



1 **Determining the hierarchical order by which the variables of sampling season, dust**  
2 **outbreaks occurrence, and sampling location, can shape the airborne bacterial**  
3 **communities in the Mediterranean basin**

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18

19 **Abstract**

20 An NGS-based taxonomic analysis was carried out on airborne bacteria sampled at ground

21 level in two periods (May and September) and two opposite localities on the North-South axis

22 of the Sardinia Island. Located in a central position of the Mediterranean basin, Sardinia

23 constitutes a suitable outpost to reveal possible immigration of bacterial taxa during

24 transcontinental particle discharge between Africa and Europe. With the aim of verifying

25 relative effects of dust outbreaks, sampling period and sampling site, on the airborne bacterial



26 community composition, we compared air collected during dust-carrying meteorological  
27 events to that coming from wind regimes not associated to long-distance particle lifting. Results  
28 indicated that: (a) a higher microbial diversity (118 orders vs 65) and increased community  
29 evenness were observed in the campaign carried out in September in comparison to the one in  
30 May, irrespective of the place of collection and of the presence or absence of dust outbreaks.  
31 (b) During the period of standard wind regimes without transcontinental outbreaks a  
32 synchronous, concerted succession of bacterial communities across distant locations of the  
33 same island, accompanied as mentioned by a parallel rise in bacterial diversity and community  
34 evenness appears to have occurred. (c) changes in wind provenance could transiently change  
35 community composition in the locality placed on the coast facing the incoming wind, but not  
36 in the one located at the opposite side of the island; for this reason the community changes  
37 brought from dust outbreaks of African origin are observed only in the sampling station  
38 exposed to south; (d) the same winds, once proceeding over land appear to uplift bacteria  
39 belonging to a common core already present over the region, which dilute or replace those that  
40 were associated with the air coming from the sea or conveyed by the dust particulate,  
41 explaining the two prior points. (e) the hierarchy of the variables tested in determining bacterial  
42 assemblages composition results: sampling period >> ongoing meteorological events >  
43 sampling location within the island.

44

## 45 **1 Introduction**

46

47 With a total volume evaluated of  $4.5 \times 10^{18} \text{ m}^3$ , terrestrial lower atmosphere represents the most  
48 extended potential biome, followed by water,  $1.3 \times 10^{18} \text{ m}^3$  (Gleick, 1993), and by soil with  
49  $6.2 \times 10^{16} \text{ m}^3$  (estimated on the basis of the deeper subsurface living bacteria currently described  
50 Szewzyk *et al.*, 1993). Concerning atmosphere, microbial cells and propagules, embody a  
51 particularly suitable conformation to take advantage of air utilization as an environment for



52 survival and dispersion. Their movement can be favored by a natural mobile reservoir of  
53 physical solid carriers represented by the air-dispersed particulate matter. Such particles range  
54 between 0.2 and 10  $\mu\text{m}$  in size (Bernstein *et al.* 2004) and average loads of 1-100  $\mu\text{g m}^{-3}$   
55 (Williams *et al.* 2002, Van Dingenen *et al.* 2004). It has been estimated that more than 5000  
56 Tg of sea salt (Tegen *et al.* 1997) and 1000-2000 Tg of soil particles, passively uplifting and  
57 transporting live cells are released every year in the atmosphere giving rise to a widely  
58 heterogeneous material conveyed from different sources (Guang *et al.* 2009; Mc Tainsh 1989,  
59 Knippertz *et al.* 2009).

60 The tropical African and Asiatic belts (Prospero *et al.* 2002, Schepansky *et al.* 2007), represent  
61 two amongst the major airlift dust sources (<http://www.who.int/>). Several studies underline that  
62 this phenomenon strongly contributes to a cosmopolitan microbial distribution (Favet *et al.*  
63 2013, Griffin 2008, Yang *et al.* 2008, Wainwright *et al.* 2003, Smith *et al.* 2010). Moreover,  
64 the correlation between specific bacterial clades and particle size (Polimenakou *et al.* 2008)  
65 opened new hypotheses on differential dispersion of taxa in relation to the dust features. High  
66 amount of bacterial 'newcomers' have been pointed out in air samples collected in occasions  
67 of foreign dust outbreaks (Maki *et al.* 2014, Rosselli *et al.*, 2015). Immigrant microorganisms  
68 classification (Sánchez de la Campa *et al.* 2013) and their effects on an autochthonous  
69 ecosystem have also been reported (Peter *et al.* 2014, Shine *et al.* 2000). Evidences of a  
70 correlation between aerosol-related biodiversity and seasons (Gandolfi *et al.* 2015) underlines  
71 the natural complexity related to this process, suggesting that effects may vary also depending  
72 on climatic periodicity. Marked seasonal patterns in airborne microbiota have also been  
73 reported in long term studies (Cáliz *et al.*, 2018). The genes that are specific to communities  
74 of bacteria inhabiting the atmosphere, referred to as aeolian lifestyle, have been studied  
75 by metagenomics approaches and include UV-induced DNA damage repair, cell  
76 aerosolization, aerotaxis, and thermal resistance (Aalismail *et al.*, 2019).



77 Europe-Mediterranean air circulation routes offer an interesting case study when focusing on  
78 airborne bacteria. The system can be represented as a multidirectional network in which  
79 biological components and weather conditions are closely related (Lelived *et al.* 2002).  
80 Extending for more than 30 degrees of latitude above the subtropical belt, Europe is crossed  
81 by middle-latitude and equatorial atmospheric systems. Mathematical models suggest that a  
82 considerable part of the air mass movements has a Northern, Atlantic source in response to the  
83 pressure generated by the Azores high (Littmann, 2000). Southern winds from Africa, prone to  
84 carry desert sand, and potentially microbes, can be determined by specific climate conditions  
85 (Kostopoulou and Jones 2007, Benkhalifa *et al.*, 2019). It has been estimated that, as a  
86 consequence, 80-120 Tg of dust per year are transported across the Mediterranean towards  
87 Europe (d'Almeida 1986; Dulac *et al.* 1996), reaching the higher troposphere layers (Alpert *et*  
88 *al.* 2004) and spilling over, until the far-Northern sides of the continent (Franzè *et al.* 1991).  
89 In order to track the biodiversity of these airways, the Italian island of Sardinia was chosen as  
90 ideal observatory point to collect airborne bacteria moving inside and outside Europe. Located  
91 in the middle of the Mediterranean Sea, this landmass is separated from Italy, France, Spain  
92 and Africa coastal baselines by distances of 120, 150, 230, and 100 nautical miles (NM)  
93 respectively (Fig. 1). Its geographical position facilitates the displacement of western high- and  
94 low-pressure air masses coming from Gibraltar and becoming the first and the last frontier for  
95 microbes entering or leaving Europe, respectively. In a prior study (Rosselli *et al.* 2015), we  
96 described a core microbiome in the bacteria cast upon the Sardinia island under different wind  
97 regimes through analyses of DNA from deposited particles. The analysis compared the trans-  
98 Mediterranean airflow with that of winds from Europe, and pinpointed a number of taxa which  
99 have records in clinical infections. In that investigation the sampling dates were all  
100 concentrated in a single period of six days (in February) and some variations of the airborne  
101 biota were observed in response to the opposite wind



102

103 **Fig. 1** Mediterranean area with Sardinia Island detail and sampling locations Sassari and  
104 Cagliari.

105

106 directions. However, the most remarkable evidence was a prevailing constancy of the microbial  
107 composition in spite of the changing winds provenances. In the present study instead we  
108 analyzed a series of events featuring a starting dust outbreak, a 109 days-long period devoid of  
109 dust-carrying winds, and a second dust outbreak. The analyses were performed in two  
110 oppositely located stations: Cagliari, on the South-East side of Sardinia, facing the African  
111 side, and Sassari in the North-West, i.e. farthest from the dust-carrying winds. The sampled  
112 particulate was analyzed by NGS sequencing of the amplified 16S rRNA genes. The main goal  
113 of the project was to verify in which hierarchical order the different variables of (a) sampling  
114 period, (b) occurrence of dust-carrying outbreaks, and (c) sampling location, could act in  
115 determining airborne bacterial communities composition.



116 **2. Materials and Methods**

117

118 **2.1 Meteorological monitoring**

119

120 Surveillance of the weather trends and conditions to anticipate dust outbreaks from Africa  
121 towards Sardinia and winds of interest was performed by routine checking of the MODIS  
122 satellite data and Meteosat imagery combined with the SKIRON forecasting model (Nickovic  
123 *et al.* 2001).

124 Europe daily synoptic conditions were analyzed on the weather charts available from the  
125 [www.eurometeo.com](http://www.eurometeo.com) and [www.metoffice.gov.uk](http://www.metoffice.gov.uk) websites.

126 The origin and the trajectory of the dust carried by winds towards Italy were inferred by the  
127 NOAA HYSPLIT model (Hybrid Single Particle Lagrangian Integrated Trajectory Model)  
128 (Draxler *et al.* 2014; Rolph 2014).

129 Monitoring was aimed at predicting two distinct conditions: i) North-African high-pressure  
130 nuclei favoring Southern winds suitable to carry and deposit dust over Sardinia (dust-enriched  
131 events); and ii) North-European high-pressure nuclei, determining northern winds referred to  
132 as ‘Controls’ (dust-negative events).

133 In addition, PM<sub>10</sub> concentration (particulate matter with a diameter of less than 10 μm) and  
134 meteorological data registered by the ARPAS (Regional Environmental Protection Agency of  
135 Sardinia) monitoring stations were taken into consideration in relation to the arrival of African  
136 air masses.

137 Information about wind direction and intensity (every 10 minutes), temperature and humidity  
138 (once per hour) were downloaded by the ISPRA website (<http://www.mareografico.it/>) and two  
139 sampling stations located in Cagliari (39.21°N, 9.11°E) and Sassari - Porto Torres (40.84°N,



140 8.40°E). Data covered a 7 months time-lapse, from March to September 2014, in order to obtain  
141 a nearly annual view to focus within the main weather instability period.

142

## 143 **2.2 Sampling**

144

145 Samples were collected on Teflon filters (Sartorius Stedim Biotech) by using a Skypost Tecora  
146 apparatus (compliant to the European legislation 96/62/gmeCE) processing 39 liters of air per  
147 minute. For each sample, date and atmospheric conditions are reported and fully described in  
148 the Results chapter and Supplementary Materials.

149 A one-day filtering step was performed for each sampling, extended to two days when a dust-  
150 outbreak became evident. A total of two filters for each collection were processed for  
151 sequencing.

152

## 153 **2.3 DNA extraction and Sequencing**

154

155 DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek Inc.) as described by  
156 the manufacturer. Quality and quantity of the extracted nucleic acid were measured using a  
157 NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.).

158 Amplification of the 16S-rRNA genes for sequencing was performed using the universal  
159 primers 27F-1492R (AGAGTTTGATYMTGGCTCAG and  
160 TACGGYTACCTTGTTACGACTT, respectively). PCR was carried out using Platinum® Taq  
161 High Fidelity DNA Polymerase (Life Technologies) in a PTC-200 Thermal Cycler (MJ  
162 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  
163 min for 30 cycles), 72°C for 10 min and 4°C on hold. The amplification of the No Template  
164 Control (NTC) was negative. Next generation sequencing was carried out at the facilities of



165 the Porto Conte Ricerche Srl (Alghero, Italy). Briefly, amplicons were quality-checked on an  
166 agarose gel and purified using the Agencourt® Ampure® XP PCR Purification Kit. One ng of  
167 DNA was processed using the Nextera XT DNA Sample Preparation Kit (Illumina Inc.) and  
168 sequenced using the HiScanSQ (Illumina Inc.) with 93bp x 2 paired-end reads. Sequences were  
169 submitted to the European Nucleotide Archive(ENA) inside the “Dust Metagenome”  
170 BioProject with the accession numbers ERX836645-56.

171

#### 172 **2.4 Data analysis**

173

174 Reads were cleaned on the basis of quality and fragments of Nextera adapters removed by  
175 Trimmomatic (Bolger et al. 2014) set at the value of 3 for leading and trailing trimming, and  
176 bases lower than 20 on a 4-base wide sliding window. Quality was confirmed by FastQC  
177 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and reads were analyzed with  
178 Qiime1.9.0 (Caporaso et al. 2010). The OTU table was created using the pick\_otus script with  
179 the Closed-reference OTU picking strategy with the Greengenes reference OTUs database  
180 clustered at 97% (ver. gg\_13\_8). The same script checked against chimeric sequences using  
181 the Broad Microbiome Utilities’ 16S Gold reference database (version microbiomeutil-  
182 r20110519). The OUT table was filtered based on the total observation count of an OTU at  
183 least of 3 and low abundance filtering of 0.005%. Finally, the OTU table was rarefied  
184 (subsampling) at 1109571 counts (equal to the sample with a lowest depth) for all the samples.  
185 Perl and the R-package Vegan were subsequently used for cladograms and distance-based  
186 clustering analyses, Ggplot, Plotrix and WindRose provided graphic support.

187 Molecular data regarding bacterial species compositional differences across different  
188 treatments were analysed by multivariate analyses (Principal Coordinate Analysis, PCoA;  
189 Principal Component Analysis, PCA, Discriminant Analysis of Principal Components DAPC),



190 and ecological indices calculation, using the Calypso online software tool (Zakrzewski et al.,  
191 2017). Prior to the analyses, the relative abundances of taxa were equalized by applying the  
192 total sum of squares scaling (TSS) normalization followed by square root transformation.

193

194

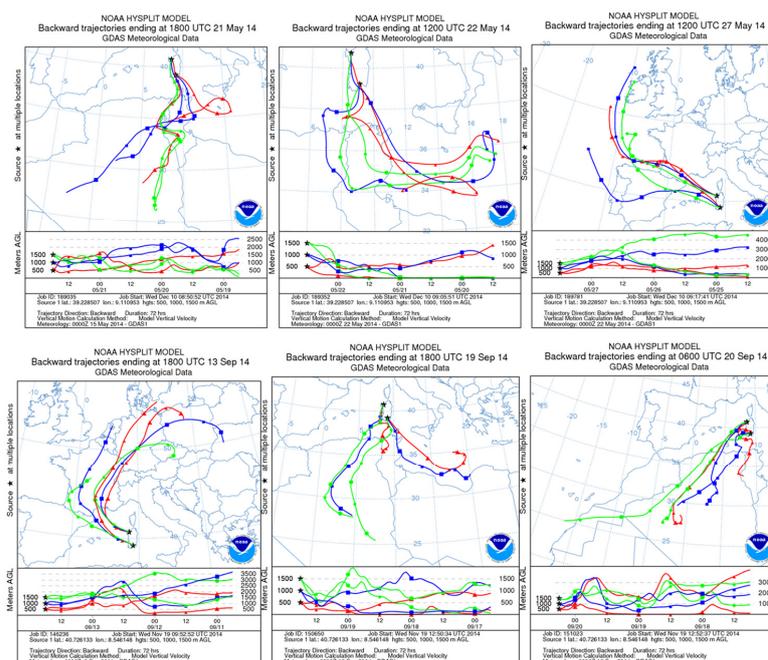
### 195 **3. RESULTS**

196

#### 197 **3.1 Meteorological events**

198 To capture the air microbiota of Sardinia and to put in evidence taxa which could be associated  
199 to specific events (winds in northbound direction prone to carry dust from shores across the  
200 Mediterranean as opposed to calm air or slow flows from the opposite quadrant), weather  
201 forecasts and other data on air circulation were regularly browsed to select suitable dates for  
202 the sampling. This allowed to integrate boundary conditions and environmental variables to  
203 assess possible correlations between these and microbial community fluctuations (Fiamma,  
204 2016).

205 Dust-carrying air masses moved over the Mediterranean on May 21<sup>st</sup> through May 22<sup>nd</sup> 2014  
206 towards north-east, covering the entire Sardinia island. PM10 concentrations throughout the  
207 second half of May at both North and South collection sites displayed increases in  
208 correspondence of the dust event (Supplementary Figure S1). Incoming dust of African origin  
209 was equally evidenced by charts reporting wind fronts and pressure (Supplementary Figure  
210 S2), and images from satellite (Supplementary Figure S3). The itinerary of particles was  
211 reconstructed by plotting 3-day backward trajectories of the air mass using a NOAA HYSPLIT  
212 model (Figure 2) which tracked the North-African zone as the source of the convective motion  
213 responsible for the dust discharge on Italy observed on May 21<sup>st</sup> -22<sup>nd</sup>.



214

215 **Fig. 2. Upper panel:** 3-Day air mass backward trajectories calculated by the NOAA HYSPLIT  
216 model ending at 18:00 UTC May 21<sup>st</sup>, 12:00 UTC May 22<sup>nd</sup> and 12:00 UTC May 27<sup>th</sup> 2014  
217 at both sampling sites. **Lower panel:** 3-Day air mass backward trajectories calculated as above,  
218 ending at 18:00 UTC September 13<sup>th</sup>, 18:00 UTC September 19<sup>th</sup> and 06:00 UTC September  
219 20<sup>th</sup> 2014 at both sampling sites (credit to: ready.arl.noaa.gov/HYSPLIT.php).

220

221

222 May 27<sup>th</sup> was selected as “clear day” featuring a weather not conducive anymore for air  
223 convection from Africa to Sardinia. Such conditions consisted in overall European low  
224 pressures as opposed to high pressures over Mauritania, Mali, Libya and Algeria  
225 (Supplementary Figures S2 and S3). Particle back-tracking supported the evidence of a slow  
226 flow of air masses only from north-western corners on May 27<sup>th</sup> (Fig.2, upper panel, rightmost  
227 image).



228 An outbreak of dust on Sardinia was recorded again in 2014 during the second fortnight of  
229 September. Low pressure from the north-western coast of Spain to Morocco was opposed to  
230 parallel high-pressure system that extended over North Africa (Libya, Algeria, and Tunisia)  
231 through Sicily. This circumstance caused the flow of dust-carrying air masses over the  
232 Mediterranean basin, reaching in particular Southern Italy and Sardinia. Air movement from  
233 the African continent made air temperature rise to values above the usual September means,  
234 with a peak on Sep. 20<sup>th</sup> in Sassari (northern sampling site) and on Sep. 21<sup>st</sup> in Cagliari  
235 (southern sampling site) (Supplementary Figure S4-a,b). In relation to this condition, from  
236 September 19<sup>th</sup> through the 21<sup>st</sup> a dust outbreak from Sahara flew over the Mediterranean and  
237 entirely covered Sardinia. The relative wind fronts and pressure values are shown in  
238 Supplementary Fig. S5. Patterns of PM<sub>10</sub> from daily records taken at both Sardinian sampling  
239 stations, also displayed a rise during the dust outbreak (Supplementary Fig. S4-c). Satellite  
240 imagery confirmed again the occurrence of incoming dust-loaded air masses from Northern  
241 Africa (Supplementary Fig. S6) consistent with their 3-day back-trajectories (Fig. 2 lower  
242 panel). Those confirmed that on September 19<sup>th</sup> - 20<sup>th</sup> air flows were from North African origin.  
243 About a week earlier instead, September 13<sup>th</sup> had featured low pressures on Italy while high  
244 pressures were recorded over the southern part of Morocco, Algeria and Mauritania. This  
245 picture was not permissive for any transport of air loads from Africa to Sardinia and the day  
246 was therefore considered as the “clear day” reference of the period. Air representative of the  
247 dust outbreak condition was thence sampled from Sep.19<sup>th</sup> through 20<sup>th</sup>, while the  
248 corresponding control air was collected on September 13<sup>th</sup>.

249

### 250 3.2 Bacterial community composition

251



252 To put in evidence microbial variation we envisaged the possibility of finding i) a local set of  
253 taxa, with specificity for one of the two sampling corners of the island, and a relative  
254 independence from the weather events, ii) those linked to the occurrence of dust outbreaks,  
255 (distinguishing in this case the first 12 hours timeframe from the second 12h one. iii) bacteria  
256 showing season-related fluctuations being specific or enriched in one or the other sampling  
257 times (May vs. September).

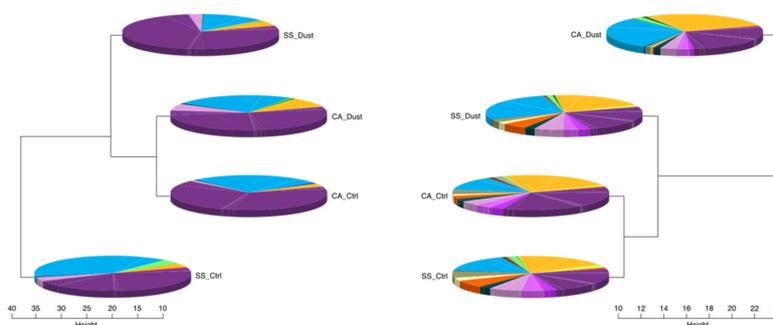
258 A synoptic view of the results at Phylum/Class level is shown in Fig. 3.

259 In terms of conserved taxa the core of those observed more regularly included classes as  
260 Gammaproteobacteria, Bacilli and orders as Actinomycetales; their maxima were seen in the  
261 May samples where those reached percentages above 90%, while their minima appeared in the  
262 Sassari controls in September with values around 50%.

263 With respect to the Actinobacteria phylum, the Actinomycetales order was the one most  
264 commonly encountered, being found in all samples; in particular it was featured in the south-  
265 facing station (Cagliari), and its numbers tended to double in relation to the dust events. The  
266 overall levels of relative abundance as well as diversity within members of the Actinobacteria  
267 phylum increased from 5.66 % values, observed in May to 13 % in September. Particularly  
268 enriched were the Gaiellales and Solirubrobacterales order within the Thermooleophilia class.  
269 The orders within the Firmicutes phylum, that dominated the May samples, resulted  
270 Lactobacillales and Bacillales. Their relative abundances were higher in the Sassari (Northern  
271 Sardinia) control samples, than in those collected in the southern point of Cagliari, with values  
272 of 37 % vs. 12 % respectively. At the same time an unchanging level of 25% was recorded in  
273 both control and dust samples in the south-facing location. In the September samples the  
274 situation was different as in both controls those orders were below 15%, while during the dust  
275 outbreak it was 25 % in both Cagliari and Sassari stations.



276 The May sampling was also characterized by a large share of Gammaproteobacteria, a class  
277 reaching 75% of the dust-related spring samples in Sassari. In particular Pseudomonadales and  
278 Enterobacteriales were constantly observed. Some taxa constituting spring-signature cases  
279 were detected in the Alteromonadales with *Marinimicrobium*, *Marinobacter* and taxon



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281

282 **Fig. 3:** Cluster Dendrogram (Euclidean distance method, complete linkage) on the identified  
283 bacterial orders. May samples (the four left column pie charts) compared sideways to the  
284 corresponding September samples (right column pie charts). Data from the two sampling  
285 stations of Sassari (SS, northern Sardinia) and Cagliari (CA, Southern Sardinia) are shown,  
286 comparing the two wind regimes ('Dust': during dust outbreaks under winds from Africa, and  
287 'Ctrl': Control, under winds from Europe). Pie colours coding (clockwise): yellow:  
288 Actinobacteria; light green: Acidobacteria; red: Verrucomicrobia; dark purple:  
289 Gammaproteobacteria; fuchsia: Deltaproteobacteria; light fuchsia: Betaproteobacteria; light  
290 pink: Alphaproteobacteria; black: Planctomycetes; orange: Nitrospirae; white: NC10; khaki:  
291 Gemmatimonadetes; blue: Firmicutes; brown: Chloroflexi; grey: Chlorobi; green:  
292 Bacteroidetes; dark green: Armatimonadetes.

293

294

295 OM-60. For the fall period instead, Xantomonadales recurred with some genera in the  
296 Sinobacteraceae family, amounting from 1.2% to 2% respectively in the dust-free controls of  
297 Cagliari and Sassari,



298 Within the Alphaproteobacteria class, Caulobacterales, with genera related to *Brevundimonas*  
299 were at 1% relative abundance level in the Cagliari samples collected during the dust episode  
300 of May.

301 The Rhizobiales order was present in both seasons with a 3% peak in spring (Cagliari, dust-  
302 related), dropping to 1.5 % in all fall analyses. In the same period Rhodospirillales showed a  
303 relative increase, particularly in the controls in Sassari where they reached 3.4%.

304 The Burkholderiales (Class Betaproteobacteria) of the population were found at 1 % in May  
305 within the dust-related sequences and at higher values, reaching 2.7% in Cagliari and 4% in  
306 Sassari, in the controls of September.

307 Some groups appeared rather season specific as the Mollicutes for May, while the Pirellulales  
308 order (in the Planctomycetes phylum) and the classes of Nitrospira and Gemmatimonadetes  
309 characterized the September sampling.

310 To better refine the bacterial deposition dynamics during the outbreaks, during the total 24h  
311 sampling time, two sampling sub-periods were set, splitting the total collecting span into two  
312 12-h lapses, by changing the filters after the first one and separating the collected material as  
313 different samples. An increase in the inflow of air particulate was observed for the 12-24 h  
314 period.

315 This set up was also functional to individuate taxa that would display high variation in relation  
316 to dust events in comparison to those who would not. The latter were considered to represent  
317 the common core of bacteria that were constantly present in samples, irrespective of the  
318 changing meteorological events. To apply this distinction, the criterion was to set a cutoff value  
319 with respect to the percent of variation occurring between the first 12 h of the collection time  
320 and the second half of it. Only the taxa which displayed a mean variation higher than ½ of the  
321 corresponding standard deviation were considered. The resulting level of variation in the two  
322 sampling stations is reported in Tab. 1 and the corresponding number of orders is displayed in



323

324

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 Sepember - Dust	1.3	0.4	5.4
Cagliari September - Dust	4.7	1.1	11.4

325

326

327 **Tab. 1:** Average, minimum and maximum percent variation between taxa counts harvested in  
328 the first 12 hours sampling period of the dust event and those harvested in the subsequent 12  
329 hours sampling period. Only taxa displaying a difference in percentages higher than half of  
330 their standard deviation were selected for the present comparison.

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332

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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%

334

335

336 **Tab 2.** Community diversity at order level of taxa occurring during dust events and of those  
337 displaying variations higher than half the standard deviation between the first 12h and the  
338 second 12h sampling period (selected orders). The percentage of orders selected upon this  
339 criterion over the total of the orders observed in samples collected during the dust events is  
340 indicated.



341 Tab. 2. The Sassari (North-facing) collection site was the one that in both seasons resulted to  
342 feature the highest number of significantly changing taxa. The identities of these are shown in  
343 Supplementary Fig.S7 (May event), and Supplementary Fig. S8 (September event). In the  
344 graphs, the first 12h lapse is plotted above the baseline and the second (12-24 h) is on the  
345 specular position below.

346 As regards the ecological indexes characterizing the communities, species diversity and  
347 evenness values were calculated, and results are shown in Tab. 3. The difference that can be  
348 appreciated is mainly relative to the series of September samples, in which all had higher values  
349 for each of the indexes when compared to the May ones. Conversely, neither the presence of  
350 dust events nor the sampling location appeared to confer relevant differences in this respect.

351 The numerical effect of the different sampling season on bacterial communities is visible in  
352 Tab. 4, comparing the mean relative abundances of the main orders in the two sampling  
353 months, grouped independently from site and meteorology events. Among the most evident  
354 phenomena. the September campaign shows the enrichment in the Actinomycetales order and  
355 in a number of others that were below detection in the May sampling. In parallel, the diminution  
356 of the formerly dominant Enterobacteriales and Pseudomonadales, and the substantial stability  
357 of the Bacilli across the compared times were observed.

358 The patterns of conservation and diversity involving the bacterial communities analyzed were  
359 subsequently inspected by multivariate approaches. Principal Component Analysis yielded an  
360 output (Fig. 4) that confirms how a separation of communities can be viewed only when  
361 considering the seasonal factor (Fig.4. A), while the variables of dust vs. calm air, or the  
362 sampling location, led to plots with heavily overlapping patterns. The May vs. September  
363 divide occurs along the horizontal axis, i.e. the one explaining the highest fraction of variation  
364 (35%). The same phenomenon is reproduced with a higher support (54 %) in a parallel

365



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

366

367

**Tab 3.** Ecological diversity and evenness indices resulting from the sequence checklist analysis

368

in the different samplings.

369

Phylum	Class	Order	Mean percentage May	Mean percentage 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

370

371

**Tab. 4.** Percent frequency of sequences belonging to the indicated orders in the averaged data

372

of all samplings (Dust and control) of each seasonal sampling period (May or September). Data

373

in which frequencies were higher than 1% in at least one of the two seasons are reported. These

374

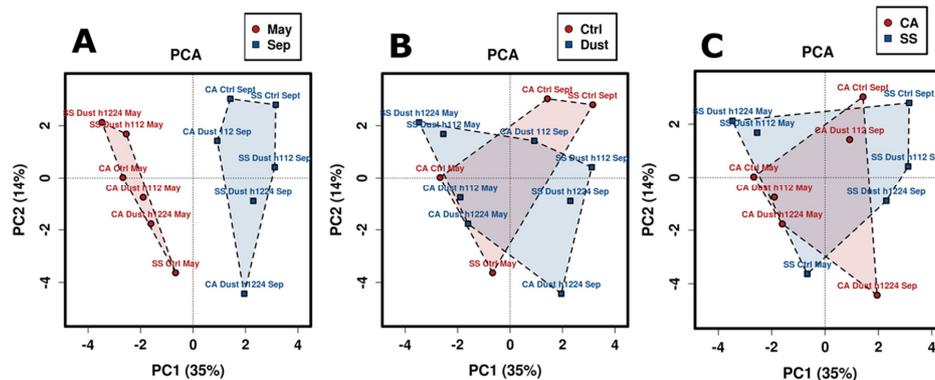
represent the 91.1% of the total sequences for the May sampling (on a total of 65 orders found)

375

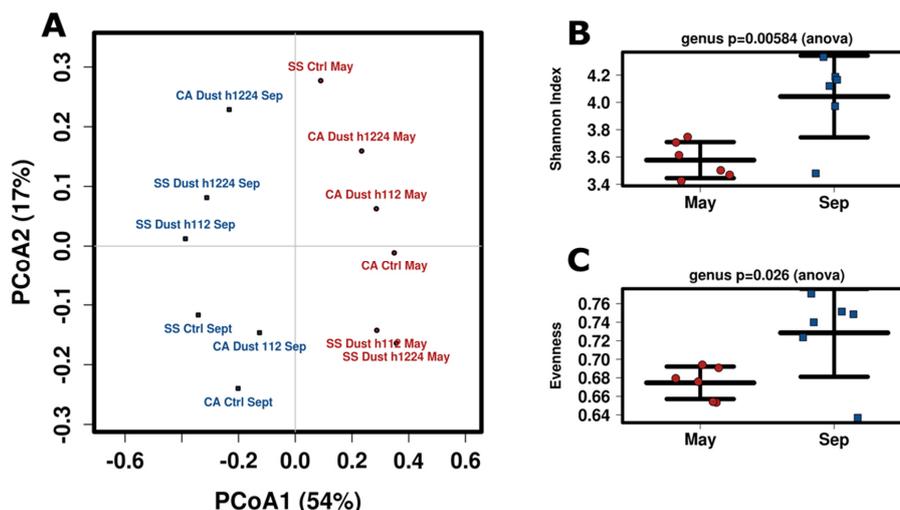
and 79.4% of the September sampling (on a total of 118 orders found).



376 ordination approach, the principal Coordinate Analysis (Fig. 5. A). In the same figure the main  
377 differences occurring in community structure between the two sampling times are further  
378 explored by reporting the ecological indexes of Shannon species diversity and community  
379 evenness resulting from grouping the data and separating them only in relation to the sampling  
380 period variable, irrespective of meteorology events and collection sites. The superiority of the  
381 September values in both indexes, and particularly for the taxa diversity, is supported by the  
382 significance of the p values of discrimination between samples thereby reported.  
383



384  
385  
386  
387 **Fig. 4. Principal Component Analysis (PCA) ordination plot of the bacterial community**  
388 **compositional data.** Polygons encompassing the positions of three different variables are  
389 drawn to visualize season (A), ongoing meteorological event (B), or sampling location (C).  
390



391  
392

393 **Fig. 5. A. Principal Coordinate Analysis.** Dataset is as in Fig. 4. **B.** Shannon index of species  
394 diversity boxplot comparison between the two season's samplings (source for calculation:  
395 square root of total sum of squares data transformation). **C.** Community evenness index  
396 comparison on the same data. The significance of differences by ANOVA is reported over each  
397 diagram.

398

399 The higher strength of clustering of the sampling date groups with respect to the alternative  
400 ones (meteorological or geographical) was verified by running a Discriminant Analysis on the  
401 Principal Component ordination (DAPC) in which the data are first transformed by PCA, from  
402 which, clusters are subsequently identified using Discriminant Analysis, thus partitioning  
403 sample variance into the between-group and within-group components. Results are shown in  
404 Fig. 6. Besides confirming the sampling season as the strongest driver of community change,  
405 the analysis further shows that the dust vs. control clustering is acting more efficiently than the  
406 Sassari vs, Cagliari sampling site comparison. This allows to draw a hierarchical ranking of  
407 the variables in shaping the bacterial airborne communities, in which, noting also the different



408 scale of the horizontal axis (Discriminant function 1) adopted for the three graphs, the order  
409 results : Season >> Meteorology > Geography.

410 In order to determine which bacterial taxa were mostly accompanying/causing those changes  
411 in a statistically significant manner, and to rank their individual importance in this  
412 phenomenon, we run an analysis of the differentially featured taxa, testing both an ANOVA  
413 variance analysis and a non parametric Wilcoxon Rank test verification of the ranking. The  
414 two tools gave coherent scores and the results of the ANOVA output are shown in  
415 Supplementary Table S1. A total of 76 taxa were found featuring p values < 0.05, from which,  
416 upon applying a stringent Bonferroni-adjusted p value correction, six of those stood above  
417 the significance cutoff, and all within minimal false discovery rate values ( FDR < 0.005). All  
418 of them were cases which were highly reduced in September in comparison to May. The taxa  
419 included as the most effective in explaining the differences (p value = 0.000019, the order  
420 Oceanospirillales, known as marine oil spill-associated bacteria (Cao et al, 2013), followed by  
421 known animal parasites as the Coxiellaceae family (Lory, 2014), marine extremophyles as the  
422 Thiohalorhabdales (Tian et al., 2017), and three species of *Pseudomonas*, including the  
423 pathogenic *P. viridiflava* (Hu et al., 1998), the decontamination-associated *P. nitritireducens*  
424 (Wang et al., 2012) and *P. alcaligenes* which is reported also a human pathogen (Suzuki et al,  
425 2013). The two corresponding analyses of differentially represented taxa by meteorology or by  
426 geography, i.e., grouping dust vs. calm air or Cagliari vs. Sassari sites did not yield any  
427 significantly supported cases under the Bonferroni-adjusted p values stringent condition (data  
428 not shown).

429

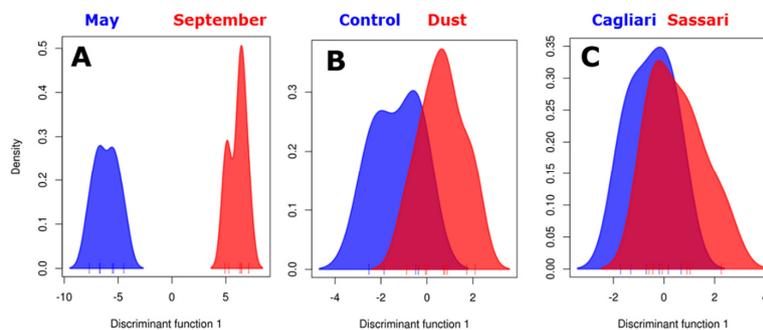
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433



434

435

436 **Fig. 6. Discriminant Analysis of Principal Components analysis.** Group partitioning  
437 involved A: season; B: ongoing meteorological event; C: sampling location.

438

439 In order to compare all communities with each other and extract further information on their  
440 degrees of divergence, the sequencing data were analyzed by individual comparisons across  
441 sites and dates. The results of each of the 66 pairwise combinations are shown in Tab. 5,  
442 displaying the Bray Curtis similarity values between each couple of communities. Color-based  
443 conditional formatting applied to the values allows to appreciate how all the comparisons  
444 involving different seasons show the most divergent scores (red shades) in comparison to those  
445 within the same season, that show much more similarity, with few exceptions related to dust  
446 events and depending on the aspect faced by the collecting site with respect to the incoming  
447 wind direction.

448

449

450

451



Different season					
<i>Different place</i>			<i>Same place</i>		
SS D1-12 May	CA D1-12 Sep	0,476	SS D1-12 May	SS D1-12 Sep	0,348
SS D1-12 May	CA D12-24 Sep	0,344	SS D1-12 May	SS D12-24 Sep	0,380
SS D1-12 May	CA Ctrl Sep	0,449	SS D1-12 May	SS Ctrl Sept	0,355
SS D12-24 May	CA D1-12 Sep	0,416	SS D12-24 May	SS D1-12 Sep	0,317
SS D12-24 May	CA D12-24 Sep	0,321	SS D12-24 May	SS D12-24 Sep	0,372
SS D12-24 May	CA Ctrl Sep	0,423	SS D12-24 May	SS Ctrl Sep	0,312
CA D1-12 May	SS D1-12 Sep	0,471	SS Ctrl May	SS D1-12 Sep	0,450
CA D1-12 May	SS D12-24 Sep	0,532	SS Ctrl May	SS D12-24 Sep	0,507
CA D1-12 May	SS Ctrl Sep	0,388	SS Ctrl May	SS Ctrl Sept	0,420
CA D12-24May	SS D1-12 Sep	0,481	CA D1-12 May	CA D 1-12 Sep	0,536
CA D12-24May	SS D12-24 Sep	0,538	CA D1-12 May	CA D12-24 Sep	0,476
CA D12-24May	SS Ctrl Sep	0,417	CA D1-12 May	CA Ctrl Sep	0,526
SS Ctrl May	CA D 1-12 Sep	0,448	CA D12-24 May	CA D 1-12 Sep	0,530
SS Ctrl May	CA D12-24 Sep	0,523	CA D12-24 May	CA D12-24 Sep	0,479
SS Ctrl May	CA Ctrl Sep	0,469	CA D12-24 May	CA Ctrl Sep	0,525
CA Ctrl May	SS D1-12 Sep	0,387	CA Ctrl May	CA D 1-12 Sep	0,450
CA Ctrl May	SS D12-24 Sep	0,464	CA Ctrl May	CA D12-24 Sep	0,416
CA Ctrl May	SS Ctrl Sep	0,355	CA Ctrl May	CA Ctrl Sep	0,453
Same season (May) Control taken after dust					
<i>Different place</i>			<i>Same place</i>		
SS D1-12 May	CA D1-12 May	0,649	SS D1-12 May	SS D12-24 May	0,736
SS D1-12 May	CA D12-24 May	0,627	SS D1-12 May	SS Ctrl May	0,545
SS D1-12 May	CA Ctrl May	0,640	SS D12-24May	SS Ctrl May	0,522
SS D12-24 May	CA D1-12 May	0,709	CA D1-12 May	CA D12-24May	0,802
SS D12-24 May	CA D12-24 May	0,655	CA D1-12 May	CA Ctrl May	0,784
SS D12-24 May	CA Ctrl May	0,713	CA D12-24 May	CA Ctrl May	0,791
CA D1-12 May	SS Ctrl May	0,618			
CA D12-24 May	SS Ctrl May	0,679			
SS Ctrl May	CA Ctrl May	0,676			
Same season (September) Control taken before dust					
<i>Different place</i>			<i>Same place</i>		
SS D1-12 Sep	CA D 1-12 Sep	0,600	SS D1-12 Sep	SS D12-24 Sep	0,718
SS D1-12 Sep	CA D12-24 Sep	0,604	SS D1-12 Sep	SS Ctrl Sep	0,705
SS D1-12 Sep	CA Ctrl Sept	0,656	SS D12-24 Sep	SS Ctrl Sep	0,616
SS D12-24 Sep	CA D 1-12 Sep	0,599	CA D 1-12 Sep	CA D12-24 Sep	0,554
SS D12-24 Sep	CA D12-24 Sep	0,655	CA D 1-12 Sep	CA Ctrl Sep	0,589
SS D12-24 Sep	CA Ctrl Sep	0,656	CA D12-24 Sep	CA Ctrl Sep	0,441
CA D 1-12 Sep	SS Ctrl Sep	0,523			
CA D12-24 Sep	SS Ctrl Sep	0,434			
SS Ctrl Sep	CA Ctrl Sep	0,705			

452

453

454 **Table 5. Bray Curtis similarity values between the bacterial communities composition**

455 **resulting from pairwise comparisons of all samples. Abbreviations: SS: Sassari; CA:**



456 Cagliari; D1-12: dust event, first 12 hour period; D12-24: dust event, second 12 hour period;

457 Ctrl: control conditions (absence of dust events); Sep: September.

458

459

#### 460 **4. DISCUSSION**

461

462 In the present study the filtered air particulate was analyzed in different seasons and under  
463 different wind regimes, using culture-independent DNA sequencing-based approaches  
464 targeting the species-diagnostic 16S-rRNA genes from the air-carried bacterial community and  
465 an Illumina next generation sequencing platform. Sites were selected also because of their  
466 opposite positions facing Africa (Cagliari) or continental Europe (Sassari). The analysis was  
467 performed within a 7-month time lapse, March to September, chosen also as it offers higher  
468 probabilities of weather shifts favoring both northern- and southern-winds (Israelevich *et al.*  
469 2012). This timeframe proved suitable to the scope as it was possible to exploit two episodes  
470 in which dust outbreaks carried by winds of African origin occurred and were preceded and  
471 followed by inversions of the air circulation offering control sampling periods with opposite  
472 features.

473 The central goal of this study was to assess which variables (sampling time of the year, dust  
474 outbreak vs. calm atmosphere, and north-facing vs. south-facing collection site) would be most  
475 effective in determining airborne community divergence or homogenization.

476 One first general aspect that can be commented is a higher diversity of the communities during  
477 the September sampling in comparison to May, independently from the dust events and from  
478 the sampling station location.

479 This phenomenon, besides the ecological values differences (Tab. 2, Tab. 3, Fig.5, and Tab.  
480 S1) can be also appreciated visually, by comparing the left and the right pie charts in Fig.3,



481 (featuring community composition at order-rank level, and the corresponding cluster analysis  
482 based on their relative percentages), and noticing the more complex color-coded pattern of the  
483 latter sampling, showing also a consistent similarity of most color sectors presence and  
484 proportions. It is not possible from these single-year data to deduce whether such increase  
485 could be part of a recurring seasonal phenomenon causing, cyclically, higher species richness  
486 after summer periods, or if what we observe could be part of a different pattern of stochastic  
487 variability.

488 Nevertheless the overall partitions of systematic groups observed in a given sampling time,  
489 irrespective of dust outbreaks or sampling corner of Sardinia, share much more similarity  
490 within the samples of that period than with any of those collected in the other season. It appears  
491 that in general, air collected during dust discharge from a Saharian wind can account for less  
492 variation over its reference control sampling than the choice of sampling that site four months  
493 apart.

494 In our prior work (Rosselli et al., 2015) we had studied community composition in the same  
495 Sardinian stations in a short period of winter (in late February) during and after a single dust-  
496 carrying event. In that study the main feature evidenced was the existence of a conserved core  
497 microbiome, encompassing 86-95 % of the taxa, to which the incoming dust would cause some  
498 detectable diversity variation but on a rather limited proportional scale. Such minor effect of  
499 the dust-lifting storms observed in winter is in fact confirmed in the present work in which the  
500 time of the year factor appears as the variable of major order in shaping community structure  
501 and richness.

502 Literature reports have pointed out differences in airborne microbial composition between  
503 seasons; peaks of fungi causing invasive infections in humans were signaled in spring whereas  
504 higher proportions of allergenic fungi were observed in fall (Yamamoto et al. 2012).



505 Consistent with the present data a higher diversity of both fungal and bacterial airborne cells  
506 in late summer and early fall has been observed in United States-based surveys (Bowers et al.  
507 2012, Bowers et al., 2013).

508 Hypotheses to explain the increase in circulating taxa widely observed in the fall sampling  
509 campaign can be formulated. In first instance one should consider whether there could have  
510 been a change in the prevailing winds origin or direction across the period that encompasses  
511 the two sampling seasons. This can be evaluated upon inspecting publically available  
512 meteorology records showing the wind roses for the two sampled localities. These data, from  
513 March to November, for the Cagliari and Sassari weather stations, are shown in Supplementary  
514 figures Fig. S9 and Fig S12, respectively. In the Cagliari plots (southern Sardinia) it can be  
515 observed that between May and September there was basically no variation of the wind  
516 patterns, with the prevailing ones blowing towards North-West, with stable intensities.  
517 Likewise in the Sassari area (Fig. S12), although some fluctuations in the strength of the  
518 westbound winds can be seen, the dominant air motion throughout the period remains the one  
519 heading South. In essence these data allow to rule out that the change in community patterns  
520 could be due to major air-driven events of taxa immigration from other insular or continental  
521 sources.

522 In addition to the wind orientation and force, data from the two stations regarding temperature  
523 and humidity of the same winds can be analyzed (Supplementary Fig. S10, Fig. S11, Fig. S13,  
524 Fig. S14). Humidity values from May to September winds tend to be rather similar, whereas  
525 air temperatures increase in line with the summer progression. These data do not account by  
526 themselves for events of species enrichment either.

527 Another aspect that can be verified is to compare the two periods in terms of PM10 particulate  
528 concentration; these are reported in Supplementary Fig. S1.C (May) and Fig. S4.C  
529 (September). Although there are obvious peaks of PM10 in correspondence with the dust



530 outbreaks dates, the basal levels of PM10 concentrations before and after those, are rather  
531 similar in the spring and fall period. This rules out the possibility of a diversity rise as linked  
532 to a general increase of such small particles trafficking over the areas.

533 The observed data reveal that, while dust-associated winds can account for some specific  
534 limited ingressions of taxa, a far more noticeable pattern appears consisting in a successional  
535 rise of taxa diversity. It is not yet possible to establish whether this occurrence could be linked  
536 to late summer in relation to the climatic conditions of the season. The second part of the  
537 summer, especially in the Mediterranean regions, is characterized by prolonged drought  
538 alternated to irregular thunderstorms. The income of a thunderstorm is accompanied by  
539 convective instability of the atmosphere and this phenomenon has been already pointed out as  
540 conducive to the emission and transport of fungal spores plumes (Burch and Levetin, 2002). A  
541 possible explanation for a richer pattern of airborne microbes after several weeks of  
542 prevailingly dry climate can be sought in the acknowledged fact that those seasonal conditions  
543 enhance the daytime height of the planetary boundary layer over Europe and continental US  
544 (Seidel et al. 2012), and that the ensuing low pressures foster the turbulence near ground and  
545 the overall convection, resulting in a frequent uplift of particles from land surfaces. In addition,  
546 it could also be postulated that the dryer and warmer summer conditions can eventually lead to  
547 partial cell dehydration in microbes lying at soil or vegetation surface, resulting in lighter cell  
548 weights more prone to be advantageously lifted by the local low layers air turbulence.

549 A further factor that can be hypothesized to have played a role in reducing the diversity of  
550 airborne community samples in May, comes from the analysis of the differentially featured  
551 taxa between the spring and the fall samplings (Tab. S1) where the strongest statistically  
552 significant differences were six taxa that resulted highly enriched in the former period and that,  
553 as cited above, included marine bacteria associated to oil spill-related oleovory phenotypes,  
554 extremophiles, and potential pathogens. These occurrences can be interpreted as possible clues



555 for a transient event of water pollution around the sampled areas that could have impacted also  
556 on the overall airlifted microbial diversity.

557 In addition to the above, a series of considerations can be drawn upon inspecting the pairwise  
558 community difference analysis, whose similarity values are shown in Tab. 5. It also needs to  
559 be recalled that, in order to examine the effect of a dust-free period, in May the control (May  
560 27<sup>th</sup>) was sampled after the dust event (May 21<sup>st</sup> – 22<sup>nd</sup>), while in September the control (Sep  
561 13<sup>th</sup>) was taken before the new dust outbreak (Sep 19<sup>th</sup> – 20<sup>th</sup>). Therefore, the summer, within  
562 which communities could undergo dust-independent changes, is in fact framed between the  
563 two control points, chosen as representative of dust-free atmosphere following a wind direction  
564 reversal. Within those months there were no dust-carrying wind outbreaks from the African  
565 land. This enabled also to verify whether a relatively long period without dust intrusions could  
566 have allowed an overall homogenization of the bacterial airborne communities over the island  
567 Sardinia.

568 The first consideration that stems from the global view of these data is once again that the most  
569 distant communities are those compared from different seasons (Tab.5, upper section) as  
570 evidenced by the red-to-yellow shades of the conditional formatting. It is worth noticing in this  
571 respect that no particular difference appears when comparing communities between those  
572 collected from the same site (right panel in the upper section) or in the cross-comparison  
573 between the two different places. Moreover, in these samplings from different seasons, the  
574 effects of the dust events in comparison to calm air with dust-free wind regimes, is not apparent,  
575 being diluted in the major season-related divergence of the communities.

576 When inspecting cases of the same season, the situation in May is representing a comparison  
577 picturing the recovery after the dust event, as the control follows the outbreak. One evident  
578 aspect in that is how the juxtapositions within the same place, feature the most similar cases  
579 (darkest shades of green) with the notable exceptions linked to the dust outbreak in Sassari,



580 which is the North-facing station (notice the two yellow-shaded values). On the contrary, such  
581 divergence does not appear at all in the South-facing Cagliari site. One interpretation of this  
582 interesting difference is that in the May control, when the air flux reversed after the dust event,  
583 the wind blowing from the northern quadrant, was conveying in Sassari air masses that came  
584 straight from the sea; while on the opposite corner (Cagliari) instead, the same air had passed  
585 over the whole Sardinia. Thus, the Northern collection station received sea-sweeping air,  
586 bringing ‘fresh’ taxa, i.e. not belonging to the Sardinian land-related common bacterial core of  
587 the season, while the southern station of Cagliari received instead land-sweeping air that had  
588 travelled all the way over the island latitudinal extension, and that therefore would have  
589 become mixed with the island-related core of biota. Thus, the sea-related entries would bring  
590 little contribution to the southern site communities after more than 100 miles of travelling and  
591 being diluted through the terra firma atmosphere. This would explain why, in May, shifting  
592 from dust outbreak to control in the Southern location, did not bring community divergence as  
593 it did in the Northern one.

594 The imprint of the common Sardinian core on homogenizing communities when the dust  
595 preceded the control, is also testified by the left panel (same month of May but different places)  
596 resulting in all green shades of medium value, showing that in such situation there was little  
597 difference also between different places.

598 An independent and indirect confirm of this interpretation is given by the situation in  
599 September. In that case, the African dust-carrying event was set to be taken after the control;  
600 this originated a reversed situation in comparison to the one observed in May; this time the  
601 place where the dust-outbreak did not bring particular change was the Northern site, Sassari,  
602 as the northbound wind from African origin had supposedly already discharged its load while  
603 passing over the land of the island from which, at the same time it would have lifted a vast  
604 portion of land-related common biota. Vice versa, in Cagliari, appreciable changes occurred



605 in relation to the dust arrival, which support the view of air blown over the sea plus dust, as the  
606 elements causing changes due to the new kinds of bacteria that hit this side at the frontal south-  
607 facing port of entry of the island. The left panel of the section (same season, September,  
608 different place) further confirms this as: (a) the strongest drivers of community divergence  
609 (yellow to orange colors) are flagged by the two comparisons between the Sassari control and  
610 the Cagliari dust situations, and the second of those, in the 12-24 hours window of the dust  
611 event is progressively more divergent than that recorded during the first 12 h (0.434 vs. 0.523  
612 similarity value). Moreover, the comparison between the two sites in the September control  
613 before dust, shows a rather high similarity (0.705), that is the highest among the September  
614 comparisons of different sites, which confirms that, before the dust outbreak, when both  
615 localities had experienced a long period devoid such phenomena, the two places had achieved  
616 a high degree of uniformity in spite of their distance. In that status, both communities were also  
617 profoundly different from their composition in May. A period of over 100 days without  
618 intrusions of dust-carrying northbound winds, appears to have accompanied an appreciable  
619 concerted successional change of the air-associated bacteria upon the Sardinian territory.

620 Essentially it appears that when airborne dust has to cross longitudinally the entire large island,  
621 it reaches the Northern sampling site (Sassari) less charged with community-changing  
622 potential, and/or, that it must have lifted bacteria from of the Sardinian common core, thus  
623 causing little variation upon their discharge over a station on the same island. On the contrary,  
624 when landing on the south-facing outpost of Cagliari, coming straight from Africa and, until  
625 that moment, having travelled over the sea only with air-lifted transcontinental dust, those air  
626 masses delivered in the south outpost of Cagliari an appreciably novel community. The  
627 geographic position of the sampling sites in relation to the wind origin appears therefore to  
628 play a major role in the patterns outcome. This supports the view that, in case of dust outbreaks,  
629 Cagliari, in the south, is at the forefront of changes that are substantially attenuated before they



630 reach Sassari; and vice versa, in case of reversed winds. A distance of >100 miles appears  
631 sufficient to absorb and buffer wind-borne taxa immigration in quantitative terms, from either  
632 side.

633

634 In conclusion, data are supportive of season related successional phenomena, involving a  
635 pattern of diffuse contemporary colonization over large portions of land, whose effect in  
636 shaping and homogenizing communities is stronger than the one conferred by occasional  
637 transcontinental discharges.

638 These clues entail novel aspects for our better understanding of microbial transport and spread  
639 across territories, of the epidemiological patterns for clinically relevant taxa, and can foster the  
640 predictive modeling of overall environmental microbiology dynamics.

641

#### 642 **Supplement.**

643

644 The supplement related to this article is available online at: [https.....](https://...)

645

#### 646 **Author contributions.**

647

648 PC conceived the project; RM, AS, AC, and PD designed the experiments and supervised the  
649 project. RR, MF, MD, GP1 and GP2 carried out the analyses and interpreted the data. AS  
650 wrote the manuscript.

651

652

#### 653 **Competing interests**

654

655 The authors declare that they have no conflict of interest.



656

657

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659

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