameters less than 2.5 and 10 µm, respectively)[10] could represent a recently evolved transfer-mechanism supporting and increasing

their natural dust-mediated dispersion and opening new ways for their epidemic diffusion.

Facing the African Continent, Europe is subjected to a large-scale dust-transportation. It has been estimated that 80–120 Teragram (Tg) of dust per year are carried across the Mediterranean towards Europe. In particular, dust transported by winds can reach an elevation up to 8 km in the atmosphere over the Mediterranean basin[11].

In order to track the biodiversity of these airways, the Italian island of Sardinia was chosen as ideal observatory point to collect airborne bacteria moving inside and outside Europe. Located in the middle of the Mediterranean Sea, distances of 120, 150, 230, and 100 Nautical miles (NM) separate this landmass from Italy, France, Spain and Africa coastal baselines respectively. Its geographical position facilitates the displacement of western high- and low-pressure nuclei coming from Gibraltar and becoming the first and the last frontier for microbes entering or leaving Europe, respectively. Moreover, the amount of aerial particulate allowed the detection of desert-dust intrusions and a direct correlation with the airborne biota and local health status (www.nasa.gov/topics/earth/features/health-sapping.html).

Every dust-carrying event can be very different from others of the same kind; in fact dust transport and concentration in the air can vary remarkably depending on the synoptic situation[12]. In general the main meteorological scenarios that originate the transport of dust towards the central and western Mediterranean Basin are characterized by the presence of four different synoptic conditions:

i) a strong North African thermal low pumps dust till the mid-troposphere, where the western side of a high pressure centered slightly westward transports the plume;

ii) the eastern side of an Atlantic trough with the western side of the associated ridge, located between Tunisia and Libya is able to originate an all-level transport;

iii) the western side of a well-structured high on the central Mediterranean basin can supply the flow for low level transport;

iv) the north-eastern side of a sea level low centred in south Libya can create the condition for a low level transport[13 14].

The geographic position and seasonal weather conditions make Italy one of the first frontiers subjected to a natural dust-transportation. Satellite data and forecasting models foretell geographical dispersion of African dust, revealing that the main sources are the Sahara and the Sahel regions, located in the north-western part of Africa. Dust events that reach Italy are more frequent in the May-November period, but can also take place during December-April[15].

In the western side of insular Italy, surrounded by the Mediterranean sea, the Sardinia island is located at 130 Nautical miles (NM) (240 km) from the North-African coastline and represents a cornerstone for the above phenomena as well as an ideal observation point for monitoring of dust transportation. Dust outbreaks in Sardinia were already described[16 17] but there is a lack of studies on the microbiological diversity of winds discharging particles from Africa.

Dust particles lifting and discharge from Africa to Europe is a recurring phenomenon linked to air circulation conditions. The possibility that microorganisms are conveyed across distances entails important consequences in terms of biosafety and pathogens spread.

In the past surveys of the environmental microbiological communities have been limited by the resolution and throughput power of sequencing methods, but nowadays these are being overcome. Efficient sequencing platforms (e.g

http://www.illumina.com) allow over 1Gigabase (1 Gb, a billion nucleotides) of sequenced DNA per single run. As the single average bacterial genome is approximately 4 Mb, it is envisaged that a single experiment will cover hundreds of bacterial genomes, along with the attached viral component, and of several tons of microbial eukaryotic genomes. The type and magnitude of data will require a correspondingly efficient job processing bioinformatics pipeline.

Metagenomic studies represent a great opportunity to investigate fungal and bacterial diversity in the air during dust events and monitor their spread locally or across the Mediterranean Sea. Moreover, metagenomic studies can facilitate monitoring of airborne microorganisms through identifying pathogenic microbes and their distribution patterns and involvement in disease outbreaks that impact plant, animal, and human health.[18]

The main gaps in knowledge are:

- to know which specific factors and events (climatic, seasonal etc.) can significantly raise the hazard level inherent to this potentiality;
- overcome the limit of non culturable microorganisms (99%) with genetic determinants of taxonomy identity (small subunit ribosomal RNA gene) and metagenomics to the whole genome level using next generation sequencing (NGS) in order also to unravel the presence of pathogenicity genes and of their variants.

Moreover, not only limited to free-living organisms, microbial dispersion can be favored by a natural mobile reservoir of physical, solid, carriers represented by the air-dispersed particulate matter. Such particles are included in a formal range between 0.2 up to 10 um[19] and average loads of 1-100 ug m[3, 20, 21].

It has been estimated that more than 5000 Tg of sea salts and 1000-2000 Tg of soil particles, passively uplifting and transporting live cells are released every year in the atmosphere giving rise to a heterogeneous, traveling, material from

different sources. Seas and deserts, in particular the tropical African and Asiatic belts[22] represent the major dust sources but an increasing amount of particulate related to human activities is released in the atmosphere (http://www.who.int/). Several studies underline that microbes take advantages from each of these phenomena, which strongly contribute to their cosmopolitan ranges and colonization abilities. Particle-associated biodiversity was described identifying possible sources of aerial-dust suitable to carry microorganisms. The detection of non-sporeforming[23] and UV-resistant bacteria and their potential survivor in the stratosphere[24] endorses the existence of natural long-range transfer mechanisms exploited by microorganisms in association with aerial dusts[25]. In addition, the correlation between specific bacterial clades and particle size[26] opened new hypotheses on differential dispersion related to the dust features besides the constraints due to the source and site of origin.

While a considerable amount of scientific research has addressed atmospheric pathways and aerosol chemistry, very few studies to determine the numbers and types of microorganisms transported within these desert dust clouds, and the roles that they may play in human health have been conducted. Previous culture-based studies have established that the bacterial community can move through the atmosphere in clouds, but no studies has demonstrated the movement of a prokaryote pathogen and linked it to the occurrence of disease. The closest known associations of dust storms and human disease of microbial origin are the outbreaks of meningitis that occur periodically in North Africa and the outbreaks of Coccidioidomycosis (also called Valley Fever) in California.[5] And the resurgence of old ones like tuberculosis and cholera, reflects changes in human ecology such as increased mobility of long-distance trade, changes in personal behavior and social organization, global changes such as deforestation and climatic changes. A vast majority of microbial species, although viable are not

culturable in laboratory media, and such condition is even more pronounced when dealing with spores and other quiescent life adaptations as frequently occurring in harsh and dry milieus as the atmosphere. Among microrganisms Fungi comprehend a heterogeneous group of saprobes, parasites or symbionts microorganisms and can be pathogens or endophytes[26]. Different groups of fungi are adapted to the environment, comprising terricolous fungi, fungi associated with plants, hyphomycetes, yeasts and microcolonial fungi[27]. Despite molecular studies of mycotic diversity have started to emerge in recent years[28–30], the composition of fungi in desert environments and even more in dust-transported is still not well-defined. The main objective of this study was to investigate whether airborne transportation of live microbes can cause immigration of pathogens across distant countries.

For this reason the study was conducted to define the identities of microorganisms carried by Saharan dust to Sardinia (Italy), which is is located exactly in the route of dust long distance transportation, representing the ideal point for collecting samples of airborne bacteria moving towards Europe.

Dust samples were collected through filters during dust events following careful metereological monitoring. As controls, we examined local atmospheric microorganisms that could adsorb to the sand filters, after 12-24 h exposure at the same sites, but in the absence of a sandstorm event. Filters collected were subjected to both culture-based and cultivation-independent methods via NGS of the 16S genes from the airborne metagenome. Data comparison and integration would provide interesting clues concerning the contribution of this mechanism of geographical dispersion to the biodiversity modulation and its possible effects on ecosystems and human health and activities.

Notably, this study representing the first application of a "whole environment" shotgun genomic approach on air-transported particles, will allow the

simultaneous analysis of the genomes of all microorganisms present in the sample, and therefore, unlike other approaches, a census on the possible presence and abundance of viruses, phages, viroids, and of yet unknown organisms.

Results would be crucial for early warning purposes regarding the prediction of disease outbreaks, or potential acts of bioterrorism.

Europe-Mediterranean air circulation routes offer an interesting case study platform when focusing on airborne bacteria. The system can be represented as a multidirectional network in which biological components and weather conditions are closely related and feature air masses from different natural sources also related to human-activity.

Materials and Methods

Meteorology forecasts.

The predictive evaluation and alert of the Saharan dust discharge events was carried out by continuous monitoring of Moderate Resolution Imaging Spectroradiometer (MODIS) satellite data and Meteosat imagery combined with SKIRON59 forecasting model. SKIRON is a version of the ETA/NCEP weather forecasting model developed from the University of Athens with a forecast horizon of 5 days. The origin and the back-trajectory plots of the dust carried by winds towards Italy were inferred by the NOAA HYSPLIT model (Hybrid Single Particle Lagrangian Integrated Trajectory Model)[60,61] Rolph, G. D. Real-time Environmental Applications and Display sYstem (READY) Website (http://ready.arl.noaa.gov). NOAA Air Resources Laboratory (2014) (date of access: 04/10/2014). Draxler, R. R. & Rolph, G. D. HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARLREADY Website (http://www.arl.noaa.gov/HYSPLIT.php). NOAA Air Resources Laboratory, College Park, MD. NOAA Air Resources Laboratory (2014) (date of access: 07/10/2014).

In addition, PM10 (particulate matter with a diameter of less than 10 μ m) and meteorological data registered by the ARPAS (Regional Environmental Protection Agency of Sardinia) monitoring stations were used to highlight the arrival of African air masses.

Sampling sites and sample collection.

The sampling was conducted in Sardinia Island which extends from 38.86° N to 41.31° N and 08.14° to 9.84° E. The selected sampling sites were located in Cagliari (39.23°N, 9.11°E), on the southern coast facing the African continent,

and Sassari (40.75°N, 8.49°E) which represents, on the opposite side, the first outpost site reached by the north-western winds. The two sampling locations are 174 km apart and the distance covers essentially the whole longitudinal territorial length of the island.

Sampling was conducted from November 2013 to December 2014 using a Skypost Tecora air-filtering apparatus with Teflon filters (Sartorius Stedim Biotech). Filters were collected every 12-24 hours in sterile boxes and transported at room temperature to the laboratory, where they were processed for culture and stored at −4°C until nucleic acids extraction.

Culture-dependent analyses

Isolation and identification of bacteria

In order to characterize in parallel the fraction of culturable biota, some of the filters collected from different dust events were placed, sample-side up, cut and cleaned with Tween 20. The filters pieces were removed and placed in selective and non-selective agar media plates and incubated at 37°C for 24–72 hours for bacteria growth. The media used were following: Blood Agar, Chocolate Agar, Mannitol salt Agar, Mac Conkey agar, Thioglycollate medium usp with resazurine for bacteria and Sabouraud dextrose agar (SDA) with chloramphenicol (0.05g/l) and gentamicin (0.1g/l) (Microbiol Diagnostici, Macchiareddu Uta Italy) and in proteose-yeast extract-glucose (PYG) medium, specific agar for Amoebae[27]. Cultured bacterial taxa were identified starting from a single colony using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF, Microflex LT Bruker), Vitek2 system, MicroScan WalkAway plus System and optical microscopy for colony morphology assessment. The MALDI TOF technology is an alternative to classical microbiological identification for bacterial isolates, whose recognition is accomplished through comparison of the

peak lists with a library containing the spectra information of species. Nevertheless it is recommended to couple its use with complementary techniques as the MALDI-TOF database is still incomplete as regards spectra of a number of species, e.g. some ofthe non-pathogenic Bacillus species. Therefore the Vitek2 Biomerieux and MicroScan WalkAway plus System Beckmann Coulter, providing automated identification based on antibiotic susceptibility profiles, was used as a suitable companion technique which can provide targeted phenotypic tests, in particular those covering aerobic endospore-forming bacteria.

Isolation and identification of filamentous fungi

The filters collected from different dust events were placed, sample-side up, cut and cleaned with Tween 20 (Tween-20 0,05%, NaCl 0,85%) and inoculated into plates of Sabouraud dextrose agar (SDA) with chloramphenicol (0.05g/l) and gentamicin (0.1g/l) (Microbiol. diagnostici, Uta, Italy). Plates were incubated at room temperature for 7-15 days. Identification of fungi was done based on the macroscopic and microscopic characteristics using standard mycological methods.

Isolated fungi were sent to the Centro di Sperimentazione e Assistenza Agricola (CERSAA, Albenga, Italy) for species molecular identification by PCR using specific primers ITS1F and ITS440 [28] targeting the internal transcribed spacer (ITS) region of the ribosomal rRNA gene.

Cultured-independent analysis.

Nucleic acid extraction and Metagenomic

All filters processed for DNA analyses were selected based on the observed weather conditions and the backward trajectories analysis. The filters were

collected in order to represent northbound dust outbreaks reaching Cagliari or Sassari (CA-Dust and SS-Dust, respectively), additionally the dust-negative controls were taken at each of the two above sampling stations under northwesterly wind (CA-Ctrl and SS-Ctrl). Two filters for each specific weather event were considered in each sampling site, two replicates per site and per condition were taken for a total of eight filters. DNA was extracted from pretreated filters using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek Inc.) and following the manufacturer's protocol. Quality and quantity of the extracted nucleic acids were assessed using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific Inc.).

For bacteria detection, amplification of the 16S-rRNA genes was performed using the universal primers 27F-AGAGTTTGATYMTGGCTCAG and 1492R-TACGGYTACCTTGTTACGACTT[29]. PCR was performed using Platinum® Taq DNA Polymerase High Fidelity (Life Technologies) in a PTC-200 ThermalCycler (MJ Research Inc.) set as follows: 95 °C for 5 min, (95 °C for 0.5 min, 51 °C for 0.5 min, 72 °C for 2 min for 30 cycles), 72 °C for 10 min and 4°C hold. NGS was done at the Porto Conte Ricerche Genomic Core Facility (www.portocontericerche.it/) (Alghero, Italy). Briefly, amplicons were quality-checked on an agarose gel and purified using the Agencourt® Ampure® XP PCR Purification Kit. One ng of DNA was processed using the Nextera XT DNA Sample Preparation Kit (Illumina Inc.) and sequenced using the HiScanSQ (Illumina Inc.) with 93 bp × 2 paired-end reads.

Sequencing Data Analysis.

A bioinformatic pipeline based on a two-step alignment-and-selection strategy was developed in order to classify and quantify each bacterial group. Illumina

reads were quality filtered and aligned against the SILVA reference database by using BWA.

Sequences covered at least for the 10% of the total length by uniquely aligned reads were firstly selected, obtaining a new dataset of putative subjects. Using these as a reference database, a second BWA alignment was performed and sequences covered at least for the 30% of the total length were then considered to give the final result. The naive 16S-rRNA genes classification attributed by the SILVA database was applied for the subjects taxonomic assignment. The percentage of the total number of uniquely-aligned reads for each sample was considered to estimate and to quantify each taxa.

Results and Discussion

In order to seize the Sardinian air microbiota and to distinguish microrganisms possibly linked to defined events (dust-carrying northbound winds from across the Mediterranean or air flows from opposite quadrant), meteorological forecasts and air circulation monitoring services were constantly observed to anticipate and choose suitable sampling dates. Two significative Dust events have been selected on the basis of meteorological conditions, occurred from November 2013 to December 2014.

The obtained results and discussion of the two analysed events will be describe in two separate chapters DUST I and DUST II. Moreover filamentous fungi and parasite (Amoeba) detection will be treated in a third chapter DUST III.

DUST I: "Microbial immigration across the Mediterranean via airborne dust".

In this part of the study, 4 filters samples (043,044,046 and 047) collected during one unique significant meteorological event from 19th to 20th February 2014 were processed for microorganisms identification by colturable and uncolturable methods and the comparison of sand filter versus control filter results were performed.

Meteorological event

The sampling was done by considering four-days time laps. Back trajectories plots were used to project the main potential source of dust and confirmed the intrusion of African air masses occurring on February 15th through 20th 2014. On the subsequent period from February 20th, the above circulation regime halted, high pressure cells over Europe caused opposite winds blowing from

northeastern origin, which passed over Italy and Sardinia and allowed to collect samples representing the dust-free control conditions. The first intrusion of African air masses occurred on the 15th-17th, mainly affecting the PM10 levels in the north of Sardinia. On the 18th and 19th February 2014 air masses loaded with dust crossed the Mediterranean northward and eastward towards Italy and Sardinia, affecting the whole island. The analysis of the PM10 daily pattern registered in February at the sampling sites showed increasing values during the dust event at both sites with a marked decrease on the 20th. The arrival of air masses loaded with dust from North Africa was confirmed by satellite imagery (Figure 1).

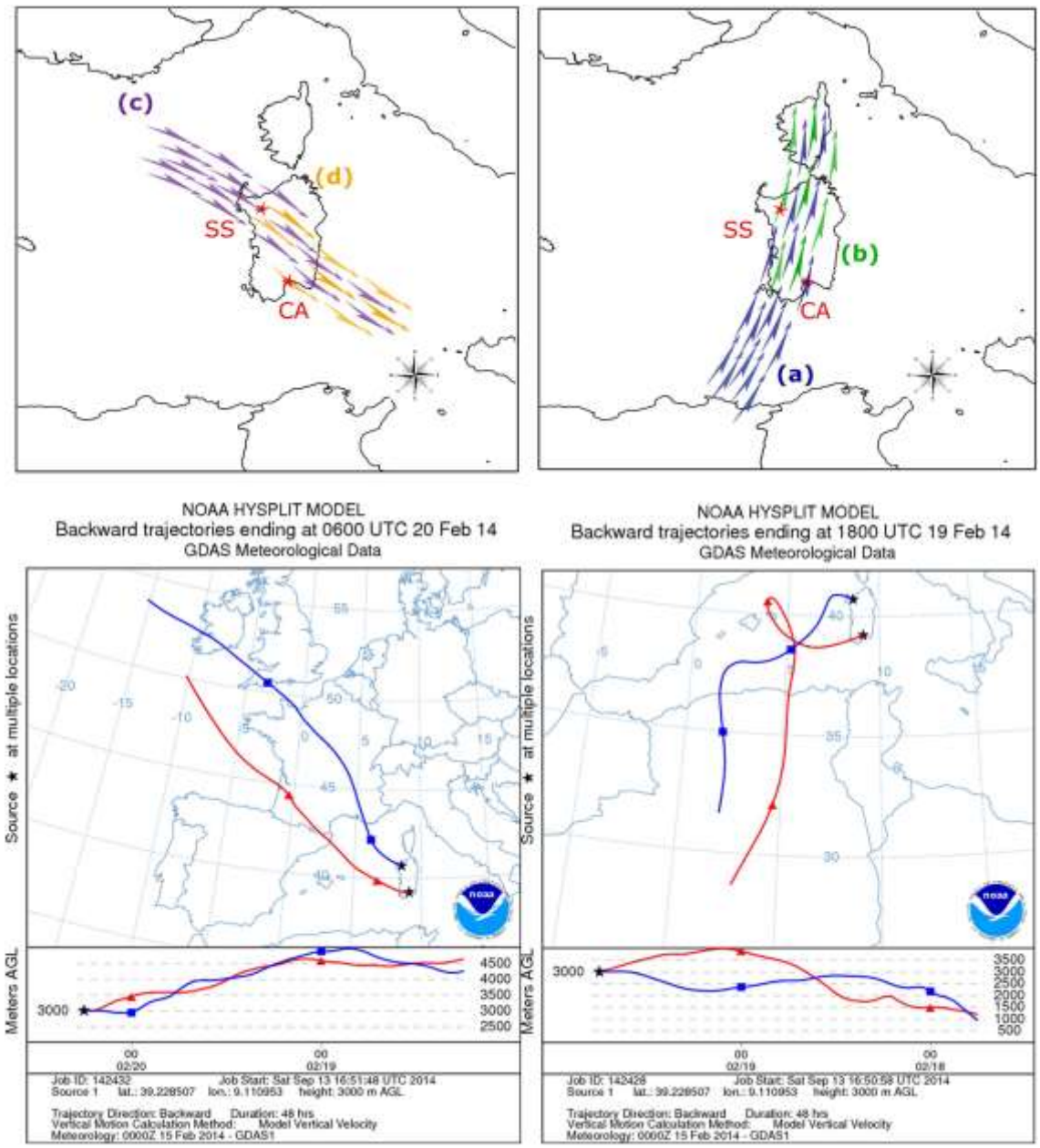

Figure 1. Reconstruction of the wind flows crossing the two sites during the sampling dates. Top left panel: control conditions event with winds blowing from northwest to south-east, Top right panel: African dust-carrying event with northbound winds. Bottom panels: corresponding particle back-trajectories simulations evaluated by the HYSPLIT model for the two sampling days. SS: Sassari sampling point; CA: Cagliari sampling point. Letters and colored arrows in the two top panels indicate four theoretically distinct pools of putatively airborne bacteria: (a) from Africa and Mediterranean sea (blue arrows); (b) the same plus those raised from inland Sardinia in northbound wind motion from Cagliari to Sassari (green arrows); (c) from continental Europe and Tyrrhenian sea (violet arrows); (d) the same plus those raised from inland Sardinia in southbound wind motion from Sassari to Cagliari (orange arrows). Source of images: top panels: this work; bottom panels: output of the public website service software HYSPLIT (http://ready.arl.noaa.gov/ HYSPLIT.php).

Exploring Diversity of Microorganisms in the Airborne dust through Culture-dependent and Culture-Independent Analysis of 16S rRNA Gene.

The results of the culture-dependent approach, gave us some informations about the upper abundance of microbial species in the atmosphere. Fungi genera were more abundantly isolated and mostly represented by Aspergillus spp., while among bacteria Bacillus spp. was the leading culturable taxon. Species identified among Bacillus spp. included *Bacillus simplex, Paenibacillus amylolyticus, Brevibacillus formosus, Bacillus megaterium, Bacillus cereus, Kocuria rosea.* There is solid evidence in the literature a small fraction of airborne microorganism is viable[30]. The recovery of only these species can be related to the genetic advantages that fungi and bacillus have over many other microorganisms, to be capable of producing spores. Spores enhance survival during transport and periods of prolonged environmental stress and for that they can growth in solid agar. Spore-forming organisms, such as Bacillus species and other Gram-positives, and Aspergillus sp. tend to dominate culture-dependent surveys of airborne microbial diversity as reported from other authors[1] .

Due to the inability to cultivate most bacterial taxa, unfortunately culture-based methods do not cover all the microbial communities and their diversity. On the other hand, culture-independent methods via NGS provided much more information regarding atmospheric sand-associated bacterial composition.

The mean quantitative results of the NGS sequencing of 16S in the different samples are shown in Table 1. The qualitative results of the sequencing annotation allowed the identification of several hundreds of operational taxonomical units per sample. The data at genus rank level are shown in Table 2

where the number of genera and the corresponding ecological indexes calculated from the community data are reported.

The most abundant phyla recorded in all samples were Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria as shown in Figure 2. A higher amount of orders belonging to Proteobacteria can be observed in the SS-Dust community. Higher percentages of Firmicutes (the Bacillales order in particular), characterized the two samples collected in Cagliari site (CA-Dust and CA-Control). The prevailing occurrence of a common core of shared taxa is shown in detail by the overlapping Venn diagrams representation in Figure 3. The highest proportions of taxa were those shared by all four samples, 92.3% and 95.9%, 85.6% and 95.6% for CA-Dust, CA-Control, SS-Dust and SS-Control, respectively, consisting in 138 genera. Conversely, the non-overlapping occurrences were all within frequencies ranging from 0.25% (CA-Control) and 3.03% (SS-Dust). Although limited in comparison to the constant core higher numbers of event-specific genera occurred for the two dust-exposed samplings, with 90 and 45 for Cagliari and Sassari, respectively. Principal Component Analysis (PCA) and cluster analysis (Figure 4) defined groups where the dust-related samplings occupied distinct positions while the two controls occurred in a closer range of the ordination plot. The cluster analysis also showed shorter distances between the two controls and longer branches separating the dust samples, in particular for the one collected in the most southern location (Cagliari). The culture independent work have been used to understand the link between atmospheric environmental conditions, like dust events, and the occurrence of particular microbial species. The taxonomical composition of the core bacterial microbiome in comparison with the locality-shared, whether event-shared, or uniquely occurring groups at phylum and class level is shown in Figure 5. Concerning the identities of specific genera within the relevant phyla, as shown

Actinobacteria were identified in all the conditions with lower percentages in the two dust-related samples, 7.5% and 3.8%, for Cagliari and Sassari respectively, and higher for the two controls, 14.8% and 11.0%. Within this major phylum the genus Propionibacterium represented the most abundant. This taxon was previously reported as desert dust particles and particles-associated pollutants but our data indicated higher percentages in the two controls. These two samples shared moreover an amount of other exclusive Actinobacteria genera. On the other hand, interestingly, higher percentages of Corynebacterium, already detected in the Saharan air dispersed-particles characterized the two dust-related samples. Members of the Bacteroidetes phylum were identified in all the datasets with percentages varying from 16% (dust) to 28% (control) in the two Cagliari samples while in the Sassari samples, these values were about double as Bacteroidetes-related reads represented from 32% to 59% of the total uniquely-aligned reads in the dust-related and in the control-sample respectively. Several authors observed their environmental abundance. In terms of taxa variability it is worth noticing in the analyzed datasets, that although different at genus level within each sample the relative percentages of the Bacteroidetes in control vs. dust samplings, remain surprisingly unaltered. About Firmicutes, the two Cagliari samples showed higher percentages, with a maximum that reaching the 57% for the dust-related samples and 30% for the negative control. Lower values were shown in Sassari, with percentages between 20% and 12% respectively. This phylum was often reported as one of the most abundant in the air-collected samples[4]. The Bacillus genus was detected but it does not represent the most abundant. High percentages of Streptococcus and Lactococcus characterized both Cagliari samples and they were always detected in lower percentages; these genera had rarely been reported as particle associated in literature. Moreover, several Firmicutes like Anoxybacillus were

identified as specific taxa characterizing the Cagliari samples. A different situation was observed for the Proteobacteria phylum: an opposite trend was evident analyzing the four samples: percentages, from 16% to 28%, characterized the two Cagliari samples, dust and control, respectively, while opposite proportions, from 39% to 16% occurred the two dust-related and control from Sassari. Notwithstanding the phyla disproportions, genera distributions within them did not show evidence of consistent trends or correlations. Interestingly, several groups were shared between the two dust-related samples. An amount of Alphaproteobacteria and Gammaproteobacteria characterized all the analyzed conditions but within this phylum several differences between dust-events and controls were detected. In particular, Alphaproteobacteria were found as dust-related samples. Taxa previously reported as desert-dust associated as Paracoccus, Sphingomonas, Methylobacterium or never detected before in these environments, as Caulobacter and Brevundimonas appeared to characterize the samples.

Some exceptions were also encountered: some Gammaproteobacteria like Citrobacter, Cronobacter, Klebsiella, Pantoea and Stenotrophomonas were shared between the Cagliari-control and the Sassari dust-related samples. These shared genera flanked an amount of other Alpha and Gammaproteobacteria that characterized all the analyzed conditions, indicating a broad distribution and their relevance in the Sardinian air-microbiome definition.

Sample	CA-Dust	SS-Dust	CA-Ctrl	SS-Ctrl
n. of paired reads	1'331'182	1'664'672	1'270'770	1'125'836
n. of uniquely aligned reads	756'655	1'051'021	780'713	644'623
PRJNA274749 (Bioproject)	SAMN03332112	SAMN03332148	SAMN03332147	SAMN03332149

Table 1. Total number of sequences and uniquely aligned reads obtained from samples by NGS

Samples	CA-Dust	CA-Ctrl	SS-Dust	SS-Ctrl
N. of Genera	345	232	293	259
Simpson Index	0.891	0.910	0.931	0.818
Shannon Index	3.204	3.086	3.487	2.717

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Table 2. Number of genera detected by 16S sequence analysis in the four cases and species diversity indexes.

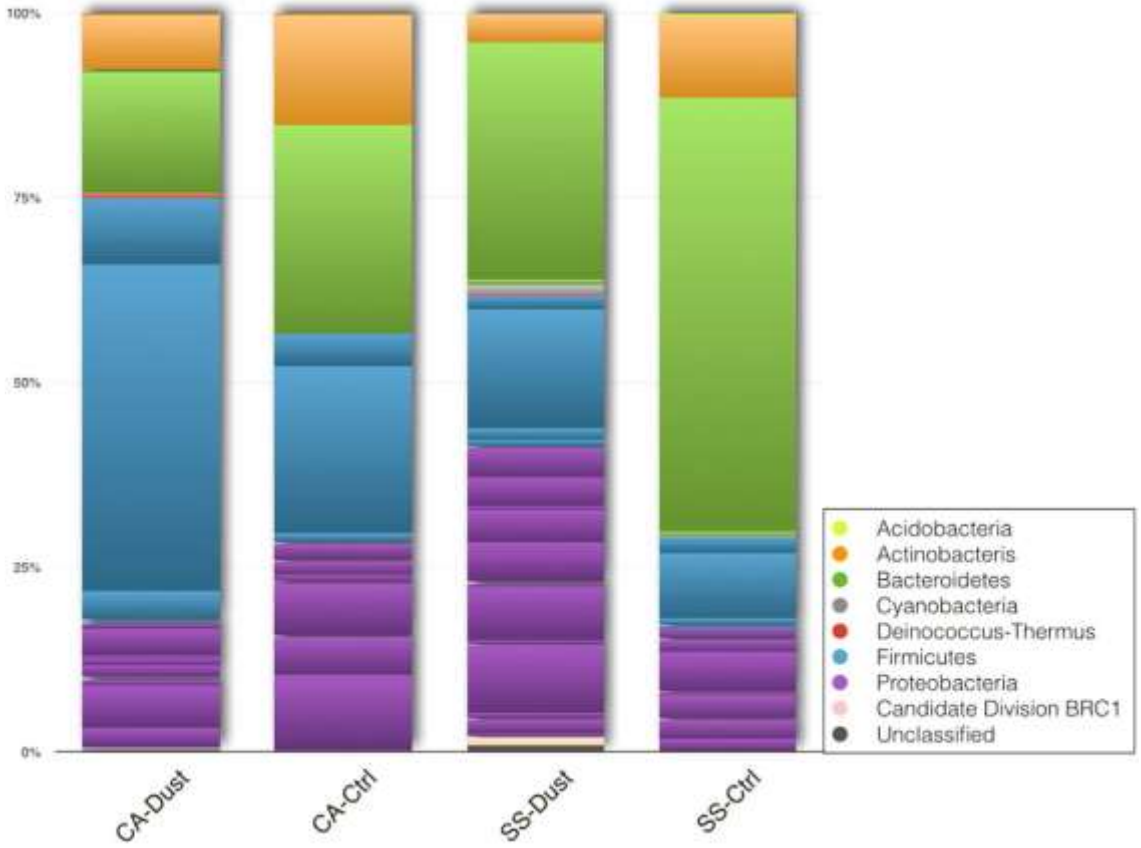

Figure 2. Percentages of sequences assignable to database-identifiable bacterial in each of the two sampling sites at each event (Control or Dust). CA: Cagliari sampling site, SS: Sassari sampling site. The slices within each phylum block of the same color indicate the number of orders found within that phylum.

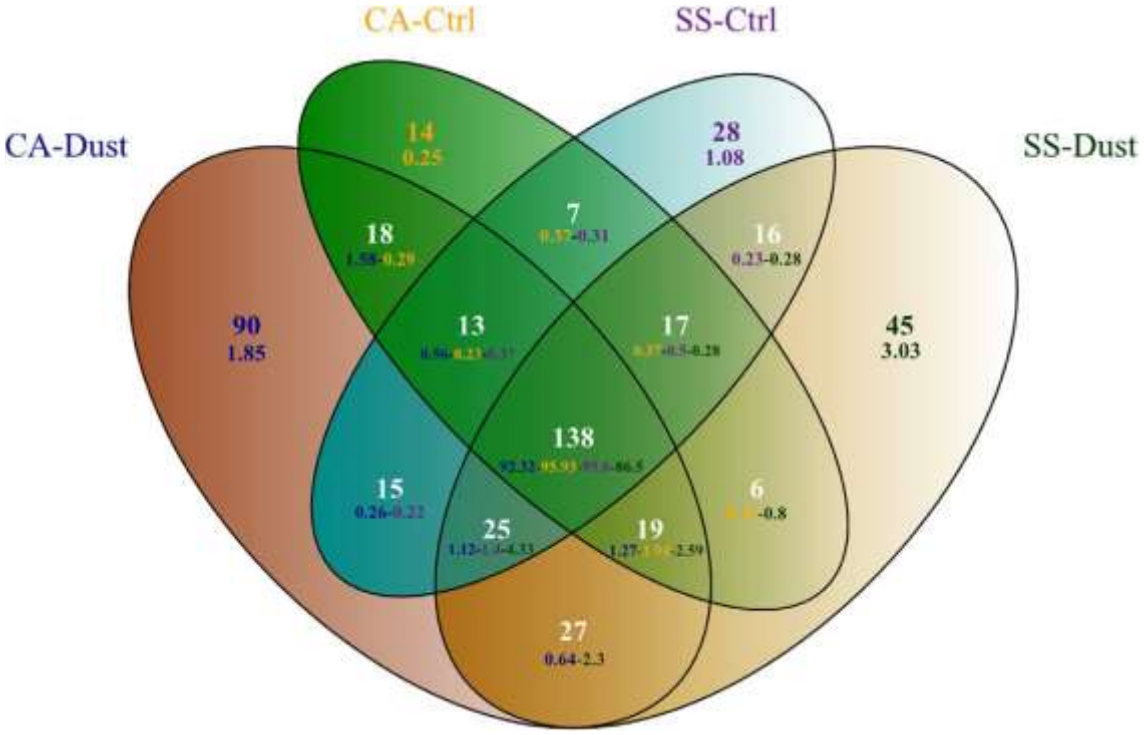

Figure 3. Venn diagram showing the number of specific or shared genera identified in each of the four locations/conditions and the relative percentages (smaller font digits).

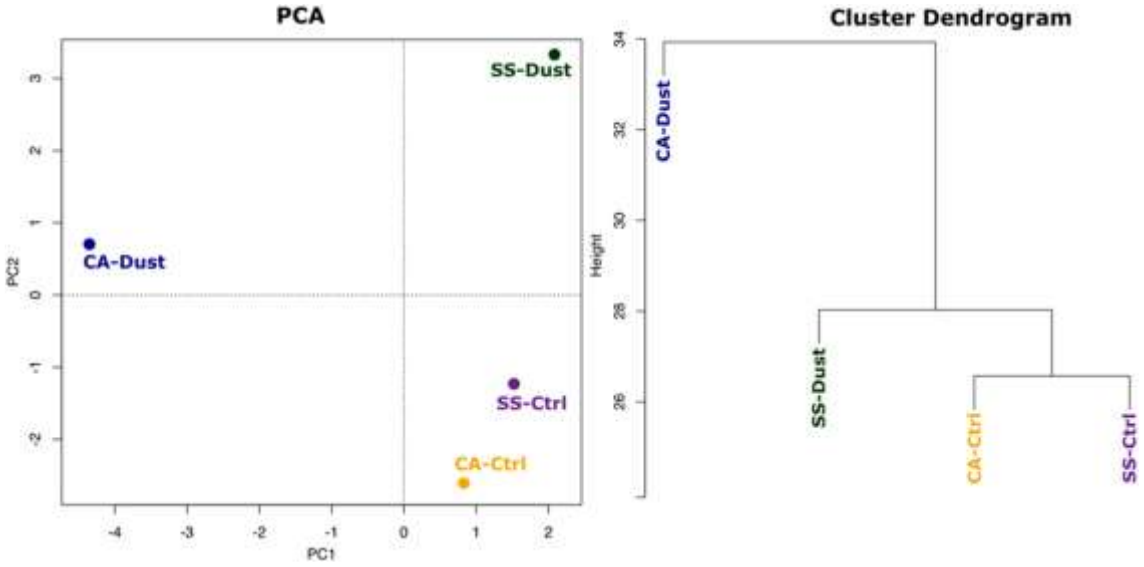

Figure 4. Principal Component Analysis (left) and Cluster Analysis based on euclidean distance, with average linkage method (right) based on the identified genera.

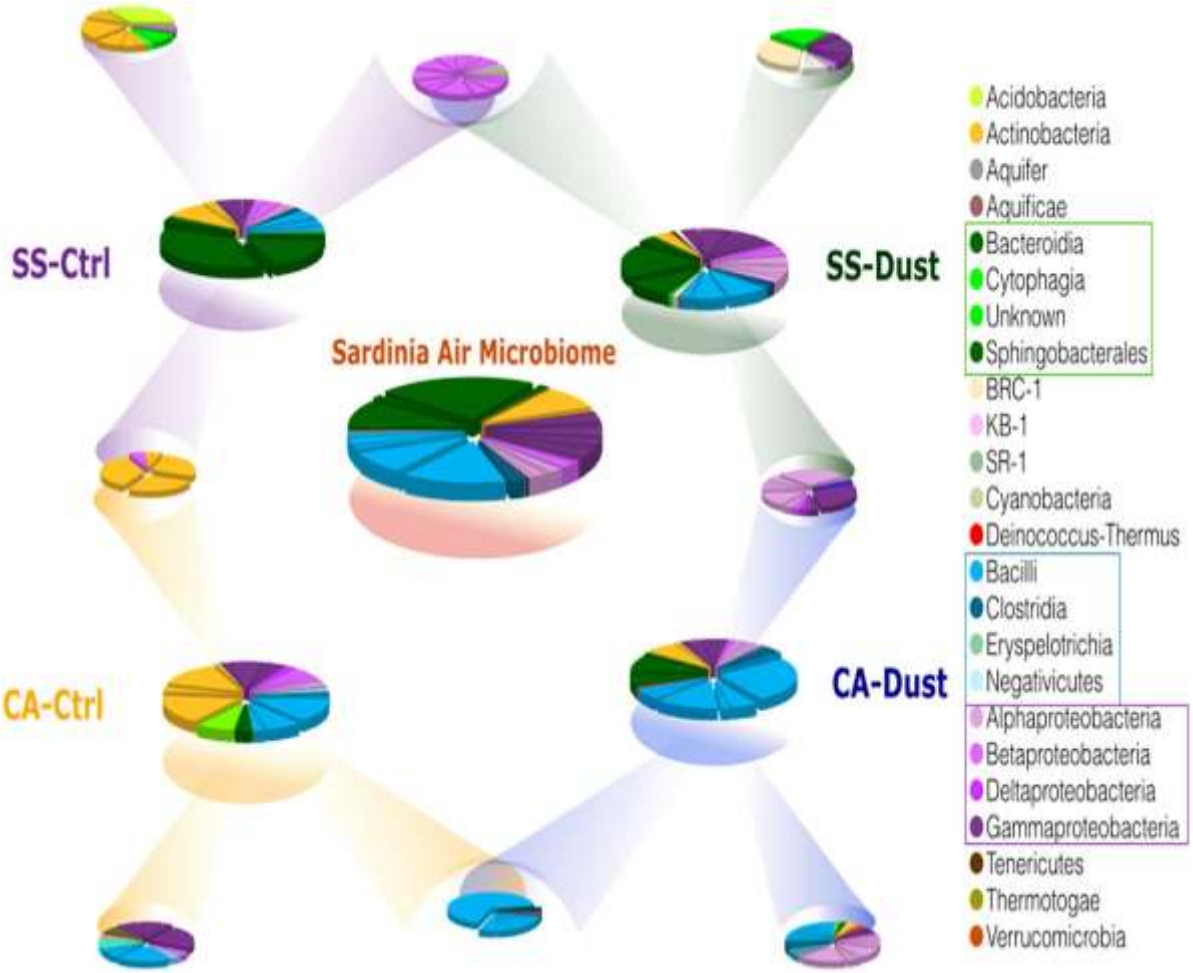

Figure 5. Proportions of identified phyla and classes in the four sampling cases. The legend on the right shows the color codes of the phyla, or, for three of them, the occurring classes listed within boxes in the legend as follows: Bacteroidetes (green tints); Firmicutes (blue tints); Proteobacteria (purple tints). The larger central pie chart shows the situation of the constant core of taxa shared by all four cases, constituting the putative Sardinia air microbiome, supposedly independent from place and weather conditions. The four inner-diamond small pies show the taxa shared pairwise by the 'locality pairs' (Cagliari or Sassari) or by the 'whether condition pairs' (dust or control). The four outer-corners small pies show the unshared (specific) fraction of each case. Pies diameters not drawn in scale with sequences frequency number.

DUST II: "Seasonal patterns rule the composition of airborne bacterial communities over the Mediterranean basin"

In this second part of the study, we analysed 6 filters (085-087-090-091-095-097) collected from 21th to 27th of May and September 2014.

In order to describe local, environmental and seasonal effects which could modulate the air-associated bacterial community composition, comparisons were performed by setting a scheme with three levels of analysis:

i) Local biodiversities occurring and possibly recurring in each of the two given sampling sites (Northern or Southern Sardinia) independently from the opposite meteorological scenarios or from the season;

ii) biota specifically appearing or variating within air collection during dust outbreaks in May or September (distinguishing also the first and the second 12h time lapses within the dust carrying event);

iii) biota displaying seasonal variations being specific or enriched in one or the other sampling seasons (spring vs. fall) (Figure 6).

Meteorological events

On May 21st-22nd 2014 air masses loaded with dust crossed the Mediterranean northward and eastward towards Sardinia, affecting the whole island. The analysis of the PM10 daily pattern registered throughout the second fortnight of May at Northern and Southern sampling stations sites showed a rising of values during the dust event in both sites (Figure 7). The arrival of air masses loaded with dust from North Africa was confirmed by pressure and wind fronts synoptic charts (Figure 8), and satellite imagery (Figure 9). Particle itinerary was tracked by reconstructing the 3-day air mass backward trajectories as calculated by the

NOAA HYSPLIT model (Figure 10) tracing the Northern African zone as origin of the convective motion for dust discharged over Italy on May 21st and 22nd.
The 27th of May was chosen as “clear day” as it was characterized by a synoptic situation that no longer favored the transport of air masses from Africa towards Sardinia. That day was indeed characterized by low pressures over Europe and high pressures over Libya, Algeria, Mali and Mauritania (Supplementary Figures 8 and 9). Analysis of the back-trajectories confirmed that on May 27th the air masses arrived from north-western regions (Figure 10 upper panel, rightmost image).
A dust outbreak occurred again in Sardinia in the second half of September 2014. The situation was characterized by a low-pressure system off the north-western coast of the Iberian Peninsula that extended towards Morocco and by the associated high-pressure system over North Africa (Algeria, Tunisia and Libya) and Sicily. This barometric configuration favored again the transport of dusty air masses towards the Mediterranean basin, especially towards Sardinia and Southern Italy. The arrival of the African air masses caused the daily mean air temperature to rise above the mean air temperature of the month of September (a peak can be observed on September 20th at the northern sampling site of Sassari and on September 21st at the Southern site of Cagliari (Figure 10-a,b). As consequence, on September 19th-21st 2014 a dust outbreak of African origin vas conveyed over the Mediterranean towards Italy and Sardinia, covering it in its entirety. Corresponding atmospheric pressure values and wind fronts are shown in Figure 12. The analysis of the PM10 daily pattern registered at the two sampling stations in Sardinia showed a rising of values during the dust event in both sites (Figure 11-c). The arrival of air loaded with dust from North Africa was again confirmed by satellite imagery (Figure 13) and by the 3-day air mass backward trajectories (Figure 10 lower panel).

The back-trajectories showed that on September 19th and 20th the air masses arrived from North Africa. Prior to this event, September 13th had been instead characterized by low pressures over Italy and high pressures over Mauritania and the southern part of Morocco and Algeria. This synoptic situation did not allow the transport of air masses from Africa towards Sardinia and the day was thence chosen as "clear day" control. Dusty air samples for this dust outbreak were therefore collected on the days 19th through 20th, while the control air was taken on September 13th.

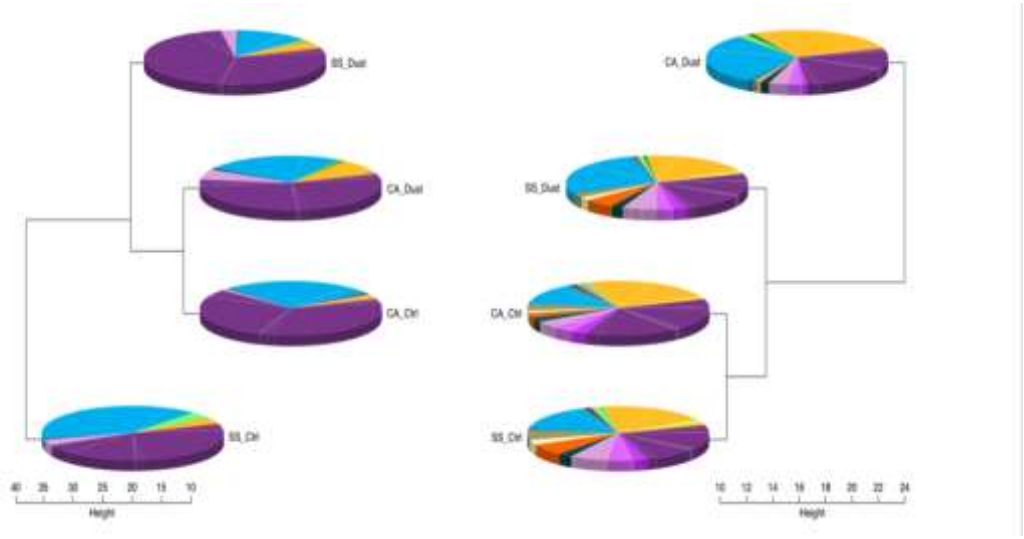

Figure 6. Cluster Dendrogram (Euclidean distance method, complete linkage) on the identified bacterial orders. May samples (the four left column pie charts) compared sideways to the corresponding September samples (right column pie charts). Data from the two sampling stations of Sassari (SS, northern Sardinia) and Cagliari (CA, Southern Sardinia) are shown, comparing the two wind regimes ('Dust': during dust outbreaks under winds from Africa, and 'Ctrl': Control, under winds from Europe). Pie colours coding (clockwise): yellow: Actinobacteria; light green: Acidobacteria; red: Verrucomicrobia; dark purple: Gammaproteobacteria; fuchsia: Deltaproteobacteria; light fuchsia: Betaproteobacteria; light pink: Alphaproteobacteria; black: Planctomycetes; orange: Nitrospirae; white: NC10; khaki: Gemmatimonadetes; blue: Firmicutes; brown: Chloroflexi; grey: Chlorobi; green: Bacteroidetes; dark green: Armatimonadetes.

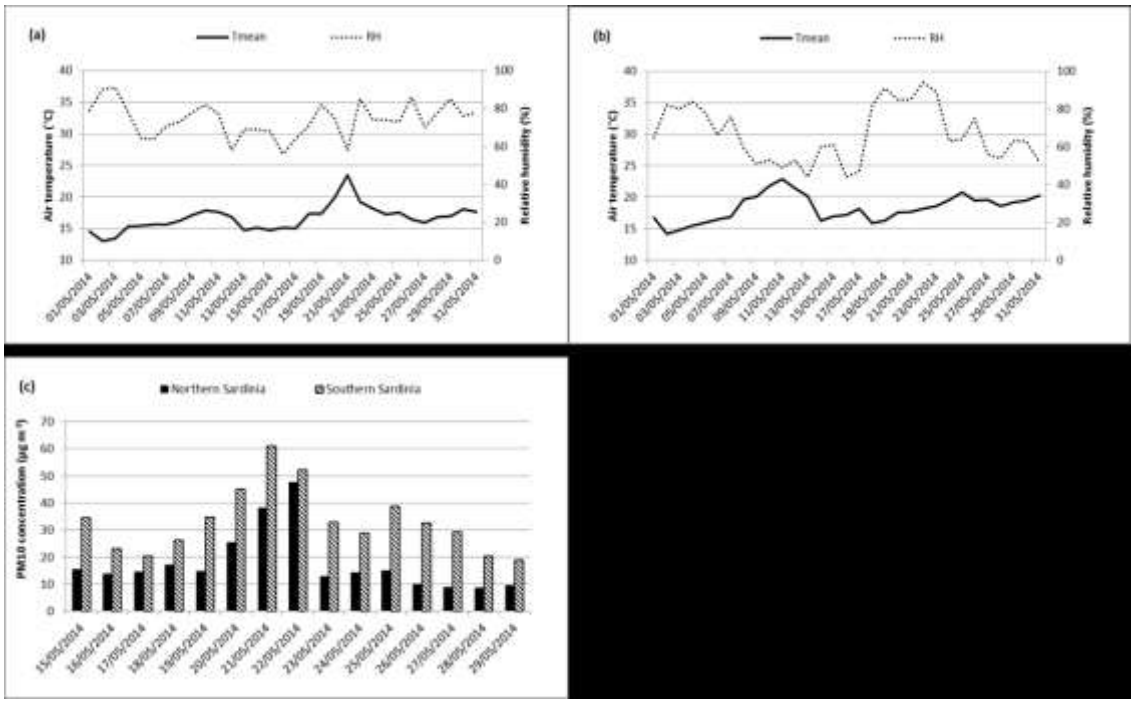

Fig. 7. Daily mean air temperature and mean relative humidity of May 2014 in (a) Sassari (Northern Sardinia) and (b), Cagliari/Domus de Maria (Southern Sardinia). (c): daily PM10 values at monitoring stations in Northern Sardinia (40.72°N, 8.55°E) and southern Sardinia (39.24°N, 9.12°E)

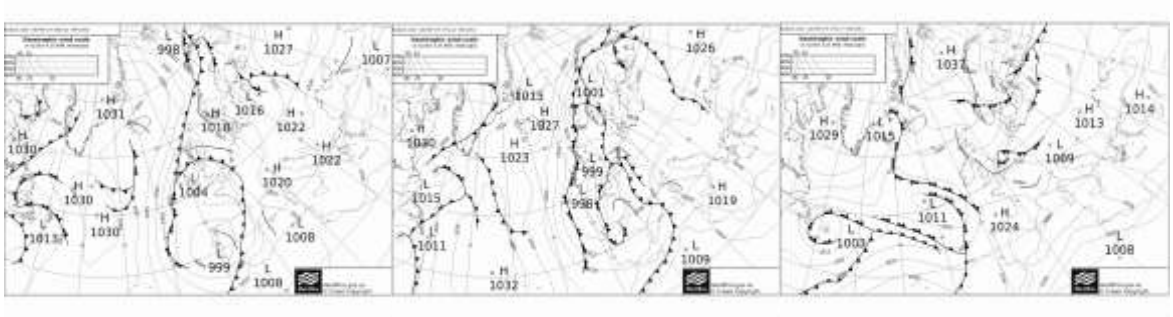

Fig. 8. Synoptic charts showing surface pressure and wind fronts for the dates of May 21st, 22nd and 27th 2014 (credit to: www.metoffice.gov.uk).

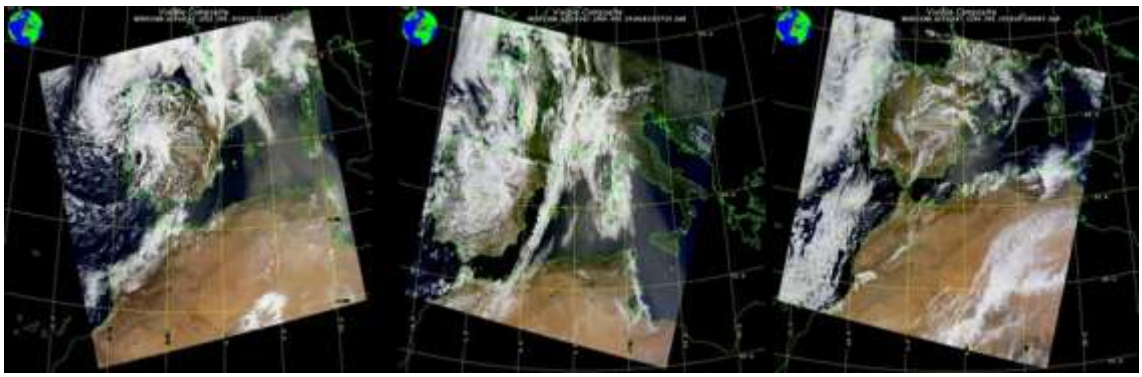

Fig. 9. Satellite images (MODIS) – May 21st, 22nd and 27th 2014 (credit to: ladsweb.nascom.nasa.gov).

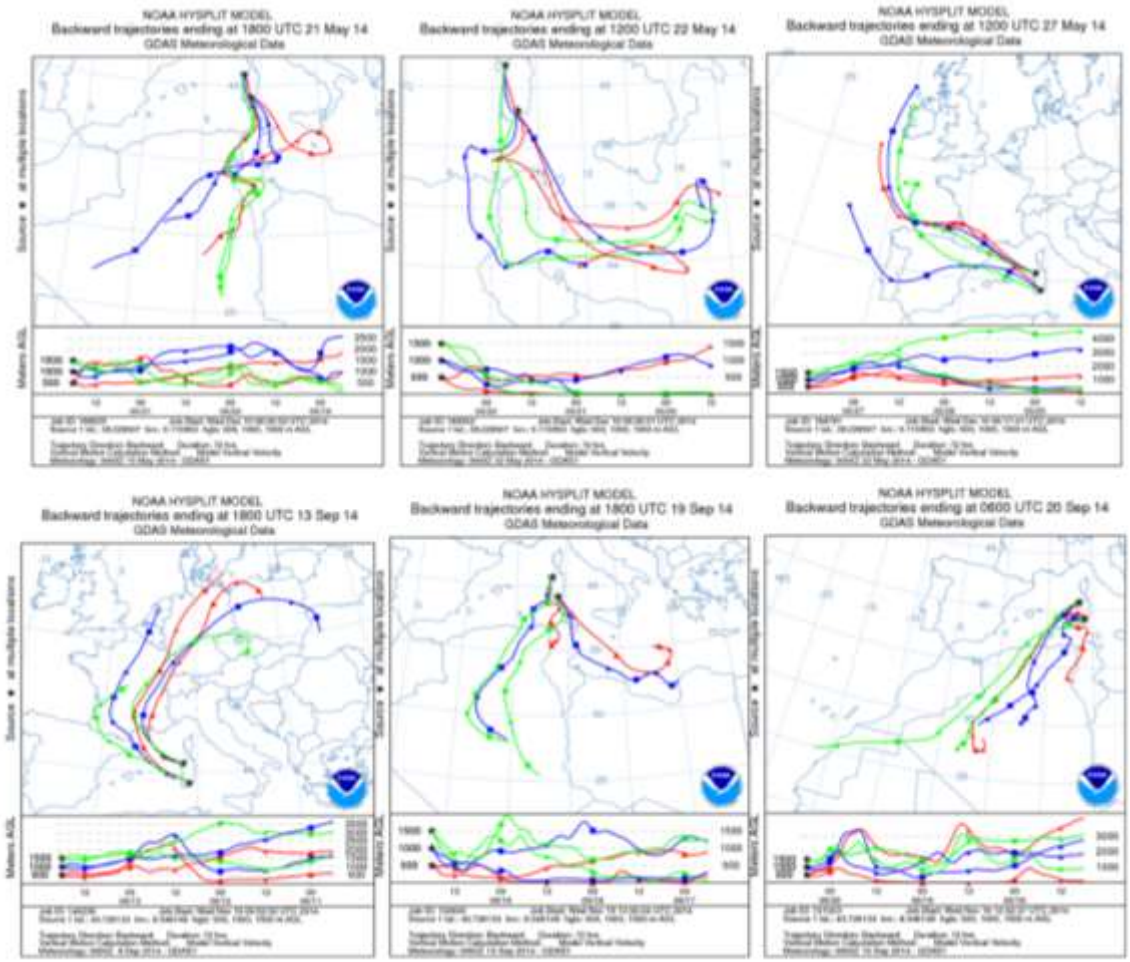

Figure 10. Upper panel: 3-Day air mass backward trajectories calculated by the NOAA HYSPLIT model ending at 18:00 UTC May 21st, 12:00 UTC May 22nd and 12:00 UTC May 27th 2014 at both sampling sites. Lower panel: 3-Day air mass backward trajectories calculated as above, ending at 18:00 UTC September 13th, 18:00 UTC September 19th and 06:00 UTC September 20th 2014 at both sampling sites (credit to: ready.arl.noaa.gov/HYSPLIT.php).

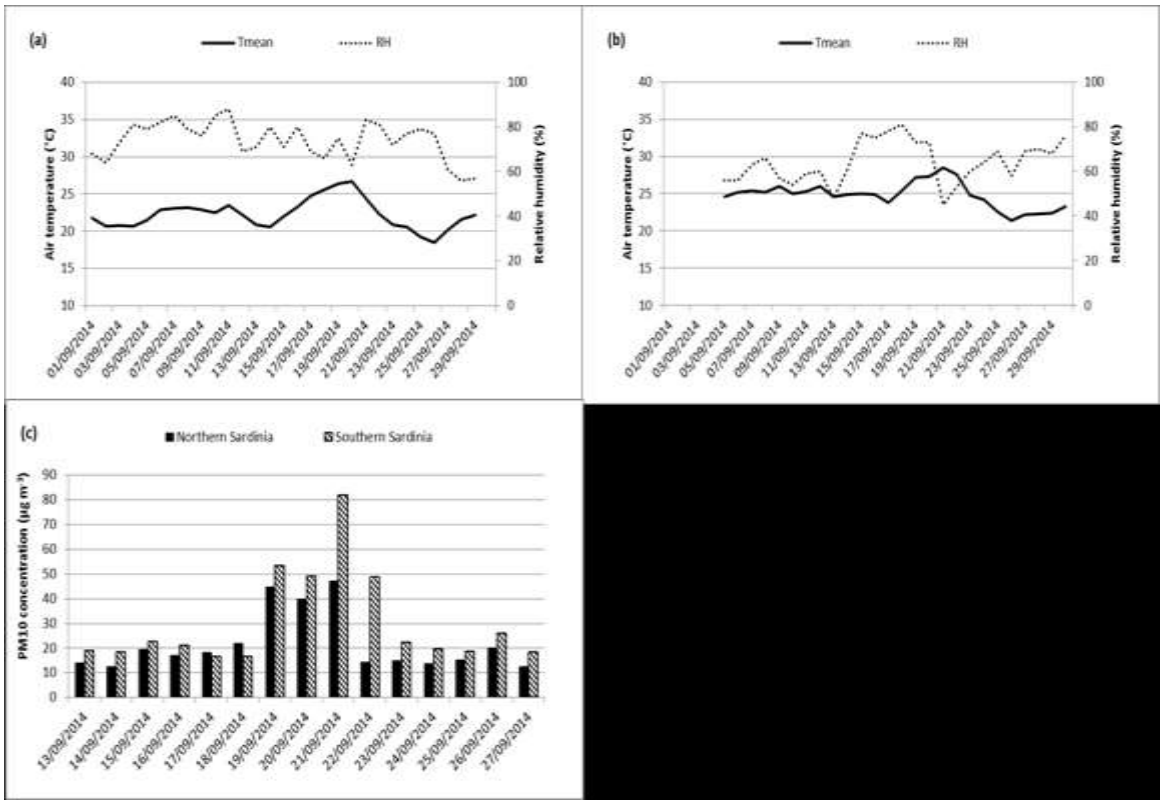

Figure 11. Daily mean air temperature and mean relative humidity of September 2014 in (a) Sassari (Northern-Sardinia) and (b), Cagliari/Domus de Maria (Southern Sardinia). (c): daily PM10 values at monitoring stations in northern Sardinia (40.72°N, 8.55°E) and southern Sardinia (39.24°N, 9.12°E)

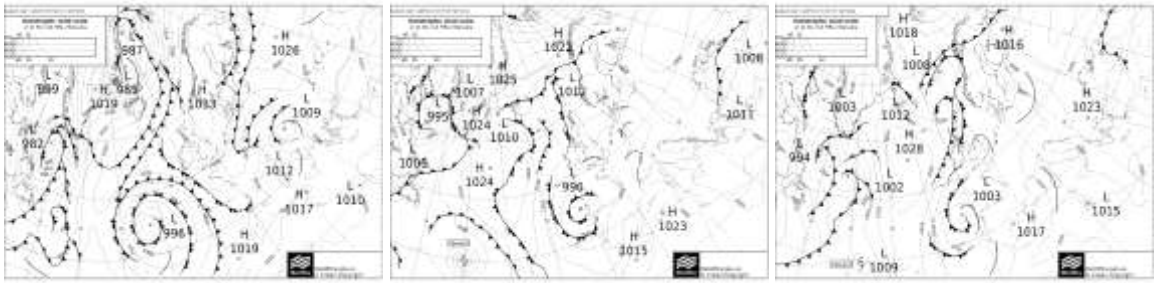

Figure 12: Synoptic charts showing surface pressure and wind fronts for the dates of September 13th, 19th and 20th 2014 (credit to: www.metoffice.gov.uk).

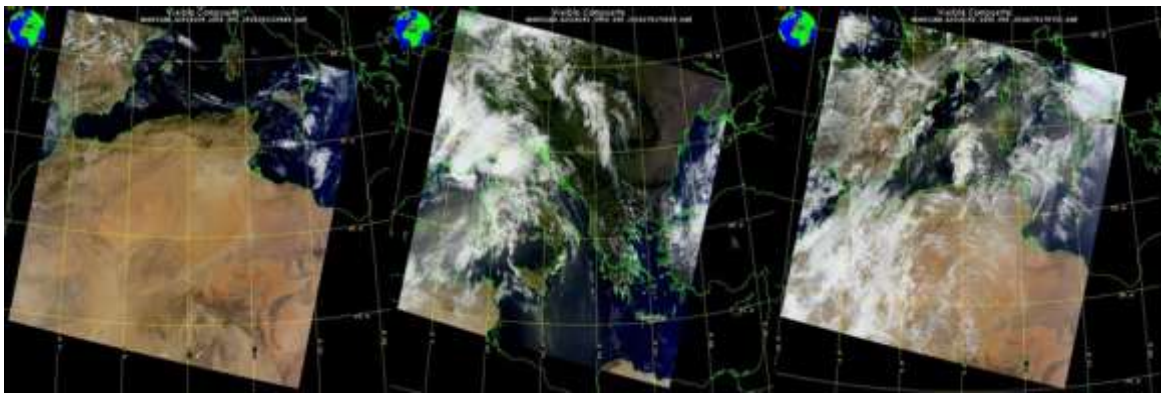

Figure 13. Satellite images (MODIS) – September 13th, 19th and 20th 2014 (credit to: ladsweb.nascom.nasa.gov).

Exploring Diversity of Microorganisms in the Airborne dust through Culture-Independent Analysis of 16S rRNA Gene

A common core of conserved taxa was observed. Each samplings, was characterized by a major background of shared orders and classes related to Gammaproteobacteria, Bacilli and Actinomycetales, which were consistently identified. These represented a minimum of 50% of the observed OTUs in the Sassari controls of September and reached values above 90% in all samples collected in May.

Regarding Actinobacteria, Actinomycetales represent the most abundant airborne order of this phylum found in all the samples considered. Higher percentages characterized the Cagliari (Southern) sites during the dust-outbreaks by doubling their amount if compared with the respective dust-free controls. Actinobacteria biodiversity increased from May to September enriching in the season-specific Thermooleophilia class with Gaiellales and Solirubrobacterales appearing at conspicuous frequencies. A soil-related 0319-7L14 Actinobacteria taxon characterized the September controls in both Sassari and Cagliari sites.

Within Firmicutes, Lactobacillales and Bacillales were defined as most abundant orders in the May samples. Higher percentages were identified in the Northern Sardinia Sassari control (37%), than in its dust-related counterpart (12%), while a constant value of about 25% characterized all the Cagliari (Southern) samples. An opposite trend arose when observing the September samples. The percentage of those orders of Firmicutes was less than 15% of the total population in the two controls, while higher values, more than the 25%, were found in both dust-related samples.

An higher biodiversity can be underlined by considering the taxa belonging to the Proteobacteria phylum. Gammaproteobacteria represented the most abundant class characterizing the May samples. It reached over 75% of the total bacteria identified in the Sassari dust-related sampling. Pseudomonadales and Enterobacteriales were always detected revealing a strong correlation between their relative abundances. Genera belonging to Alteromonadales as Marinimicrobium, Marinobacter and taxon OM-60 were identified as monthly signature characterizing May samples while Shewanella, that appeared in all the spring sequences, reached similar values in the dust-related September samples. Xantomonadales can be considered also monthly signatures characterizing the late summer sampling, in particular with a group of unidentified genera belonging to the Sinobacteraceae family, representing from 1.2% to 2% of the total bacterial population in the Cagliari and Sassari dust-free controls, respectively.

Focusing on Alphaproteobacteria members, Caulobacterales were always detected reaching 1% of the total bacterial population with Brevundimonas-related genera in the Cagliari May dust-related sample. Rhizobiales-related organisms characterized both May and September, Cagliari and Sassari samples reaching 3% in the May Cagliari dust-related sampling and maintaining an average of 1.5% in all the September situations. These showed also increased Rhodospirillales percentages from 1% in the Cagliari dust-related to a 3.4% in the Sassari controls. In general, higher values could be observed in the Sassari collections as belonging to an unidentified family.

Lower percentages, less than the 1% of the total population, were defined for the May related Betaproteobacteria where Burkholderiales members become representative in the dust-related reads only. Higher percentages were observed

in the September samples where higher amounts characterized the controls with 2.7% and 4% for Cagliari and Sassari, respectively.

Organisms representing the soil-related Betaproteobacteria MND-1 and unclassified co-orders were found in these samples while a 0.7% of Neisseria-related sequences was represented only in the Cagliari dust-related sequences.

By looking the other bacterial clades, specific groups were identified as possible candidates suited to define local- and weather-related signatures. Mollicutes characterized the May samples while the orders of Pirellulales (phylum Planctomycetes), and the classes of Gemmatimonadetes and Nitrospira qualified as September-related taxa.

In order to define in more detail a possible effect related to the dust outbreaks, during those events the overall sampling period of 24 h was divided in two steps: filters from the first 12h time lapse were retrieved and new filters were employed for the second 12h lapse during which an increased air-particulate inflow was observed.

From this two-step analysis sequencing data were used to extrapolate bacterial taxa which would display considerable amounts of variation during dust-carrying events, and distinguish them from the less fluctuating groups. The latter could thus be regarded as the common microbiota present in the sampled sites irrespective of the dusty winds. In order to operate such distinction a threshold was applied considering the percent variation occurring between the first and the second half of the collecting period and taking into consideration only taxa whose mean variation was higher than ½ of their corresponding standard deviation.

The variation level upon applying such cut off criterion in the two collection sites and in the two seasons is shown in Table 3, and the resulting number of taxa at order rank level is reported in Table 4. A higher number of orders was positively

selected by the applied threshold for the samples collected in Sassari, in both seasons.

To observe in detail which taxa showed increases and of which extent along the dust outbreaks of May and September, values of the first 12h are reported as histogram bars above the baseline and are opposed by those of the second 12h sampling plotted below the same line (Figure 1 and 1). In order to assess the ecological features of the airborne discharged community the main diversity indices and the evenness value were calculated from the sequencing data and are reported in Table 3. While no significant changes in species diversity can be traced neither in relation to dusty vs. control winds, nor across the two localities, a major difference stands out when comparing the spring data to the fall ones, with all indicators of richness and evenness scoring consistently higher in the latter. The prevalence of the seasonal effect in relation to taxonomy is shown in Table 4 in which the mean of percentages of samplings within the May or September period (irrespective of location and wind provenances) are reported. From such table the main trends of these differences can be further appreciated. The rise of Actinomycetales in September and the appearance of a number of orders not detected in May is appreciable as well as the substitutional decline of the Enterobacteriales and Pseudomonadales which had led the scores in the much less biodiverse spring sampling. Bacilli show instead a much more stable and persisting behavior across the two periods.

Sample	Avg variation %	Min variation %	Max variation %
Sassari May - Dust	1.4	0.05	6.7
Cagliari May - Dust	2.1	0.5	5.0
Sassari September - Dust	1.3	0.4	5.4
Cagliari September - Dust	4.7	1.1	11.4

Table 3: Average, minimum and maximum percent variation between taxa counts harvested in the first 12 hours sampling period of the dust event and those harvested in the subsequent

12 hours sampling period. Only taxa displaying a difference in percentages higher than half of their standard deviation were selected for the present comparison.

Site and period	Total Orders	Selected Orders	% of Total Orders
Sassari May	56	16	28%
Sassari September	103	28	28%
Cagliari May	52	11	21%
Cagliari September	87	14	16%

Table 4. Community diversity at order level of taxa occurring during dust events and of those displaying variations higher than half the standard deviation between the first 12h and the second 12h sampling period (selected orders). The percentage of orders selected upon this criterion over the total of the orders observed in samples collected during the dust events is indicated.

Month, Event, Place	Simpson 1-D	Shannon H	Evenness
May Dust SS h 1-12	0.771	2.062	0.151
May Dust SS h 12-24	0.740	1.902	0.156
May Dust CA h 1-12	0.833	2.175	0.183
May Dust CA h 12-24	0.833	2.205	0.197
May *Ctrl SS	0.794	2.064	0.164
May Ctrl CA	0.778	1.900	0.142
Sep. Dust SS h 1-12	0.928	3.187	0.260
Sep. Dust SS h 12-24	0.914	3.015	0.240
Sep. Dust CA h 1-12	0.887	2.792	0.212
Sep. Dust CA h 12-24	0.838	2.339	0.176
Sep. Ctrl SS	0.948	3.438	0.311
Sep. Ctrl CA	0.936	3.292	0.286
May: mean ±SD	0.79 ± 0.04	2.05 ± 0.13	0.17 ± 0.02
September: mean ±SD	0.91 ± 0.04	3.01 ± 0.40	0.25 ± 0.05

Table 5. Ecological diversity and evenness indices resulting from the sequence checklist analysis in the different samplings. (*Ctrl =Control)

Phylum	Class	Order	Mean % May	Mean % September
Proteobacteria	Gammaproteobacteria	Enterobacteriales	27.40	11.55
Proteobacteria	Gammaproteobacteria	Pseudomonadales	26.67	9.90
Firmicutes	Bacilli	Lactobacillales	18.67	15.96
Actinobacteria	Actinobacteria	Actinomycetales	5.66	13.36
Firmicutes	Bacilli	Bacillales	4.46	6.56
Proteobacteria	Gammaproteobacteria	Alteromonadales	2.77	0.76
Proteobacteria	Gammaproteobacteria	Xanthomonadales	1.51	2.63
Proteobacteria	Gammaproteobacteria	Aeromonadales	1.51	0.96
Proteobacteria	Alphaproteobacteria	Rhizobiales	1.35	1.59
Bacteroidetes	Sphingobacteria	Sphingobacteriales	1.00	1.04
Proteobacteria	Alphaproteobacteria	Rhodospirillales	0.06	2.02
Actinobacteria	Acidimicrobiia	Acidimicrobiales	0.05	2.01
Nitrospirae	Nitrospira	Nitrospirales	0.01	3.38
Actinobacteria	Thermoleophilia	Gaiellales	0.00	2.81
Actinobacteria	Thermoleophilia	Solirubrobacterales	0.00	2.37
Gemmatimonadetes	Gemm-1	Gemm-1	0.00	2.52

Table 6. Percent frequency of sequences belonging to the indicated orders in the averaged data of all samplings (Dust and control) of each seasonal sampling period (May or September). Data in which frequencies were higher than 1% in at least one of the two seasons are reported. These represent the 91.1% of the total sequences for the May sampling (on a total of 65 orders found) and 79.4% of the September sampling (on a total of 118 orders found).

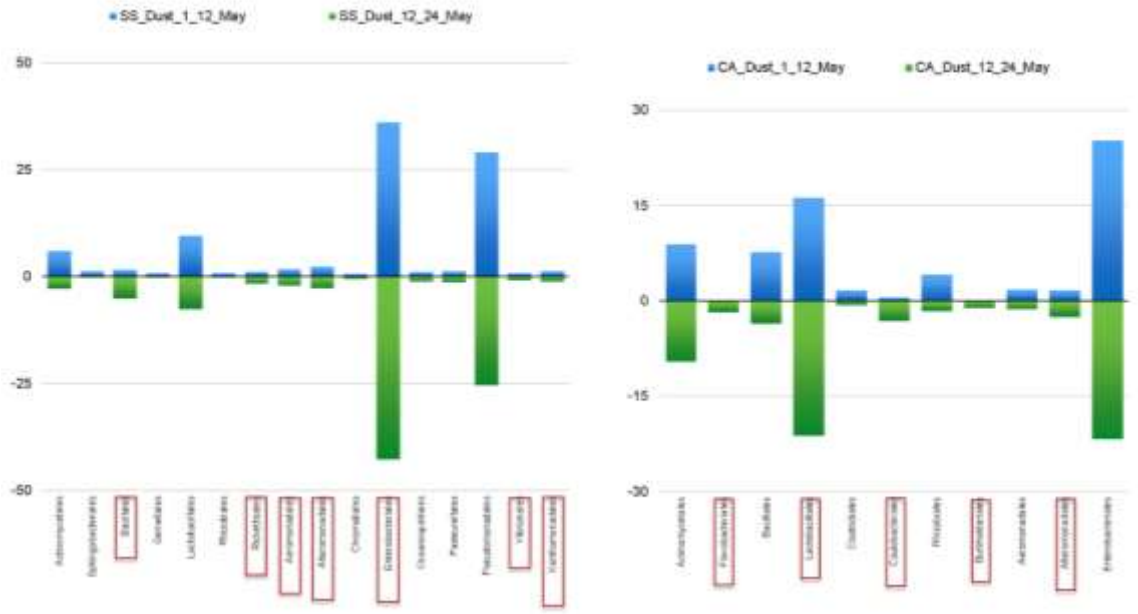

Figure 14. Percent of sequences belonging to orders observed in May samples in the first 12h (above baseline) or in the second 12h sampling period (below baseline) during the dust-carrying events in the two sites (Sassari: left; Cagliari: right). Boxes encage taxa which increase along with the dust storm.

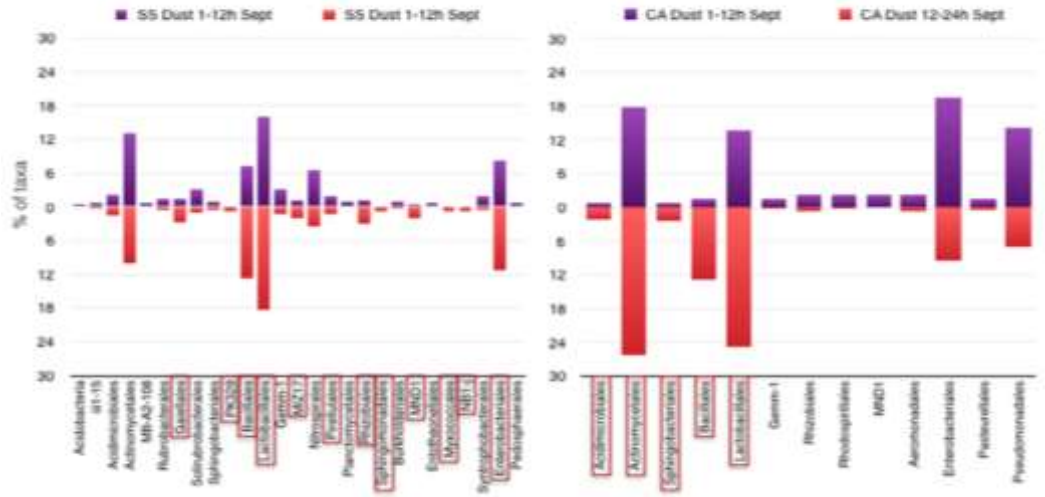

Figure 15. Percent of sequences belonging to orders observed in September samples in the first 12h (above baseline) or in the second 12h sampling period (below baseline) during the dust-carrying events in the two sites (Sassari: left; Cagliari: right). Boxes encage taxa which increase along with the dust storm.

DUST III: Exploring Diversity of Fungi and the presence of Amoebae in the airborne dust

In this part of the study we analysed 70 samples collected from air dust filters during November 2013-December 2014, in correspondence of some metereological events. For sampling Teflon filters were used and collected using sterile plastic sheets. After sampling, filters were processed under sterile conditions for coultivable method using Sabouraud agar medium for 15-30 days at room temperature. The other parts of the same filters were stored at 4°C until nucleic acid extraction for metagenomics analysis. The fungal community of dust sand samples was characterized by culture-dependent method by microscopy/morphology identification and culture-independent analysis via molecolar approach by sequencing of internal transcribed spacer (ITS) region.

The results highlighted a significative presence of fungi in airborne sand samples and in the environments suggesting their possible role and implications on human health. The culturable method evidenced the presence of filamentous fungi in the two/third part of dust filters collected during a period of a year.

In total twelve species of Ascomycota were isolated mainly from sand filters (*n*=22), borderlines (*n*=19), and controls (n 12) (Figure 16-18). The Aspergillus was the most represented genera, and its presence has been suggested to be related to direct or indirect contamination originating from organic sources and climate variations.

Our results showed a large fungal biodiversity during the four seasons. The prevalence and major variability of species were observed during the Spring and the Autumn.

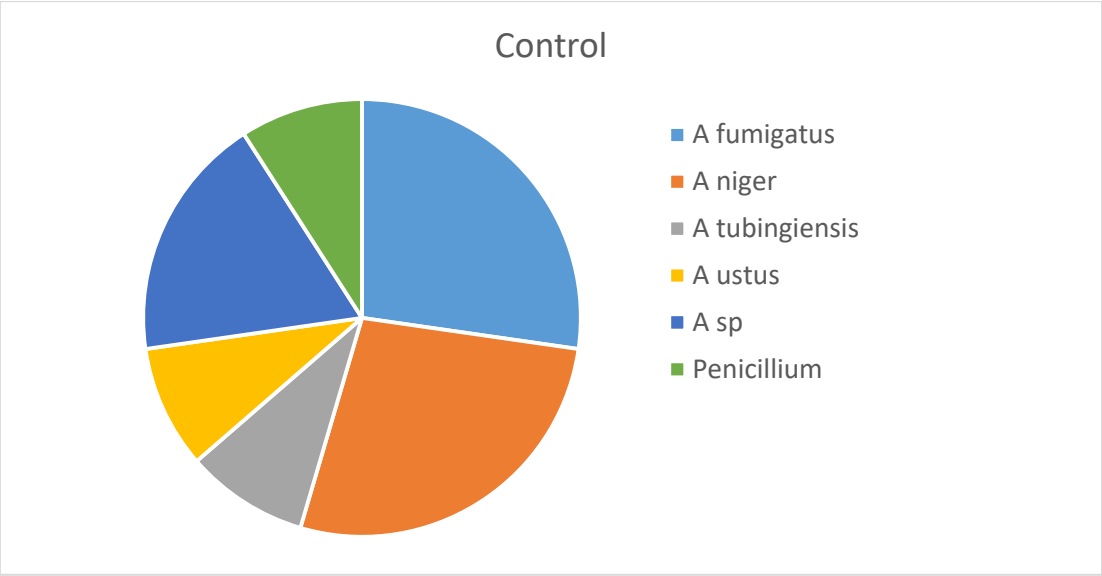

Figure 16: Filamentous fungi species identified in control samples

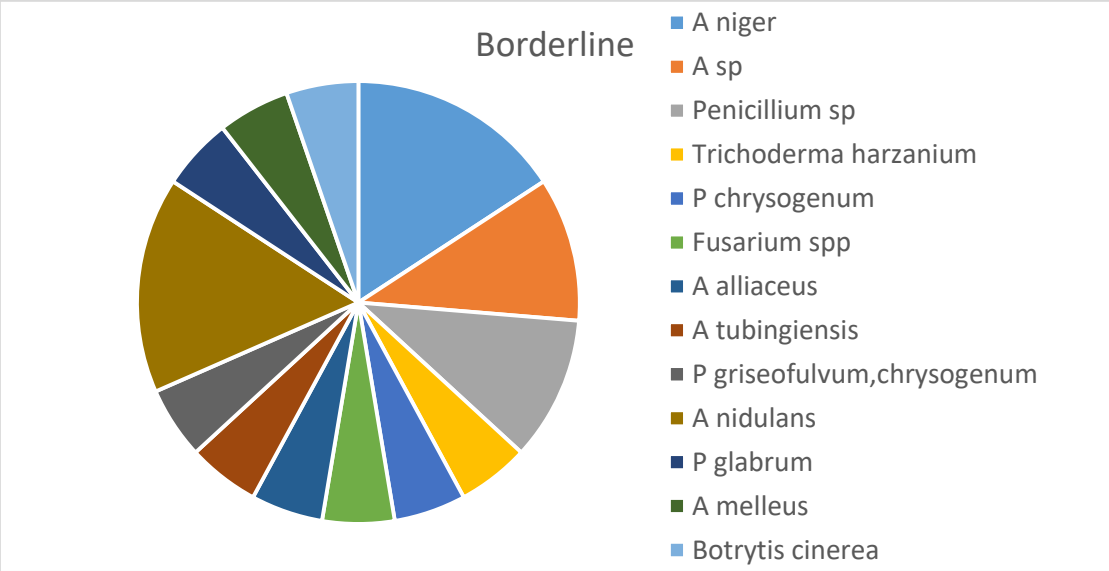

Figure 17: Filamentous fungi species identified in borderline samples

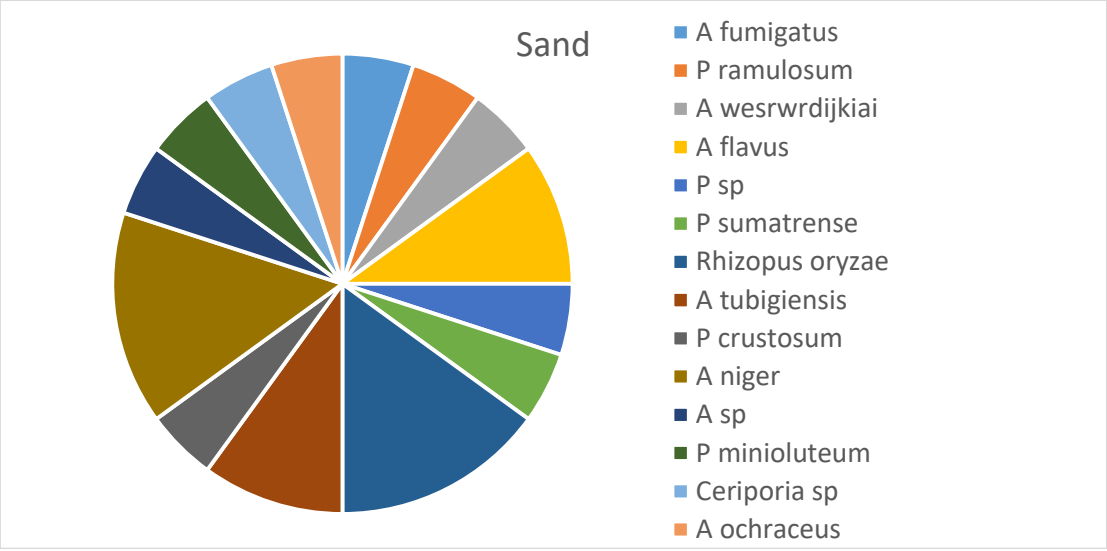

Figure 18: Filamentous fungi species identified in sand samples

Results were confirmed by metagenomic analysis on four dust filters (N° 142,143,148,152), collected during two dust events occurred in Sassari in October and November 2014. Ascomycota represented 1,5% of all microorganims detected while 0,52% of all microorganisms were Basidiomycota. Metagenomics data, confirmed the presence of the fungi during the dust event, but only at genera level, due to the limitation of this method, in accordance with other published studies [4].

In this case the traditional culturable method gave better results compared with the metagenomic method. Fungi are important residents of dust and their survival is longer than that of bacteria due to their capacity to form resistant spores.Moreover ITS sequences is able to identified fungi at species level while metagenomics approach is able to identified the genera.

Isolation of Amoebae in Dust event

Free-living amoebae (FLA) are unicellular protozoa widely distributed on all continents that are able to survive and replicate in the environment without a host and are able to cause opportunistic infections in humans and other animals.[31] FLA are present in a large variety of natural habitats like humid soil, rivers , lakes , swimming pool and external environments.

The free-living amoebae like Acanthamoeba and Naegleria species are found in a large variety of natural habitats. The cystic form persist in the environments for many years, permitting them to resist a different temperature and disinfectant. Several species are able to cause opportunistic infections.[32] However as far as we know, FLA have been never isolated from air-wind transported dust particles.

In this study during the Autumn period, the richest in sandy events, we were able to isolate an amoebae from air-filter N142 (October 2014). Briefly, the filters

were inoculated in PYG Medium and after few days samples were observed at the inverted microscope. The positive sample (142) for amoebae was subjected to total DNA extraction by Dneasy Blood and Tissue kit (Qiagen) and obtained DNA was positively amplified using specific primers for FLA Amoebae[33] as shown in Figure 19. Further the DNA extracted from the same filter was subjected to the same amplification confirming the result (Figure 19).

On the contrary, metagenomics reads did not confirm the presence of Amoebae, this can be related to the poorness of Amoebae sequences in database that were not enough to identified them. The figure below shows PCR and microscopy results.

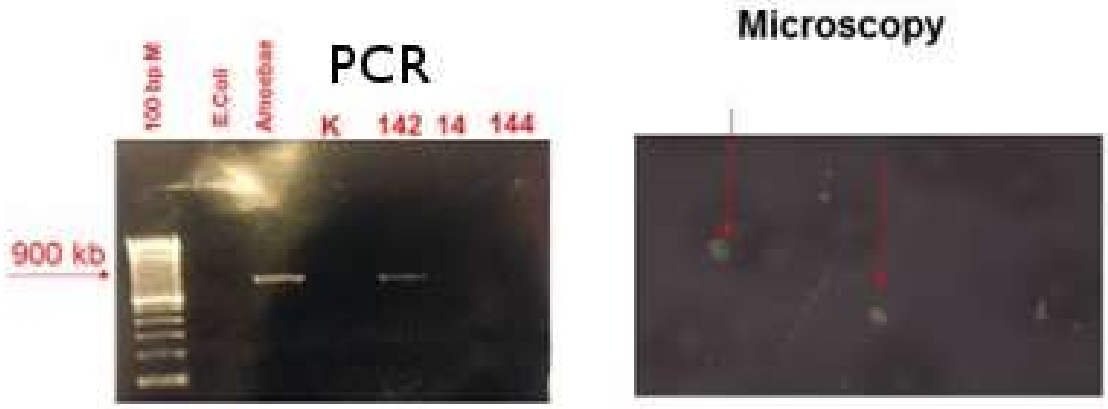

Figure 19: Amoebae identification by PCR and by Inverted Microscope

General Discussion and concluding remarks

Among the factors that contribute to the rapid spread of new infections or to the re-emergence of old diseases a major one can be the transcontinental passive transport of airborne pathogenic microrganisms carried over by dust and sand particles. Due to their intrinsically light weight, to the capability of enduring extreme environmental conditions, upon appropriate atmospheric turbulence circulations, cells, spores, virus particles can fly across different parts of the globe in spite of the traditionally viewed, oceanic or mountain range barriers. Such phenomena can assume an even more pregnant importance in view of increasing threats of deliberate warfare and bioterrorism action from given countries towards others.

As a first consideration, the analysis revealed that a wide group of phyla, recurrently consistent in spite of site and meteorology conditions, configures as a global Sardinian air microbiome. Exceptions were related to the intra-phylum biodiversity and the shared taxa between groups of samples. Notwithstanding the persisting core, it was possible to observe that biodiversity increased in relation to dust-carrying events. The atmosphere-dispersed particulate matter, constituted by a mixture of particles, containing variable fractions of natural solids and pollutants [34] represents a carrier able to passively convey an array of different microorganisms. As a consequence, emission of particle-associated bacteria, fungi and viruses determined by the transcontinental displacement via meteoric circulation (water particles and ensuing rainfall), constitutes an effective means of spreading for pathogens and a continuous process of expansion of their biogeographical ranges. The relevance as causative agents of pathologies and few informations about their dust-mediated moviments, justified this study. Unlikely, the low amount of DNA extracted, did not allow to

envisage on this particular fractions of microorganisms like viruses and parasites. Future research focused on the analysis of viruses and parasites, will increase the knowledge of airborne dust composition, the understanding of the microrganisms roles in this environment and their potential impact on ecosystems and human health.

This study represents the first to use both culture dependent and culture-independent methods to identify microrganisms bacteria in the long distance dust transport. Airborne microorganisms are exposed to extremes conditions during transport, including UV radiation, excursion temperatures and climatic changes. Microorganisms possessing adaptive strategies were expected plentiful in dust samples, in our samples it was observed by culture-method abundances of sporulating bacteria and fungi which is also in accordance with other previous reports.

Metagenomic analysis revealed that a wide group of phyla, recurrently consistent in spite of site and meteorology conditions, configures as a global Sardinian air microbiome. Exceptions were related to the intra-phylum biodiversity and the shared taxa between groups of samples. Notwithstanding the persisting core, it was possible to observe that biodiversity increased in relation to dust-carrying events. Data comparison and integration could provide interesting clues concerning the contribution of this mechanism of geographical dispersion to the biodiversity modulation and its possible effects on ecosystems and human health and activities. A prevailing constancy of the microbial composition in spite of the changing winds provenances was the most remarkable evidence. In the present study we tested two variables: (a) wind origin (African vs. Continental European) and season of sampling (late spring vs. early fall) aiming at verifying which of the two factors would translate in the

highest shift in bacterial community composition. Prokaryotes are the most widespread life forms on Earth. The secret of the evolutionary success of Bacteria and Archaea is correlated to the simple cell organization where a single chromosome occurs for the basal physiological functions and mobile elements guarantees a quick response to the environmental variations.

This study represent the basis for future investigations of the diversity and function of microbial communities in the airborne dust.

Appendix

In this thesis I present experiments and results previously published in the ISME (International society for microbial ecology) and Scientific report.

http://www.nature.com/articles/srep16306

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