DOI OF PUBLISHED VERSION:

http://dx.doi.org/10.1016/j.scitotenv.2017.03.199

Influence of seasonality and air mass origin on airborne bacterial community structure and particulate matter chemical composition in the Po Valley, Italy

Elena Innocente[a], Stefania Squizzato[a,b], Flavia Visin[a], Chiara Facca[a], Giancarlo Rampazzo[a], Valentina Bertolini[c], Isabella Gandolfi[c], Andrea Franzetti[c], Roberto Ambrosini[c], Giuseppina Bestetti[c]

[a]Department of Environmental Sciences, Informatics and Statistics (DAIS), Università Ca' Foscari Venezia, Campus Scientifico, Via Torino 155, 30172 Mestre (VE), Italy

[b]Center for Air Resources Engineering and Science, Clarkson University, Box 5708, Potsdam, NY 13699-5708, USA

[c]Department of Earth and Environmental Sciences (DISAT) –University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milano, Italy

Corresponding author:

Elena Innocente, PhD

e-mail: elena.innocente@unive.it

Department of Environmental Sciences, Informatics and Statistics (DAIS), Università Ca' Foscari Venezia, Campus Scientifico, Via Torino 155, 30172 Mestre (VE), Italy

Tel: +39 041 2348639

Abstract

The integration of chemical and biological data in aerosol studies represents a new challenge in atmospheric science. This new approach aims to couple chemical composition of particulate matter (PM) with airborne bacterial community structure in order to gain a clearer and deeper comprehension of biogeochemical cycles in the atmosphere. In this view, this study aimed to investigate the relationships occurring between bacterial populations and PM chemical composition in one of the most polluted and urbanized areas in Europe: the Po Valley (Italy). Moreover, seasonality, long- and short-range transports were also evaluated to investigate the influence on airborne bacterial communities.

PM samples were collected in two cities of the Po Valley (Milan and Venice) characterized by different meteorological conditions and atmospheric pollutant sources. Samples were analysed for water-soluble inorganic ions (WSIIs) and bacterial community structure. Chemical and biological data were jointly processed by using redundancy discriminate analysis (RDA), while the influence of atmospheric circulation was evaluated by using wind ground data and back-trajectories analysis. Results showed strong seasonal shifts of bacterial community structure in both cities, while a different behaviour was observed for air mass circulation at Milan ad Venice : long-range transport significantly affected bacterial populations in Milan whereas, local ground wind had more influence in the Venetian area.

Keywords: PM, airborne bacteria, WSIIs, ground wind circulation, back-trajectories analysis.

1. Introduction

The increasing awareness on the poor air quality conditions in the industrialized countries and urbanised areas has promoted of studies focusing on chemical composition, source distribution, and

health effects of atmospheric particulate matter (PM). An increasing interest in understanding origins, dispersion, transformation and fate of air pollutants emerged, in order to prevent damages on ecosystems, cultural heritage and human health (Heinrich et al., 2013; Pope and Dockery, 2006).

Recently, studies on the atmospheric PM have been extended to bioaerosol, a complex mixture of viable and non-viable microorganisms and other biomass (Gandolfi et al., 2013). The reason of this increasing interest on bioaerosol is mainly due to the presence in the atmosphere of endotoxins and pathogenic bacteria that are of great concern for their harmful effects on human health (Kim and Kim, 2007; Mueller-Anneling et al., 2004; Peccia et al., 2008). In fact, it has been recognized that bioaerosol could be a direct cause of infectious (tuberculosis, pneumonia, etc.) and non-infectious diseases (irritation, inflammation) (Peccia and Hernandez, 2006). Furthermore, it is well known that bacteria could affect ecosystems and agriculture productivity (Shinn et al., 2000).

The microbial communities of different atmospheric environments (urban, rural, remote, etc.) have been extensively investigated so far, and some potential local sources of airborne bacteria have been identified (Bowers et al., 2009, 2011a, 2011b). Furthermore, the abundance of total bacteria and of some specific populations of interest (for instance, pathogens) has been evaluated through different methods (Bertolini et al., 2013; Fahlgren et al., 2010). In recent years, studies were conducted to investigate differences among the bacterial communities sampled at different altitudes and to evaluate the influence of pollution on community structures at different atmospheric levels (near-surface, troposphere, etc.) (Maki et al., 2013; Zweifel et al., 2012). Therefore, the characterization of bioaerosol in specific circumstances has been widely explored.

The effect of both origin and local provenience of air masses on community assembly processes of airborne bacteria has been extensively assessed in previous studies. Bacterial long-range transport has been studied through the characterisation and the origin of tropospheric bacteria (Smith et al. 2012; DeLeon-Rodriguez et al. 2013; Maki et al. 2013) and through the assessment of the impact of dust events on the airborne microbial populations (Hervàs et al. 2009; Jeon et al. 2011; Polymenakou et al. 2008; Mazar et al. 2016; Meola et al., 2015). Previous literature

consistently indicated that bacterial tropospheric transport and dust events significantly shape airborne microbial communities. Short-range bacterial transport in the atmosphere has been previously evaluated through the analysis of air with known local provenience such as vegetated area (Lymperopoulou et al., 2016) and marine/coastal environments (Seifried et al., 2015), or inferred by the similarity between the structures of airborne communities and those obtained from local potential sources like soil, lakes, leaves and faeces (Bowers et al., 2011a,b;Bertolini et al., 2013). However, in all the above-cited papers, only qualitative insights into these bacterial dispersion processes were provided and quantitative assessment of the contribution of short- and long-range bacterial transports to the airborne bacterial community structures is still lacking.

In this study, a PM sampling campaign was carried out in two cities located in the Po Valley (Northern Italy), one of the most polluted and urbanized areas in Europe (Larsen et al., 2012). Samples were analysed for bacterial community structure, obtained by sequencing a fragment of the 16S rRNA gene, and for water-soluble inorganic ions (WSIIs). Data were processed to assess the effect of PM composition on the abundance of specific bacterial populations and to evaluate the influence of environmental conditions on the structure of bacterial communities associated to PM. To this end, air mass movements were investigated both at local and regional scale by using wind ground data and back-trajectories analysis, respectively. Relationships between bacterial communities and environmental conditions (seasons and air mass origin) were investigated by using RDA analysis, a widely applied chemometric technique to analyse biological data coupled to environmental data (Wakelin et al., 2006; Feinstein et al., 2009;Wang et al., 2012).

The obtained results integrated those reported in a previous paper, in present work relationship between water soluble inorganic ions and bacteria population were investigated, highlighting also differences due to long- and short- range transport.

2. Materials and methods

2.1 Study area and sampling sites

The presence of several pollution emission sources and unfavourable weather conditions in the Po Valley (Northern Italy) generally determine high PM and gaseous pollutant concentrations in this area, especially in cold seasons, when low temperatures, weak winds, and thermal inversion occur (Larsen et al., 2012; Masiol et al., 2012a; Canepari et al., 2014). Several studies showed that local topography and meteorological conditions, natural and anthropogenic emissions and regional transport processes make this area one of the most polluted in Europe (e.g.,Carnevale et al., 2010; Larsen et al., 2012).

Three sampling sites were located in Venice, a coastal city in the eastern part of the Po Valley, influenced by the presence of a lagoon and a wide industrial area (Rampazzo et al., 2008; Masiol et al., 2012b). A fourth sampling site was located in Milan, a big city in the middle of the Valley, characterised by highly dense residential and commercial premises and a very high volume of vehicular traffic (Marcazzan et al., 2001) (Figure 1):

- (I) Venice, Via Lissa (VL) (45°29’12.4’’N, 12°13’20.3’’E) is an urban background site settled in a high density residential zone of Mestre, at 50 m from main traffic roads and few meters from the railway (Masiol et al., 2014);
- (II) Venice, downtown (VD) (45°25’54.8’’N 12°19’13.2’’E) is an urban background site in the historical city centre,in the middle of the lagoon;
- (III) Venice, Malcontenta (MC) (45°26’18.6’’N, 12°12’13.3’’E) is an industrial site in the area of Porto Marghera, downwind from the industrial settlement (Squizzato et al., 2014).
- (IV) Milan, Via Cozzi (MI) (45°30’35.4’’N, 9°12’38.5’’E) is an urban traffic site in the northern part of the city.

2.2 Sample collection

Samples were collected during four periods representative of different seasons to evaluate PM levels, chemical composition and bacterial community structures in different environmental

conditions: July 2011 (summer), October 2011 (autumn), January 2012 (winter) and March-April 2012 (spring).

Daily samples (24 h) were collected simultaneously at each site(32 samples at MC, VL and MI; 24 samples at VD), on quartz fibre filters (Whatman QMA) by using high volume samplers equipped with PM_{10} (at MC, VL and MI) and total suspended particulate (TSP, at VD) inlets at 30 m^3 h^{-1} (VL MC and VD) and 12m^3 h^{-1} (MI). Filters were UV sterilized before sampling. All collected filters were stored at -20°C until further processing as suggested by Heinrich et al. (2003).

At VL and MC sites, PM_{10} samples were also collected on quartz fibre filters (Whatman QMA, Ø 47 mm) by using a low volume sampler in order to measure PM_{10} concentration via gravimetric determination. At MI site, PM_{10} data were provided by ARPA Lombardia (Environmental Protection Agency of Lombardy Region).

2.3 DNA extraction and building of Illumina 16S rRNA fragment libraries

Total DNA was extracted from the quartz fibre filters using the FastDNA Spin for Soil kit (MP biomedicals, Solon, OH, USA). A quarter of each filter was cut into small pieces and loaded into the bead tube of the FastDNA Spin kit for Soil after adding 1M $CaCO_3$ in order to increase the pH, and shaking at 200 rpm for 60 min. The remaining steps of the DNA extraction were performed according to the manufacturer's instructions (Gandolfi et al., 2015).

For Illumina HiSeq sequencing, the V5-V6 hypervariable regions of the 16S rRNA gene were amplified, pooled and purified as previously reported (Bertolini et al., 2013). Multiplexed sequencing of all the pooled samples were performed on a single Illumina HiSeq 1000 lane, using a paired-end 2 × 100 base-pair protocol and the 4.0 sequencing chemistry. The cluster extraction and base-calling processing analyses were performed by using the Illumina CASAVA Analysis software, version 1.8. Illumina HiSeq 1000 sequencing was carried out at BMR Genomics, Padua, Italy.

2.4 Sequence analysis

Sequence data were deposited at the European Nucleotide Archive (ENA) with the study accession number PRJEB7001 (sample accession numbers from ERS528729 to ERS528823).

Reads from sequencing were demultiplexed according to the indexes. UPARSE pipeline was used for the following elaborations (Edgar, 2013). Forward reads were quality filtered with default parameters. Suspected chimeras and singletons sequences (i.e. sequence appearing only one time in the whole data set) were removed both from the whole dataset and from each sample file. Operational Taxonomic Units (OTUs) were defined on the whole data set clustering the sequences at 97% similarity and defining a representative sequence for each cluster. A subset of 10,000 random sequences was chosen from each sample and the abundance of each OTU was estimated by mapping the sequences of each sample against the representative sequence of each OTU at 97% similarity. Taxonomic classification of the OTU representative sequences was obtained using RDP classifier (Wang et al., 2007)

2.5 Chemical analysis

Water-soluble inorganic ions (WSIIs) were extracted in ultrasonic bath for 60 min in 20 mL of ultrapure water (resistivity 18 MΩ cm). Water temperature was kept <35 °C to prevent artefacts and evaporation, then samples were stored at 4°C until ion determination. After filtration through microporous PTFE membranes (PALL Acrodisc CR, pore size 0.45 mm),four cations (Ca^{2+}, Na^{+}, Mg^{2+}, NH_4^{+}) and three anions (NO_3^{-}, Cl^{-}, SO_4^{2-}) were analysed by ion chromatography (IC, Dionex DX500), using a guard column (DionexIonpac CG12 for cations and AG14 for anions), a separation column (DionexIonpac CS12 for cations and AS14 for anions), a self-regenerating suppressed conductivity detector (Dionex ED40) and a gradient pump (Dionex GP40). An isocratic flow of 1.2 mL min^{-1} 3.5 mM Na_2CO_3/$NaHCO_3$ base eluent was used for anion detection, whereas an isocratic flow 1 mL min^{-1} 20 nM CH_3SO_3H acid eluent was used for cation analyses (Squizzato et al., 2012).

Accuracy of analysis was evaluated using standard reference material NIST 1648 "Urban particulate matter". All recoveries were in the range of 85-100%.

2.6 Statistical methods: Redundancy Discriminant Analysis

Differences in the structure of bacterial communities at different sampling sites were investigated by Redundancy Discriminant Analysis (RDA). The Hellinger transformation was applied to OTU relative abundance before the analyses. Singletons were removed before all the analyses.

As described in Yergeau et al. (2009), relative OTU abundance was used as "species data", whereas sampling site was used in the analysis as the "environmental" variable. This first analysis revealed significant differences among sampling sites (which were expected on the basis of the results of a previous work).Therefore, all the following analyses were run on data from each site separately. This analysis was followed by post-hoc pairwise comparisons between sampling sites performed by running separate RDAs on each pair of sampling locations and adjusting significance of these tests according to the False Discovery Rate (FDR) procedure of Benjamini and Yekutiely (2001).

2.6.1 RDA between bacterial communities and chemical composition

The structure of bacterial communities was investigated in relation to the chemical composition applying the RDA analysis performed by using CANOCO 4.5 (Wakelin et al., 2006; Feinstein et al., 2009; Wang et al., 2012; Vidal-Liñán et al., 2014; Brix et al., 2012; Braga Bertini et al., 2014). Chemical data were employed in the analysis as "environmental" variables. Environmental variables significantly related and that best explained OTUs were determined by Monte Carlo tests with 499 unrestricted permutations (Chen et al., 1997; Lindström, 2000). Since data were not normally distributed, a log transformation of data was performed before the analyses. Only OTUs that showed fitness over 20% to RDA analysis were considered relevant, related to chemical data and represented in the results. These OTUs were assigned the RDP taxonomic classification at the deepest level possible.

223

224 *2.6.2 RDA between bacterial communities and environmental conditions*

225 RDAs on data from each sampling site were run by separately entering season, back-trajectories and
226 local wind clusters as "environmental" variables. These analyses were performed with the VEGAN
227 package (Oksanen et al., 2016) of R 3.1.2 (R Core Team 2015).

228 The variation partitioning (VarPart) in canonical analyses was used to investigate the amount of
229 variation in airborne bacterial communities explained separately and jointly by each predictor
230 (season, back-trajectories cluster and local wind clusters), while controlling for the effect of the
231 other ones. Overall, the implemented framework of analysis is similar to that fully described in
232 Borcard et al. (2011).

233 Predictors were preliminarily investigated to understand if they were redundant. Since all predictors
234 entered in our models were factors, redundant (aka aliased) variables could occur only when a level
235 of one factor always occurred concomitantly to a level of a second factor, thus determining the
236 impossibility of disentangling their effects (redundant contrasts). For example, the only air mass
237 originating from area n. 5 was sampled in Malcontenta on 1 and 2 February 2012. The same air
238 mass was the only one coming locally from area n. 1. Thus, it is impossible to assess whether any
239 variation of airborne bacterial communities was due to an effect of origin or local provenience of
240 this air mass. Thus, origin and local provenience of air masses could not be both included in the
241 VarPart unless samples with a unique combination of origin and local provenience areas were
242 excluded, in order to remove redundant contrasts.

243 RDAs were not affected by this problem because they were run by including each predictor
244 separately. However, in order to run all the analyses on the same datasets, we performed also RDAs
245 by removing samples collected from air masses with a unique combination of origin and local
246 provenience.

247

248 ***2.7 Atmospheric circulation analysis***

To understand the origin of air masses that could influence microbiological and chemical composition of PM in study areas, back-trajectories cluster analysis was applied to identify the origin of air masses. The NOAA HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory) model (Draxler and Rolph, 2015) was used to compute -96 hour backward trajectories using GDAS1 (Global Data Assimilation System) meteorological data at starting height of 150 m over ground level by using MI and MC as starting points. A single back-trajectory was calculated once on sampling days and calculation was limited to -96 h because accuracy of back trajectory reconstruction decreases with distance and time due to model assumptions and spatial and temporal resolution of the meteorological data. Moreover, clustering back-trajectories reduces errors associated to single trajectories (Stohl, 1998). Cluster analysis was performed by using the HYSPLIT tool in order to classify all daily backward trajectories in a small number of groups of similar history (Makra et al., 2011), i.e. similar advection path and velocity. Origin area of air masses was therefore defined as the area where each air mass was located 96 h before reaching the sampling site. At all sites a proper number of clusters was set on the basis of total spatial variance analysis.

It is also well known that at receptor site air pollutant levels are influenced not only by dispersion and long-range transport in atmosphere, but also by local ground winds, resulting in changes of pollutant concentrations in relation to wind direction (Squizzato and Masiol, 2015). Therefore, a cluster analysis on wind data was performed to group days with similar ground circulation pattern. The hourly data of wind speed and direction were decomposed in their scalar components *u* and *v* relative to the North–South and West–East axes. Then, a hierarchical cluster analysis was performed by using the Ward's method and the squared Euclidean distance measure, on the daily resultant vector of air movement obtained from the sum of hourly values(Squizzato and Masiol, 2015).

WSIIs and bacterial community data were then coupled with back-trajectories and local wind clusters, in order to investigate the influence of long-range transport and local wind.

3. Results and discussion

3.1 PM and WSIIs concentrations

Table 1 summarizes the average concentrations of $PM_{10}$and WSIIs over the whole period and for each season.

PM_{10} concentrations were quite similar at all the sampling sites (49 μg m^{-3}, 37 μg $m^{-3}$and 53μg m^{-3} on average at VL, MC and MI, respectively). That similarity was probably due to the orographic and climatic conditions of Northern Italy, that promote pollutant stagnation enhancing homogeneous PM levels. This situation is exacerbated during cold periods: the typical atmospheric stability and thermal inversion cause the formation of cold masses at the ground level. Moreover, humidity is often high in this area, generating fog in winter and intermediate seasons, with low dispersion and increasing concentration of pollutants (Pecorari et al., 2013). In fact, the maximum concentration is reached in winter at all sites equal to126 μg m^{-3}, 88 μg m^{-3} and 157 μg m^{-3}at VL, MC and MI, respectively.

Generally, MI samples exhibited the highest concentrations of nitrate and sulphate all over the period (8.4 μg m^{-3} and 4.2 μg m^{-3}on average of NO_3^- and SO_4^-, respectively). VD was characterized by the highest levels of Cl^-(2.2 μg m^{-3}) and Na^+ (1.8 μg m^{-3}), probably due to the proximity of the sampling site to the Adriatic Sea and the Venice Lagoon and to the size of the sampled particles (TSP).

3.2 Correlations between ions and bacterial populations

RDA analysis was performed in order to investigate the potential relationships between ion concentrations and OTU abundance. Only correlations between ion concentrations and OTU abundances that were significant in two or more sites were described. An example of RDA analysis is reported in Figure 2 whereas all the other graphs are reported as supplementary materials (Figure SI1, SI2 and SI3).

Sulphate was removed from RDA analysis in all sites, because inflation factor, used to eliminate collinear environmental variables (Sikkink et al., 2007), was too high (>20) and could affect RDA analyses. For the same reason sodium was removed in MC and VD : in this case inflation factor is high probably due to the strong correlation of Na^+ concentrations with those of Cl^- (r=0.95; r=0.81 in VD and in MC, respectively) reflecting the marine origin of these ions (Querol et al., 2006). The cumulative percentage variance of species-environment correlations explained by the first two axes was 63.1% in VL, 53.7% in MC, 61.6% in MI and 74.5% in VD.

$NH_4^+$was correlated with *Dyella*(OTU 16) in MI and VD and *Herbaspirillum*(OTU 20) in MI and VL. All these bacteria are typical of soil (Xie and Yokota, 2005, Baldani et al., 1986).Furthermore, NH_4^+ was correlated with *Ralstonia* (OTU1, OTU41) in VD and MI and with *Betaproteobacteria* (OTU 119) in VL and MC. Calcium ion is considered a crustal ion originated from erosion and resuspension of soil (Perrone at al., 2010). In MI and MC, Ca^{2+} concentration was correlated with abundance of soil bacteria such as *Hymenobacter* (OTU38) (Oren et al., 2006) and *Sphingomonas* (OTU 19) (White et al. 1996); this relationship could suggest a common origin from soil of both these bacteria and Ca^{2+}.

On the contrary, calcium ion was inversely correlated with abundance of *Propionibacterium* (OTU 33), *Delftia* (OTU 11, OTU70, OTU95, OTU142), *Escherichia/Shigella* (OTU169, OTU802, OTU1027) and *Stenotrophomonas* (OTU51)in MC and VD sites.

3.3 Influence of environmental conditions on PM and bacterial populations

Results from cluster analysis on wind data and back-trajectories and RDAs between bacterial populations and environmental variables are discussed in this section.Sampling days were grouped on the basis of season, the provenience of air masses considering both local and distant transports and then the changes on bacterial populations considered more relevant (relative abundance> 1%) and WSIIs data for each group were discussed. Changes in ion composition and bacterial

community for each group were first investigated and, subsequently, RDAs analyses were run by entering separately season, local air masses and air masses provenience.

3.3.1 Spatial and seasonal variability of ions and bacterial communities

As shown in previous studies (e.g., Perrone et al., 2010; Squizzato et al., 2013), ionic composition of PM varies on seasonal basis. Among the analysed ions, NO_3^- dominated in PM during spring, autumn and winter whereas sulphate represented the most abundant ion in summer (Figure SI4). This behavior can be explained by the semi-volatility of ammonium nitrate (Terzi et al., 2010; Squizzato et al., 2013). Generally, sulphate and nitrate concentrations resulted significantly correlated between Milan and Venice (r = 0.5, p-value < 0.05) with statistically similar concentrations along the analysed period on the base of Kruskal-Wallis non-parametric test (p-value > 0.05). Other ions presented different patterns and concentrations among sites and seasons.

Bacterial communities were dominated by 28 OTUs that exceed the 1% of relative abundance in all sampling sites (abundant OTUs) (Figure SI5-SI8). The most abundant OTU in all sampling sites and all seasons belonged to the genus *Ralstonia* (OTU 1), which is known to be a plant-associated microorganism (Schonfeld et al., 2003). This OTU was particularly abundant in MC where it represented from 18% to 82% of the abundant OTUs in spring and winter, respectively.

Spring samples from VL, VD and MC showed similar bacterial communities with a dominance of *Ralstonia* (OTU 1) and marine bacteria belonging to the family Rhodobacteraceae (OTUs 2, 9, 12, 15, 34 and 35) (Hwang and Cho 2008; Dang et al., 2008). In Milan, spring samples were characterized by the presence of soil bacteria (*Acinetobacter* (OTU 3), *Acidovorax* (OTU 4)) and by *Lactobacillus* (OTU 25), *Actinomycetales*(OTU 26) and *Staphylococcus* (OTU 13).

During summer, *Bordetella* (OTU 14) increased at all sites. VL was also characterised by an increase of *Mesorhizobium* (OTU 10), *Propionibacterium* (OTU 7) and *Delftia* (OTU 11).

Similarly, MI showed samples enriched in *Propionibacterium* (OTU 7 and 33) and also in *Acidovorax* (OTU 4) and *Acinetobacter*(OTU 3).

Samples collected at VL site were dominated, in autumn, by bacteria typical of soil such as *Acetobacter* (OTU 22) and *Herbaspirillum* (OTU 20) (Baldani et al., 1986) whereas VD samples were characterised by higher abundances of *Bordetella* (OTU 14) and *Staphylococcus* (OTU 13) and MI samples were enriched in *Sphingomonas* (OTU 19), *Pelomonas* (OTU 23) and *Dyella* (OTU 16)

Winter samples were characterized at VL sites by other soil bacteria such as *Acinetobacter* (OTU 3), *Acidovorax* (OTU 4), *Sphingobium* (OTU 30) (Li et al., 2011; Krizova et al., 2014; Takeuchi et al., 2001) and also by marine bacteria such as *Alteromonadaceae* (OTU 5) (Kwak et al., 2012). At MI site, winter samples significantly differed from the other seasons for the increase of *Dyella* (OTU 16), *Herbaspirillum* (OTU 20) and *Pseudomonas* (OTU 18).

3.3.2 Local ground wind circulation

Five groups of days characterized by different ground circulation patterns were defined by cluster analysis (Figure 3) both in Milan and Venice area. Investigated areas presented strong differences in wind regimes. Venice exhibited the sea-land breeze regime common to most coastal areas. It represents a complex atmospheric circulation pattern, with wind blowing from north-east and south-east, affecting weather, climate dynamics and the formation and transport of pollutants between sea and mainland (Masiol et al., 2010). Milan showed relative lower average wind speed (1.8 m s^{-1}) but less wind calm hours (1%) compared to Venice (2.5 m s^{-1}, 5%) and wind blowing from east and south.

Table 2 reports the average ion concentrations and Figure 4 shows the relative abundance of the 28 most common bacterial OTUs in each wind group. In the Venice area strong winds from NE (group 1, mean speed 6.8 m s^{-1}) brought to generally low concentrations of all ions except sulphate

376 at VL and MC site. Group 2 exhibited the lowest mean speed (1.4 m s^{-1}) and was associated to an
377 increase of some ions, in particular nitrate and ammonium at all Venice sites.

378 An increase of marine and crustal components can be observed in group 3 at VL and MC,
379 whereas nitrate increased at VD. Group 4 showed a wind rose similar to that referred to the full
380 period, resulting in ion concentrations quite similar to the average PM composition. Sea salt
381 components (Cl^- and Na^+) strongly increased in group 5, characterized by strong winds from SE
382 (3.0 m s^{-1}), at all Venice sites. Moreover, an increase in sulphate concentration can be observed at
383 VD. Among analysed ions, chloride presented statistically significant differences at all Venice sites
384 (p-value < 0.05) whereas nitrate statistically changed only at VL and MC sites. At MC site also
385 sulphate exhibited concentrations statistically different among the identified groups.

386 At MI, PM composition showed a weaker wind direction dependence: although the
387 prevalent wind direction changed between the wind groups, ion concentrations seemed more
388 correlated to the wind speed. Group 1, 2 and 3 exhibited the highest mean speed (2.1 m s^{-1}, 3.0 m s^{-1}
389 and 2.1 ms^{-1}, respectively) and the lowest ion concentrations whereas an increase be observed in
390 group 4 and 5, characterised by the lowest mean speed. Statistically significant differences were
391 observed only for nitrate and calcium ion (p-value < 0.05).

392 The relative abundance of considered bacterial populations showed the most evident
393 differences at the Venice sites (Figure 4) and in particular at VD. Marine bacteria
394 (*Rhodobacteraceae*, OTU 2, 12, 15 and 35) increased at all sites in group 3, 4 and 5 when wind
395 blew from SE with a statistically significant difference at VD (p-value < 0.05).

396 At VD site, group 1 is characterized by a strong increase of *Acinetobacter* that may be due to the
397 resuspension of soil particles enhanced by the high wind speed. Furthermore, soil bacteria
398 (*Mesorhizobium*, OTU 10 and *Sphingobium* OTU 30) showed significant differences across the
399 groups.

400 At VL, an increase on *Acinetobacter* (OTU 3), a soil bacterium, can be observed in group 2 and 3
401 when a western wind component is present. Moreover, *Propionibacterium* (OTU 7) and

Ochrobactrum (OTU 32) exhibit significant differences among the wind groups. At MC, only *Staphylococcus* (OTU 13) showed significant differences.

At MI sites west winds (group 2) were associated to an increase in *Sphingobium* (OTU 30) and *Bordetella* (OTU 14). *Acinetobacter* increased in group 1 and 5 and *Herbaspirillum* (OTU 20), another soil bacterium, increased in group 3, 4 and 5. However, these increases did not seem related to a specific wind direction. Moreover, statistically significant difference was observed for *Rhodobacteraceae* (OTU 2) and *Herbiconiux* (OTU 24).

3.3.3 Long-range transport

Identified clusters on back-trajectories are shown in Figure 5. The 50% of Back trajectory were characterized by local air masses that passed across the Po Valley, but the other 50% in Both the analysed areas, Milan and Venice, were characterised by distant air masses, coming north-west Europe (United Kingdom) and Germany,. Moreover, a south-west group can be observed at MI site coupled to air masses originated in southern France (Provence), whereas in Venice air masses from southern Italy and Eastern Europe were also present. The effects of long-transport processes on PM and its composition and sources were discussed in previous studies both in Milan (Kukkonen et al., 2005; Lonati et al., 2007) and Venice area (Masiol et al., 2012a; Masiol et al., 2012b; Squizzato et al., 2012; Squizzato et al., 2014; Squizzato e Masiol, 2015). In this study, statistically significant differences on WSIIs concentrations were detected at all sites for Cl^- (VL, MC, VD and MI), Mg^{2+} and Na^+ (MC and VD), NH_4^+ and SO_4^{2-} (MI).

Average WSIIs concentrations for each back-trajectory cluster are shown in Table 3 and Figure 6 reports the relative abundance of the 28 most common bacterial OTUs in each cluster.

Group 1 gathered together days characterised by local air masses that spent most of the time over the Po Valley. PM composition is quite similar to that referred to the full period and bacterial populations did not present significant changes compared to the full period at Venice sites whereas at MI an increase of *Acinetobacter* (OTU 3), a soil and water microorganism (Li et al., 2011;

Krizova et al., 2014), could be observed. At MI, abundances of *Acinetobacter* (OTU 3), *Acidovorax* (OTU 4), *Propionibacterium* (OTU 7) and *Sphingobium* (OTU 30) were statistically higher compared to other groups (p-value < 0.05).

At VL, MC and MI, air masses from Germany (Group 2) were characterized by higher concentrations of NH_4^+, NO_3^- and SO_4^{2-}. At MI, higher concentrations of chloride were also observed but only 20% of chloride was attributable to marine origin; thus, the greatest part could be attributed to anthropogenic activities as previously observed in Turin by Malandrino et al. (2013). Among the sites, only at VL and MI significant increases of some bacterial OTUs were reported: *Gammaproteobacteria* (OTU 21) and *Bordetella* (OTU 14) at VL and *Herbaspirillum* (OTU 20), *Pseudomonas* (OTU 18) and *Acetobacter* (OTU 22) at MI. *Pseudomonas* and *Herbaspirillum* were classified as water or soil bacteria from Xie and Yokota (2005).

In Venice area air masses coming from South Italy and Adriatic Sea (Group 3) were characterized by an enrichment in marine ions, Cl^-, Na^+ and Mg^{2+}, marine bacteria as *Rhodobacteraceae* (OTU 2 and 35), nitrogen fixing bacteria originated from soil (*Mesorhizobium*, OTU 10), and *Propionibacterium* (OTU 33) (Kaspar 1982; Jarvis et al., 1997; Xu et al., 2007). These bacteria presented abundances statistically different at MC (OTU 33) and VD (OTU 2, 35 and 10).

At MI, air masses from Provence were associated to Group 3. In this group, chloride concentration is lower than in the other groups, but 48% of chlorine, calculated as proposed in Pakkanen (1996), was of marine origin. Bacteria such as *Acinetobacter* (OTU 3), *Alteromonadaceae* (OTU 5) and *Acetobacter* (OTU 22) were specific of this cluster and could be addressed to a marine origin (Kaspar, 1982; López-Pérez and Rodriguez-Valera, 2014).

Air masses coming from United Kingdom and crossing France composed Group 4. At MI this group presented days slightly enriched in soil or river sediment bacteria such as *Actinomycetales* (OTU 26), *Sphingobium* (OTU 30) (Takeuchi et al., 2001; Ushiba et al., 2003), or bacteria isolated from animal, human and environmental samples (*Bordetella*, OTU 14) (Wang et

al., 2007). Different types of bacteria such as *Staphylococcus* (OTU 13), *Actinomycetales* (OTU 26), *Lactobacillus* (OTU 25), showed a slightly increase at VD whereas *Acidovorax* (OTU 4) and *Acinetobacter* (OTU3) increased both at VD and VL site. *Acidovorax* and *Acinetobacter* could be addressed to a soil or water origin (Trujillo et al., 2005), *Lactobacillus* and *Actinomycetales* were associated to plant debris and *Staphylococcus* is a well know human pathogen (CorbiereMorot-Bizot et al., 2004).

The last cluster grouped air masses coming from Eastern Europe and it was only detected in the Venice area associated with an increase in sulphate concentrations at VL and MC. *Sphingomonas* (OTU 19) is peculiar of this group showing the highest abundances and resulting statistically different from other groups at all sites. It can be found in a lot of environments such as water, soil, associated with plants and clinical specimens and it is also pathogen for humans and animals (White et al., 1996). *Pseudomonas* (OTU 18), *Ochrobactrum* (OTU 32) and *Alteromonadaceae* (OTU 5) exhibited an increase in the relative abundances at MC, whereas *Acetobacter* (OTU 22) increased at VD. *Ochrobactrum* and *Pseudomonas* can be found in different environments such as soil, water or clinical specimens (Trujillo et al., 2005).

3.3.4 Environmental conditions and bacterial community structures

RDA analyses run by entering separately season, long distance air masses and local air masses, showed that season was a significant factor in all sampling sites whereas local air masses were significant at all sites except at MI (Table 4). Conversely, long distance air masses significantly affected the structure of the bacterial communities only at Milan sampling site.

Bacterial community structure differed significantly among sampling sites ($F_{3,115}$ = 2.305, P= 0.001), with significant differences among all sites, as indicated by post-hoc tests ($F_{1,55} \geq 1.551$, $P_{FDR} \leq 0.005$ in all cases) (Table 4). These results confirmed the seasonal differences among the communities of airborne bacteria already reported in previous studies (Gandolfi et al. 2013; Smets et al. 2016). Results seem also to suggest that local air masses influenced the microbial community

assembly in Venice, whereas long distance air masses affected the microbial communities in Milan bioaerosol.

However, if the amount of variation in air bacterial communities explained separately by each predictor (e.g.:[S|O+P]) were investigated while controlling for the effect of the other ones, results show that the independent contributions of both origin and local provenience of air masses were not significant. This probably reflects the fact that both origin and local provenience of air masses had a strong seasonal correlation which prevents disentangling the effect of the variables and suggests that longer and more intensive sampling campaigns should be planned to detect and quantify the effect of bacterial transport processes. Conversely, season *per se* explained a significant fraction of variation in the structure of bacterial communities observed in all sampling locations. It is worth noting that season is a categorical variable that accounts for variation in a number of environmental conditions which are known to affect the structure of airborne microbial communities, such as temperature, humidity, solar radiation, and sources of air PM. It is therefore reasonable that some effects on airborne bacterial populations due to local and distant air masses were captured by the variable season.

4. Conclusions

This study investigated the relationships occurring between bacterial populations, PM chemical composition and environmental conditions in one of the most polluted and urbanized area in Europe: the Po Valley (Italy). The effects of season and long- and short-range transport of bacteria via air mass movements was investigated by using different chemometric tools. The main findings can be summarised as follows:

- Results showed a correlation between NH_4^+, NO_3^- and bacteria considered involved in nitrogen cycle.

- Marine bacteria such as *Rhodobacteraceae* were more abundant in air masses with a marine origin. Other species were not clearly influenced by long-range transport, in opposition to the ionic composition.
- Such difference in taxonomic composition was probably due to the characteristics of sampling sites rather than to the influence of air mass origin, both local and distant. Moreover, it is therefore reasonable that some effects on airborne bacterial populations due to local and distant air masses were captured by the variable season.
- This evidence could suggest that, while PM composition is influenced by long-range transport, bacterial populations are affected, besides transport, by other factors (i.e., season and sampling site location).

Acknowledgments

The authors would thank ARPAV managers and technicians, for their support and samplers use, ARPA Lombardia for Milan PM_{10} data, Prof. Emanuele Argese, Lorena Gobbo, Prof. Francesco Pinna and Dr. Michela Signoretto for their analytical support.

References

Baldani, J.I., Baldani, V.L.D., Seldin, L., Dobereiner, J., 1986. Characterization of *Herbaspirillum seropedicae* gen. nov. sp. nov. Aroot- associated nitrogen-fixing bacterium. Int. J. Syst. Evol. Micr. 36, 86-93.

Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29, 1165–1188.

Bertolini, V., Gandolfi, I., Ambrosini, R., Bestetti, G., Innocente, E., Rampazzo, G., Franzetti, A., 2013. Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. Appl. Microbiol. Biotechnol. 97, 6561–6570.

Borcard, D., Gillet, F., Legendre, F.,2011. Numerical Ecology with R. Springer, New York.

Bowers, R.M., Lauber, C.L., Wiedinmyer, C., Hamady, M., Hallar, A.G., Fall, R., Knight, R., Fierer, N., 2009. Characterization of airborne microbial communities at a high-elevation site and their potential to act as atmospheric ice nuclei. Appl. Environ. Microbiol. 75, 5121–5130.

Bowers, R.M., McLetchie, S., Knight, R., Fierer, N., 2011a. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. ISME J. 5, 601–612.

Bowers, R.M., Sullivan, A.P., Costello, E.K., Collett, J.L., Knight, R., Fierer, N., 2011b. Sources of bacteria in outdoor air across cities in the midwestern United States. Appl. Environ. Microbiol. 77, 6350–6356.

Braga Bertini, S.C., Azevedo, L.C.B., de Carvalho Mendes, L., Cardoso, E.J.B.N., 2014.Hierarchical partitioning for selection of microbial and chemical indicators of soil quality. Pedobiologia 57, 293–301.

Brix, R., López-Doval, J., Ricart, M., Guasch, H., Lopez de Alda, M., Muñoz, I., Orendt, C., Romaní, A.M., Sabater, S., Barceló, D., 2012. Establishing potential links between the presence of alkylphenolic compounds and the benthic community in a European river basin. Environ. Sci. Pollut. Res. 19,934–945.

Canepari, S., Astolfi, M.L., Farao, C., Maretto, M., Frasca, D., Marcoccia, M., Perrino, C., 2014. Seasonal variations in the chemical composition of particulate matter: a case study in the Po Valley. Part II: concentration and solubility of micro- and trace-elements. Environ. Sci. Pollut. R. 21, 4010-4022.

Carnevale, C., Pisoni, E., Volta, M., 2010.A non-linear analysis to detect the origin of PM_{10} concentrations in Northern Italy. Sci. Total. Environ. 409,182-191.

Chen, Z.-S., Hsieh, C.-F., Jiang, F.-Y., Hsieh, T.-H., Sun, I.-F., 1997. Relations of soil properties to topography and vegetation in a subtropical rain forest in southern Taiwan. Plant. Ecol. 132, 229–241.

555 Corbiere Morot-Bizot, S., Talon, R., Leroy, S., 2004. Development of a multiplex PCR for the
556 identification of *Staphylococcus* genus and four staphylococcal species isolated from food. J.
557 Appl. Microbiol. 97,1087-1094.
558 Dang, H., Li, T., Chen, M., Huang, G., 2008. Cross-Ocean Distribution of *Rhodobacterales*
559 Bacteria as Primary Surface Colonizers in Temperate Coastal Marine Waters. Appl. Environ.
560 Microbiol. 74,52-60.
561 DeLeon-Rodriguez, N., Lathem, T.L., Rodriguez-R, L.M., Barazesh, J.M., Anderson, B.E.,
562 Beyersdorf, A.J., Ziemba, L.D., Bergin, M., Nenes, A., Konstantinidis, K.T., 2013.
563 Microbiome of the Upper Troposphere: Species Composition and Prevalence, Effects of
564 Tropical Storms, and Atmospheric Implications. P. Natl. acad. Sci. USA. 110,2575–2580.
565 Draxler, R.R. and Rolph, G.D., 2015. HYSPLIT (HYbrid Single-Particle Lagrangian Integrated
566 Trajectory). Model access via NOAA ARL READY Website
567 (http://ready.arl.noaa.gov/HYSPLIT.php). NOAA Air Resources Laboratory, Silver Spring,
568 MD.
569 Edgar, R.C., 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat.
570 Methods. 10, 996-998
571 Fahlgren, C., Hagström, A., Nilsson, D., Zweifel, U.L., 2010. Annual variations in the diversity,
572 viability, and origin of airborne bacteria. Appl. Environ. Microbiol. 76, 3015-25.
573 Feinstein, L.M., Sul, W.J., Blackwood, C.B., 2009. Assessment of Bias Associated with Incomplete
574 Extraction of Microbial DNA from Soil. Appl. Environ. Microb. 75, 5428-5433.
575 Gandolfi, I., Bertolini, V., Ambrosini, R., Bestetti, G., Franzetti, A., 2013. Unravelling the bacterial
576 diversity in the atmosphere. Appl. Microbiol. Biotechnol. 97, 4727–4736.
577 Gandolfi, I., Bertolini, V., Bestetti, G., Ambrosini, R., Innocente, E., Rampazzo, G., Papacchini,
578 M., Franzetti, A., 2015 Spatio-temporal variability of airborne bacterial communities and their
579 correlation with particulate matter chemical composition across two urban areas. Appl.
580 Microbiol. Biot. 99,4867–4877.

581 Heinrich, J., Pitz, M., Bischof, W., Krug, N., Borm, P.J.A., 2003. Endotoxin in fine ($PM_{2.5}$) and
582 coarse (PM2.5–10) particle mass of ambient aerosols. A temporo-spatial analysis. Atmos.
583 Environ. 37, 3659–3667
584 Heinrich, J., Thiering, E., Rzehak, P., Krämer, U., Hochadel, M., Rauchfuss, K.M., Gehring, U.,
585 Wichmann, H., 2013. Long-term exposure to NO_2 and PM_{10} and all-cause and cause-specific
586 mortality in a prospective cohort of women. Occup. Environ. Med. 70, 179-186.
587 Hervàs, A., Camarero L.,Reche I., Casamayor E.O., 2009. Viability and Potential for Immigration
588 of Airborne Bacteria from Africa That Reach High Mountain Lakes in Europe. Environ.
589 Microbiol. 11,1612–1623.
590 Hwang, C.Y., Cho, B.C., 2008. *Ponticoccuslitoralis* gen. nov., sp. nov., a marine bacterium in the
591 family Rhodobacteraceae. Int. J. Syst. Evol. Micr. 58, 1332-1338.
592 Jarvis, B.D.W., Van Berkum, P., Chen, W.X., Nour, S.M., Fernandez, M.P., Cleyet-Marel, J.C.,
593 Gillis, M., 1997. Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium*
594 *mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int. J. Syst.
595 Bacteriol. 47, 895–898.
596 Jeon, E.M., Kim, H.J., Jung, K., Kim, J.H., Kim, M.Y., Kim, Y.P., Ka, J.-O., 2011. Impact of Asian
597 dust events on airborne bacterial community assessed by molecular analyses. Atmos. Environ.
598 45, 4313-4321.
599 Kaspar, H.F., 1982.Nitrite reduction to nitrous oxide by Propionibacteria: Detoxication mechanism.
600 Arch. Microbiol. 133:126-130.
601 Kim, K. Y., Kim, C. N., 2007. Airborne microbiological characteristics in public buildings of
602 Korea. Build. Environ. 42, 2188-2196.
603 Krizova, L., Maixnerova, M., Sedo, O., Nemec, A., 2014. *Acinetobacter bohemicus sp.*nov.
604 widespread in natural soil and water ecosystems in the Czech Republic. Syst. Appl. Microbiol.
605 37,467-473.

606 Kukkonen,J., Pohjola, M., Sokhi, R.S., Luhana, L.,Kitwiroon, N.,Fragkou, L.,Rantamaki M., Berge,
607 E., Ødegaard,V., HavardSlørdal, L., Denby,B., Finardi, S.,2005. Analysis and evaluation of
608 selected local-scale PM_{10} airpollution episodes in four European cities: Helsinki,
609 London,Milan and Oslo. Atmos. Environ. 39, 2759-2773
610 Kwak, M.J., Yeon Song, J., Kwon Kim, B., Chi, W.J., Kwon, S.K., Choi, S., Chang, Y.K., Hong,
611 S.K., Kim, J.F., 2012. Genome sequence of the agar-degrading marine
612 bacterium*Alteromonadaceae sp*. strain G7. J. Bacteriol. 194, 6961-6962.
613 Larsen, B.R., Gilardoni, S., Stenström,K., Larseniedzialek, J., Jimenez, J., Belis, C.A., 2012.
614 Sources forPM air pollution in the Po Plain, Italy: II. Probabilistic uncertainty characterization
615 andsensitivity analysis of secondary and primary sources. Atmos. Environ. 50, 203–213.
616 Legendre, P. and Legendre, L., 1998. Numerical ecology. 2nd. Elsevier Science, Amsterdam, NL.
617 Li, D., Rothballer, M., Schmid, M., Esperschutz, J., Hartmann, A., 2011. *Acidovorax radicis sp.*
618 nov., a wheat-root-colonizing bacterium. Int. J. Syst. Evol. Micr. 61,2589–2594.
619 Lindström, E.S., 2000. Bacterioplankton community composition in five lakes differing in trophic
620 status and humic content. Microbial. Ecol. 40,104-113.
621 Lonati, G., Ozgen, S. and Giugliano, M., 2007. Primary and secondary carbonaceous species in
622 PM2. 5 samples in Milan (Italy). Atmos. Environ. 41, 4599-4610.
623 López-Pérez, M., & Rodriguez-Valera, F., 2014. The family Alteromonadaceae. In The Prokaryotes
624 (pp. 69-92). Springer Berlin Heidelberg.
625 Lymperopoulou, D.S., Adams, R.I., Lindow, S.E., 2016. Contribution of Vegetation to the
626 Microbial Composition of Nearby Outdoor Air. Appl. Environ. Microbiol. 82, 3822-3833.
627 Maki, T., Kakikawa, M., Kobayashi, F., Yamada, M., Matsuki, A., Hasegawa, H., Iwasaka, Y.,
628 2013. Assessment of composition and origin of airborne bacteria in the free troposphere over
629 Japan. Atmos. Environ. 74, 73–82.

630 Makra, L., Matyasovszky, I., Guba, Z., Karatzas, K., Anttila, P., 2011. Monitoring the long-range
631 transport effects on urban PM_{10} levels using 3D clusters of backward trajectories. Atmos.
632 Environ. 45, 2630–2641.
633 Malandrino, M., Di Martino, M., Giacomino, A., Geobaldo, F., Berto, S., Grosa, M.M., Abollino,
634 O., 2013. Temporal trends of elements in Turin (Italy) atmospheric particulate matter from
635 1976 to 2001. Chemosphere 90,2578–2588.
636 Marcazzan, G.M., Vaccaro, S., Valli, G., Vecchi, R., 2001. Characterisation of PM_{10} and $PM_{2.5}$
637 particulate matter in the ambient air of Milan (Italy). Atmos. Environ. 35,4639-4650.
638 Masiol, M., Rampazzo, G., Ceccato, D., Squizzato, S., Pavoni, B., 2010. Characterization of PM10
639 sources in a coastal area near Venice (Italy): an application of factor-cluster analysis.
640 Chemosphere 80, 771-778.
641 Masiol, M., Squizzato, S., Ceccato, D., Rampazzo, G., Pavoni, B,. 2012a. A chemometric approach
642 to determine local and regional sources of PM10 and its geochemical composition in a coastal
643 area. Atmos. Environ. 54,127-133.
644 Masiol, M., Squizzato, S., Ceccato, D., Rampazzo, G., Pavoni, B., 2012b. Determining the
645 influence of different atmospheric circulation patterns on PM10 chemical composition in a
646 source apportionment study. Atmos. Environ. 63, 117-124.
647 Masiol, M., Squizzato, S., Rampazzo, G., Pavoni, B., 2014. Source apportionment of PM2.5 at
648 multiple sites in Venice (Italy): Spatial variability and the role of weather. Atmos. Environ.
649 98,78-88
650 Mazar, Yinon, Eddie Cytryn, YigalErel, and YinonRudich. 2016. Effect of Dust Storms on the
651 Atmospheric Microbiome in the Eastern Mediterranean.Environ. Sci. Technol. 50, 4194–4202.
652 Meola, M, Lazzaro, A, Zeyer, J., 2015. Bacterial Composition and Survival on Sahara Dust
653 Particles Transported to the European Alps. Front.Microbiol. 6, 1454.
654 Mueller-Anneling, L., Avol, E., Peters, J.M., Thorne, P.S., 2004. Ambient endotoxin concentrations
655 in PM_{10} from Southern California. Environ. Health. Perspect. 112,583-588.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs. E., Wagner, H., 2016. vegan: Community Ecology Package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan.

Oren, A., 2006. The genera *Rhodothermus*, *Thermonema*, *Hymenobacter* and *Salinibacter*. In Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, editors. The Prokaryotes Volume 7: Proteobacteria: Delta, Epsilon Subclass. Springer. pp 712-738.

Pakkanen, T.A, 1996. Study of formation of coarse particle nitrate aerosol. Atmos. Environ. 30, 2475-2482.

Peccia, J., Hernandez, M., 2006. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. Atmos. Environ. 40,3941-3961.

Peccia, J., Milton, D.K., Reponen, T., Hill, J., 2008. A Role for Environmental Engineering and Science in Preventing Bioaerosol-Related Disease. Environ. Sci. Technol. 42,4631-4637.

Pecorari, E., Squizzato, S., Masiol, M., Radice, P., Pavoni, B., Rampazzo, G., 2013. Using a photochemical model to assess the horizontal, vertical and time distribution of $PM_{2.5}$ in a complex area: Relationships between the regional and local sources and the meteorological conditions. Sci. Total. Environ. 443,681-691.

Perrone, M.G., Gualtieri, M., Ferrero, L., Porto, C.L., Udisti, R., Bolzacchini, E. and Camatini, M., 2010. Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. Chemosphere 78, 1368-1377.

Polymenakou, P.N., Mandalakis, M., Stephanou, E.G., Tselepides, A., 2008. Particle size distribution of airborne microorganisms and pathogens during an Intense African dust event in the eastern Mediterranean. Environ. Health Perspect. 116, 292–6.

Pope, C., Dockery, D., 2006. Health effects of fine particulate air pollution: Lines that connect. J. Air Waste Manage. Assoc. 56, 709–742.

682 Querol, X., Alastuey, A., Moreno, T., Viana, M.M., Castillo, S., Pey, J., Rodrıguez, S., Artinano,
683 B., Salvador, P., Sanchez, M., Dos Santos, S.G., Garraleta, M.D.H., Fernandez-Patier, R.,
684 Moreno-Grau, S., Negral, L., Minguillon, M.C., Monfort, E., Sanz, M.J., Palomo-Marin, R.,
685 Pinilla-Gil, E., Cuevas, E., De la Rosa, J., Sanchez de la Campa, S., 2006. Spatial and temporal
686 variations in airborne particulate matter (PM_{10} and $PM_{2.5}$) across Spain 1999–2005. Atmos.
687 Environ. 42,3964-3979.
688 R Core Team (2015). R: A language and environment for statistical computing. R Foundation for
689 Statistical Computing, Vienna, Austria.
690 Rampazzo, G., Masiol, M., Visin, F., Pavoni, B., 2008. Gaseous and PM_{10}-bound pollutants
691 monitored in three environmental conditions in the Venice area (Italy). Water. Air. Soil. Poll.
692 19,161-176.
693 Seifried, J.S., Wichels, A., Gerdts, G., 2015. Spatial Distribution of Marine Airborne Bacterial
694 Communities. MicrobiologyOpen 4,475–90.
695 Shinn, E.A., Smith, G.W., Prospero, J.M., Betzer, P., Hayes, M.L., Garrison, V., Barber, R.T.,
696 2000. African dust and the demise of Caribbean coral reefs. Geophys. Res. Lett. 27,3029-3032.
697 Schonfeld, J., Gelsomino, A., Van Overbeek, L.S., Gorissen, A., Smalla, K., Van Elsas, J.D., 2003.
698 Effects of compost addition and simulated solarisation on the fate of *Ralstonia*
699 *solanacearum*biovar 2 and indigenous bacteria in soil. Fems Microbiol. Ecol. 43,63-74.
700 Sikkink, P.G., Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Monitoring for change: Using generalised
701 least squares, non-metric multidimensional scaling, and the Mantel test on western Montana
702 grasslands. In Zuur AK, Ieno EN, Smith GM, editors. Analysing Ecological Data. Springerpp.
703 463-484.
704 Smets, W., Moretti,S., Denys, S, Lebeer, S., 2016. Airborne bacteria in the atmosphere: Presence,
705 purpose, and potential. Atmos. Environ. 139, 214-221

706 Smith, D.J., Jaffe, D. a, Birmele, M.N., Griffin, D.W., Schuerger, A.C., Hee, J., Roberts, M.S.,
707 2012. Free tropospheric transport of microorganisms from Asia to North America. Microb.
708 Ecol. 64, 973–85.
709 Squizzato, S., Masiol, M., Innocente, E., Pecorari, E., Rampazzo, G., Pavoni, B., 2012. A procedure
710 to assess local and long-range transport contributions to PM 2.5 and secondary inorganic
711 aerosol. J. Aero. Science46, 64-76.
712 Squizzato, S., Masiol, M., Brunelli, A., Pistollato, S., Tarabotti, E., Rampazzo, G., Pavoni, B.,
713 2013. Factors determining the formation of secondary inorganic aerosol: a case study in the Po
714 Valley (Italy). Atmos. Chem. Phys. 13,1927-1939.
715 Squizzato, S., Masiol, M., Visin, F., Canal, A., Rampazzo, G., Pavoni, B., 2014. The $PM_{2.5}$
716 chemical composition in an industrial zone included in a large urban settlement: main sources
717 and local background. Environ. Sci.: Processes Impacts.16,1913-1922.
718 Squizzato, S., Masiol, M., 2015. Application of meteorology-based methods to determine local and
719 external contributions to particulate matter pollution: A case study in Venice (Italy).Atmos.
720 Environ. 119; 69–81.
721 Stohl, A., 1998. Computation, accuracy and applications of trajectories - a review and bibliography.
722 Atmos. Environ. 32,947–966.
723 Takeuchi, M., Hamana, K., Hiraishi, A., 2001. Proposal of the genus *Sphingomonas*sensustricto and
724 three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of
725 phylogenetic and chemotaxonomic analyses. Int. J. Syst. Evol. Micr. 51,1405-1417.
726 Terzi, E., Argyropoulos, G., Bougatioti, A., Mihalopoulos, N., Nikolaou, K., Samara, C., 2010.
727 Chemical composition and mass closure of ambient PM_{10} at urban sites. Atmos. Environ.
728 44,2231–2239.
729 Trujillo, M.E., Willems, A., Abril, A., Planchuelo, A.M., Rivas, R., Ludeña, D., Mateos, P.F.,
730 Martínez-Molina, E., Velázquez, E., 2005. Nodulation of *Lupinusalbus* by Strains of
731 *Ochrobactrum lupini* sp. nov. Appl. Environ. Microbiol. 71,1318-1327.

732 Ushiba, Y., Takahara, Y., Ohta, H., 2003. *Sphingobium amiense* sp. nov., a novel nonylphenol-
733 degrading bacterium isolated from a river sediment. Int. J. Syst. Evol. Micr. 53,2045-2048.
734 Vidal-Liñán, L., Bellas, J., Etxebarria, N., Nieto, O., Beiras, R., 2014. Glutathione S-transferase,
735 glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour
736 areas. Sci. Total. Environ. 471,107-116.
737 Wakelin, S.A., Colloff, M.J., Harvey, P.R., Marschner, P., Gregg, A.L., Rogers, L.S., 2006. The
738 effects of stubble retention and nitrogen application on soil microbial community structure and
739 functional gene abundance under irrigated maize. FemsMicrobiol. Ecol. 59,661-670.
740 Wang, F., Grundmann, S., Schmid, M., Dörfler, U., Roherer, S., Munch, J.C., Hartmann, A., Jiang,
741 X., Schroll, R., 2007. Isolation and characterization of 1,2,4-trichlorobenzene mineralizing
742 *Bordetella sp*. and its bioremediation potential in soil. Chemosphere 67,896-902.
743 Wang. Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for
744 rapidassignment of rRNA sequences into the new bacterial taxonomy. Appl EnvironMicrobiol.
745 73,5261-5267
746 Wang, T., Cai, G., Qiu, Y., Fei, N., Zhang, M., Pang, X., Jia, W., Cai, S., Zhao, L., 2012. Structural
747 segregation of gut microbiota between colorectal cancer patients and healthy volunteers. Isme
748 J. 6,320-329.
749 White, D.C., Sutton, S.D., Ringelberg, D.B., 1996. The genus *Sphingomonas*: physiology and
750 ecology. Curr. Opin. Biotech. 7, 301-306.
751 Xie, C.H. and Yokota, A., 2005. *Dyella japonica* gen. nov., sp. nov., a c-proteobacterium isolated
752 from soil. Int. J. Syst. Evol. Microbiol. 55,753-756.
753 Xu, H., Davies, J., Miao, V., 2007. Molecular Characterization of Class 3 Integrons from *Delftia*
754 *spp*. J. Bacteriol. 198,6276-6283.
755 Yergeau, E., Schoondermark-Stolk, S.A., Brodie, E.L., Dejean, S., DeSantis, T.Z., Goncalves, O.,
756 Piceno, Y.M., Andersen, G.L., Kowalchuk, G.A., 2009. Environmental microarray analyses of
757 Antarctic soil microbial communities. Isme J. 3,340-351.

Zweifel, U.L., Hagström, Å., Holmfeldt, K., Thyrhaug, R., Geels, C., Frohn, L.M., Skjøth, C. a., Karlson, U.G., 2012. High bacterial 16S rRNA gene diversity above the atmospheric boundary layer. Aerobiologia 28, 481-498.

Table captions

Table 1. Seasonal and all period (all) mean (μgm^{-3}) and LOD (μgm^{-3}) of WSIIs and PM_{10} (μgm^{-3}) determined in sampling sites.

Table 2. Average concentration of WSIIs for each group identified by cluster analysis on wind ground data compared to the full period average.Concentrations are expressed in $\mu g\ m^{-3}$.

Table 3. Average concentration of WSIIs for each group identified by cluster analysis on back-trajectories compared to the full period average.Concentrations are expressed in $\mu g\ m^{-3}$.

Table 4. Results from RDA and partial RDAs. [S], [O] and [P]: fractions of total variation in community structure explained by season S, origin O, and local provenience P of air masses. First lines report the results of RDA run by entering each predictor separately. Following lines report marginal effects for each predictor included in the model. [S|O+P], [O|S+P] and [P|S+O]: variation fractions identifying pure effects of, respectively, season, origin and local provenience of air masses calculated by partial RDAs. Significance of RDAs and partial RDAs was assessed by a randomization procedure (Legendre andLegendre 1998).

Figure captions

Figure 1. Sampling sites and areas of interest.

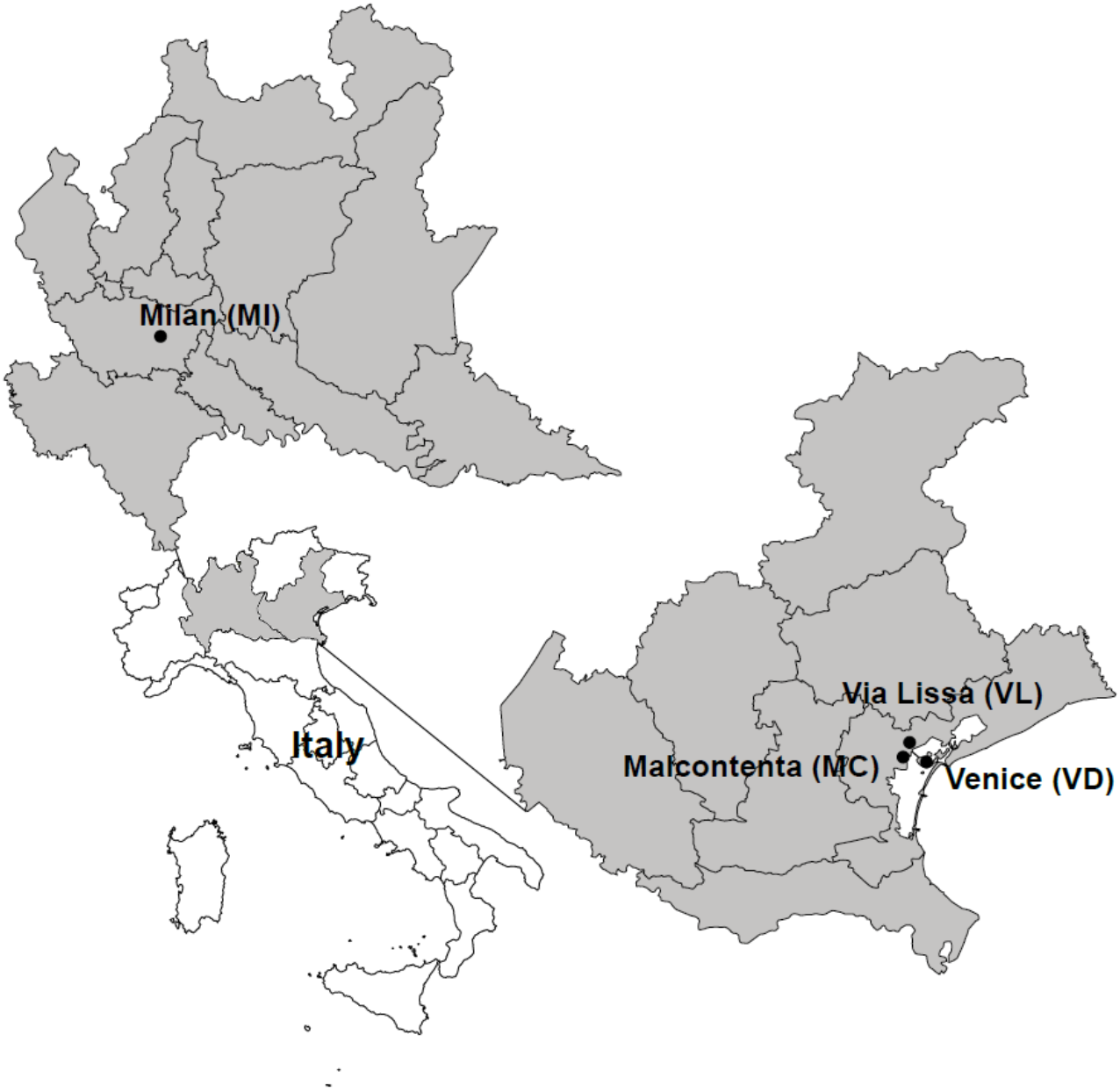
Figure 2. Example of RDA analysis between bacteria and WSIIs for VL samples.

Figure 3. Wind rose computed for each group identified by qHCA at Venice and Milan sites.

Figure 4. Bacterial community structures for each cluster of wind data

Figure 5. Clusters obtained by back-trajectories cluster analysis.

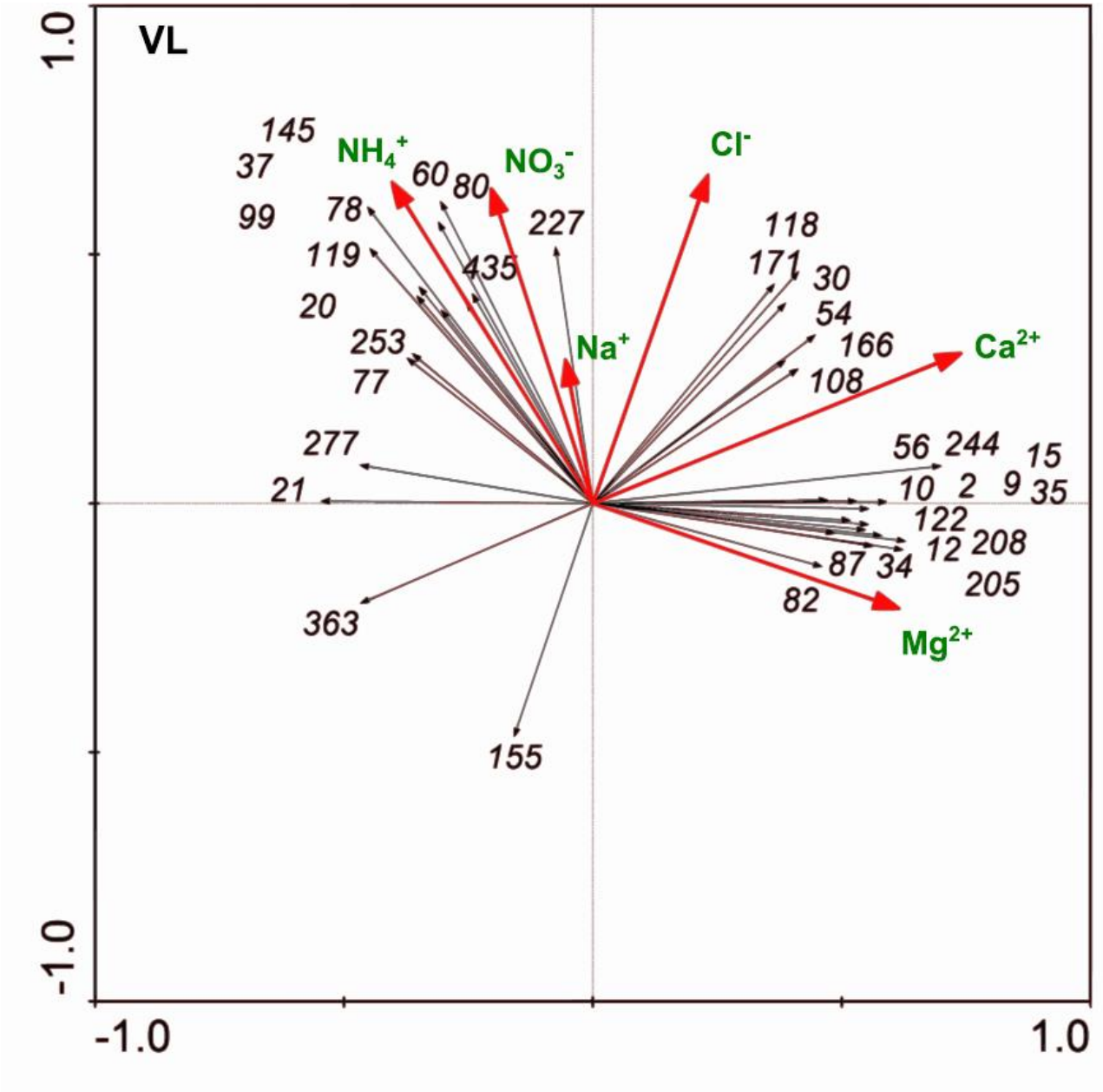
Figure 6. Bacterial community structures for each back-trajectories cluster.



791

792 Fig.1

793

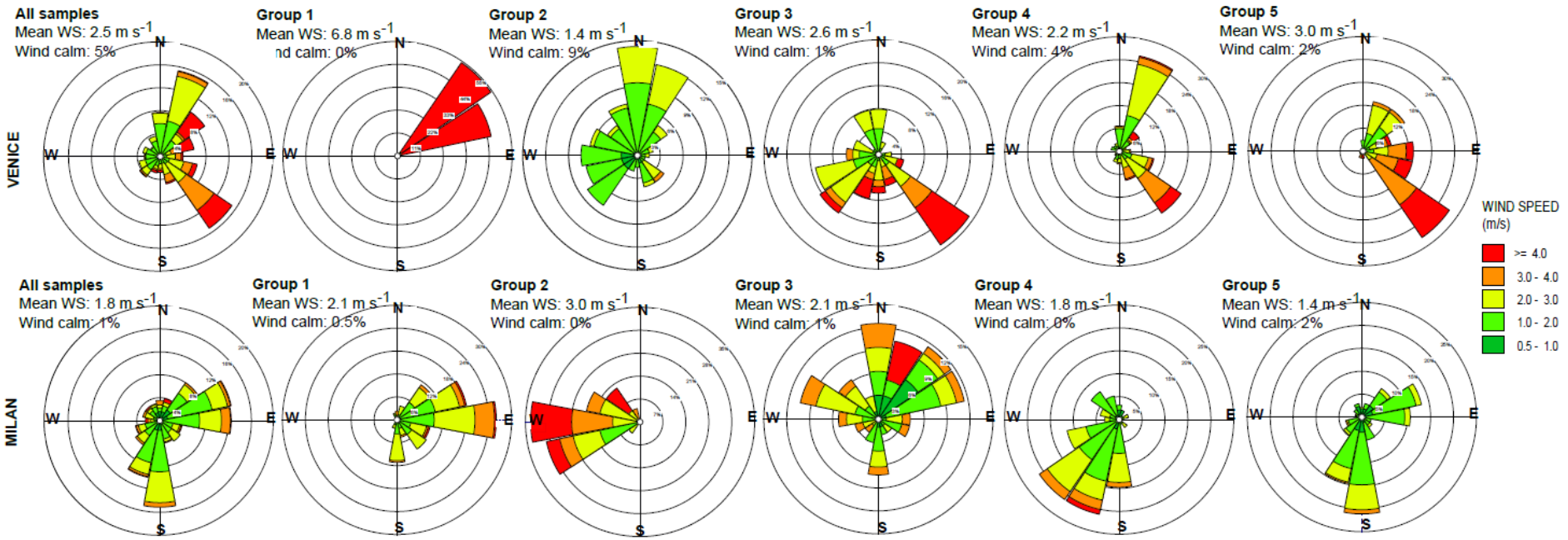


794

795 Fig.2

796

797


All samples
Mean WS: 2.5 m s-1
Wind calm: 5%
Group 1
Mean WS: 6.8 m s-1
nd calm: 0%
Group 2
Mean WS: 1.4 m s-1
Wind calm: 9%
Group 3
Mean WS: 2.6 m s-1
Wind calm: 1%
Group 4
Mean WS: 2.2 m s-1
Wind calm: 4%
Group 5
Mean WS: 3.0 m s-1
Wind calm: 2%
VENICE
All samples
Mean WS: 1.8 m s-1
Wind calm: 1%
Group 1
Mean WS: 2.1 m s-1
Wind calm: 0.5%
Group 2
Mean WS: 3.0 m s-1
Wind calm: 0%
Group 3
Mean WS: 2.1 m s-1
Wind calm: 1%
Group 4
Mean WS: 1.8 m s-1
Wind calm: 0%
Group 5
Mean WS: 1.4 m s-1
Wind calm: 2%
MILAN
N
E
S
W
WIND SPEED
(m/s)
>= 4.0
3.0 - 4.0
2.0 - 3.0
1.0 - 2.0
0.5 - 1.0

798

799 Fig.3

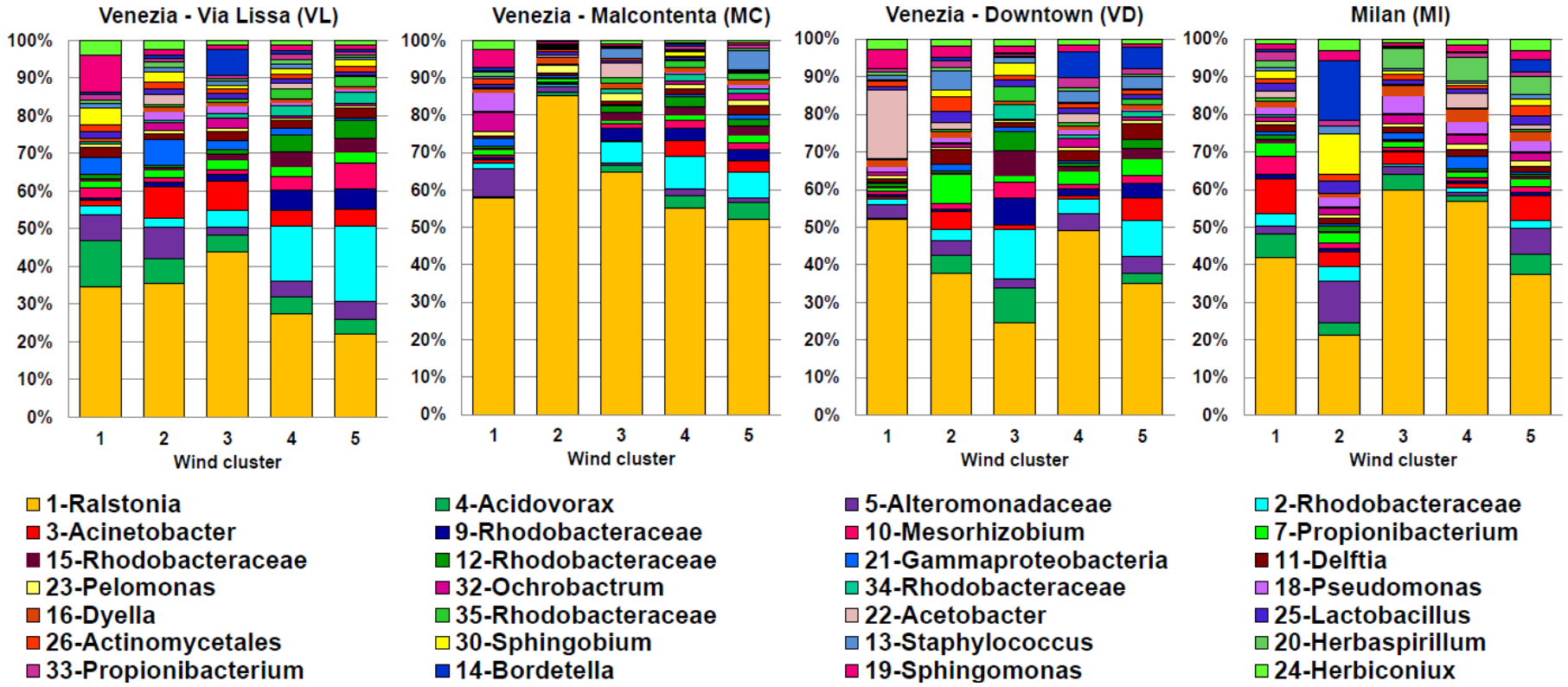
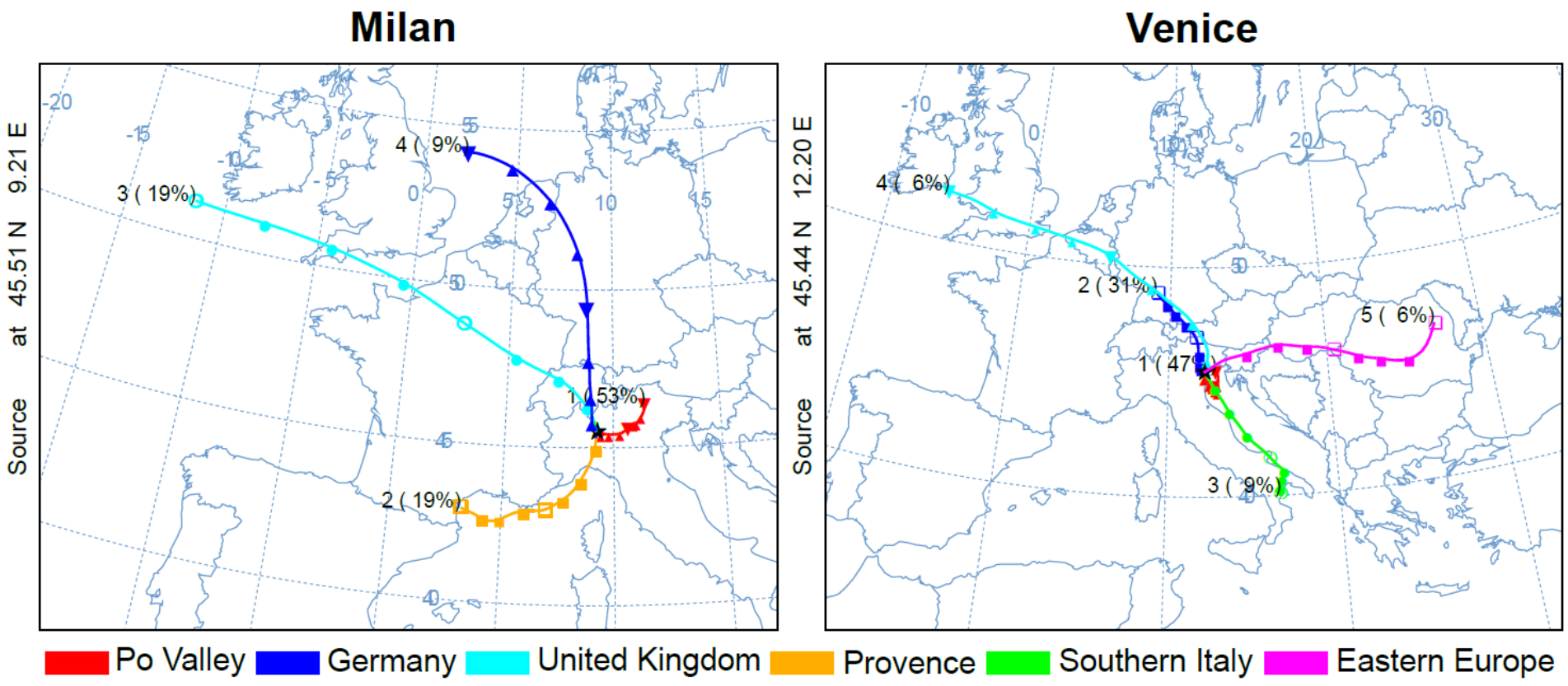

Venezia - Via Lissa (VL)
Venezia - Malcontenta (MC)
Venezia - Downtown (VD)
Milan (MI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
4
5
Wind cluster
1-Ralstonia
3-Acinetobacter
15-Rhodobacteraceae
23-Pelomonas
16-Dyella
26-Actinomycetales
33-Propionibacterium
4-Acidovorax
9-Rhodobacteraceae
12-Rhodobacteraceae
32-Ochrobactrum
35-Rhodobacteraceae
30-Sphingobium
14-Bordetella
5-Alteromonadaceae
10-Mesorhizobium
21-Gammaproteobacteria
34-Rhodobacteraceae
22-Acetobacter
13-Staphylococcus
19-Sphingomonas
2-Rhodobacteraceae
7-Propionibacterium
11-Delftia
18-Pseudomonas
25-Lactobacillus
20-Herbaspirillum
24-Herbiconiux

Fig4



803

804 Fig.5

805

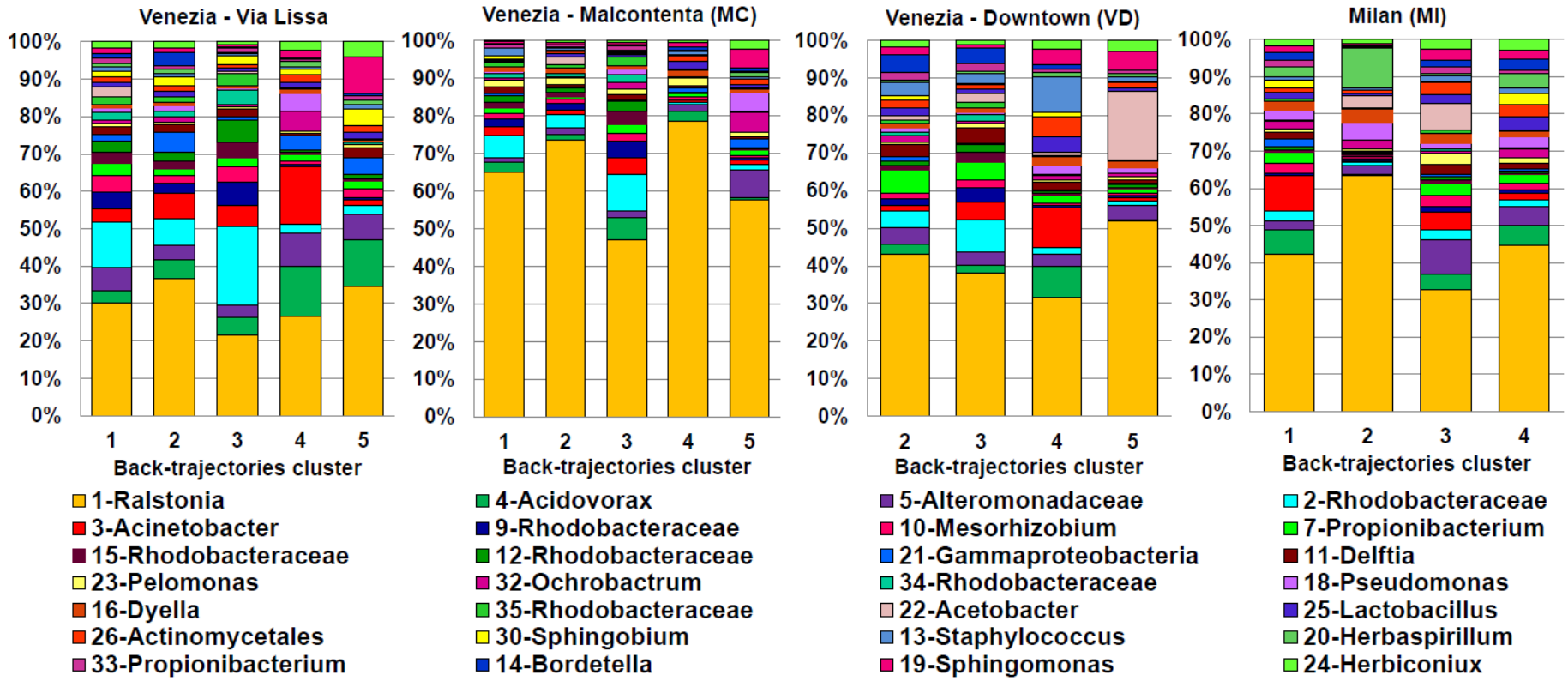
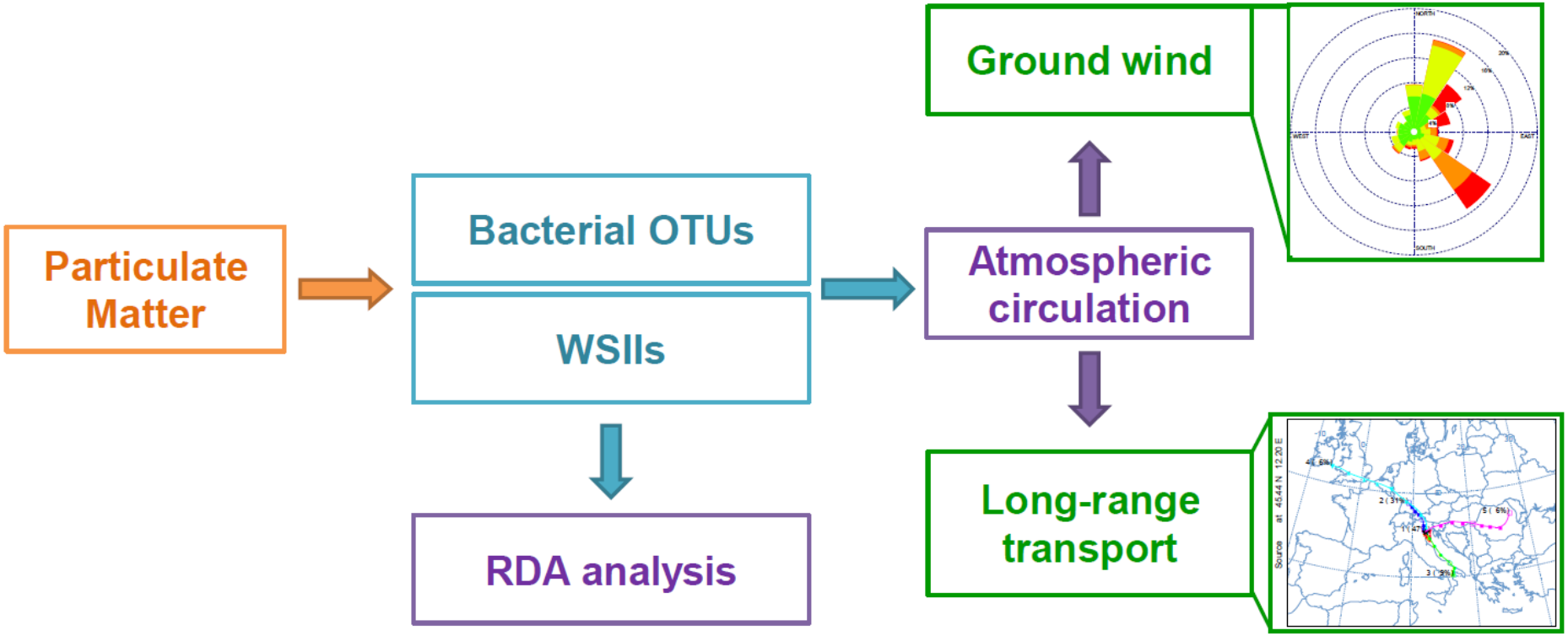

Venezia - Via Lissa
Venezia - Malcontenta (MC)
Venezia - Downtown (VD)
Milan (MI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1 2 3 4 5
2 3 4 5
1 2 3 4
Back-trajectories cluster
1-Ralstonia
3-Acinetobacter
15-Rhodobacteraceae
23-Pelomonas
16-Dyella
26-Actinomycetales
33-Propionibacterium
4-Acidovorax
9-Rhodobacteraceae
12-Rhodobacteraceae
32-Ochrobactrum
35-Rhodobacteraceae
30-Sphingobium
14-Bordetella
5-Alteromonadaceae
10-Mesorhizobium
21-Gammaproteobacteria
34-Rhodobacteraceae
22-Acetobacter
13-Staphylococcus
19-Sphingomonas
2-Rhodobacteraceae
7-Propionibacterium
11-Delftia
18-Pseudomonas
25-Lactobacillus
20-Herbaspirillum
24-Herbiconiux

Fig.6



808

809 Graphical abstract