

Diversity and hydrocarbon-degrading potential of epiphytic microbial communities on *Platanus x acerifolia* leaves in an urban area

Peer-reviewed author version

Gandolfi, Isabella; Canedoli, Claudia; IMPERATO, Valeria; Tagliaferri, Ilario; GKOREZIS, Panos; VANGRONSVELD, Jaco; Schioppa, Emilio Padoa; Papacchini, Maddalena; Bestetti, Giuseppina & Franzetti, Andrea (2017) Diversity and hydrocarbon-degrading potential of epiphytic microbial communities on *Platanus x acerifolia* leaves in an urban area. In: ENVIRONMENTAL POLLUTION, 220, p. 650-658.

DOI: 10.1016/j.envpol.2016.10.022

Handle: <http://hdl.handle.net/1942/23254>

**Diversity and hydrocarbon-degrading potential of epiphytic microbial communities on
Platanus x acerifolia leaves in an urban area**

Authors: Isabella Gandolfi^a, Claudia Canedoli^a, Valeria Imperato^a, Ilario Tagliaferri^a,
Panagiotis Gkorezis^b, Jaco Vangronsveld^b, Emilio Padoa Schioppa^a, Maddalena Papacchini^c,
Giuseppina Bestetti^a, Andrea Franzetti^a

^aDept. of Earth and Environmental Sciences, University of Milano-Bicocca, Milan, Italy

^bCentre for Environmental Sciences – Hasselt University, Hasselt, Belgium

^cINAIL, Dipartimento Innovazioni Tecnologiche e Sicurezza degli Impianti, Prodotti ed
Insediamenti Antropici, Rome, Italy

Corresponding author: Isabella Gandolfi, Dept. of Earth and Environmental Sciences,
University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan (Italy) –
isabella.gandolfi@unimib.it

Abstract

Plants and their associated bacteria have been suggested to play a role in air pollution mitigation, especially in urban areas. Particularly, epiphytic bacteria might be able to degrade atmospheric hydrocarbons. However, phyllospheric bacterial communities are highly variable depending on several factors, e.g. tree species, leaf age and physiology, environmental conditions. In this work, bacterial communities hosted by urban *Platanus x acerifolia* leaves were taxonomically characterized using high throughput sequencing of 16S rRNA gene, and their temporal and spatial variability was assessed by comparing samples collected from different locations in the city of Milan (Italy) and in different months. The diversity of alkane hydroxylase (*alkB*) phylotypes harboured by phyllospheric bacteria associated to urban *Platanus* trees was also evaluated. Results revealed that temporal changes, which are related to seasonality, acted as a stronger driver both on *Platanus* phyllospheric community structure and on *alkB* phylotype diversity than sampling location. Biodiversity of bacterial communities decreased along the growing season, leading to a strong dominance by the genus *Stenotrophomonas*. On the contrary, diversity of hydrocarbon-degrading populations increased over the months, although it resulted lower than that reported for other habitats. It was therefore hypothesized that atmospheric hydrocarbons might play a key role in the selection of phyllospheric populations in urban areas.

Capsule: Increasing diversity of hydrocarbon-degrading bacterial populations hosted by urban *Platanus* leaves suggests that atmospheric hydrocarbons might select phyllospheric populations.

Keywords: phyllosphere, phylloremediation, hydrocarbons, alkane hydroxylase, air purification

Introduction

Air pollution is a matter of global concern, especially in urban areas, due to the harmful effects of atmospheric pollutants on human health and on the environment. Current emission reduction methods and mitigation strategies are not adequate to fully meet the World Health Organization (WHO) guidelines for air pollutants (Ali et al., 2012; Weyens et al., 2015). Since most of the environmental problems in urban areas are generated at local level, often one of the most effective ways to deal with them is through local solutions (Bolund and Hunhammer, 1999). Plants have been suggested to effectively contribute to reduce air pollution levels and offsetting greenhouse gas emissions in cities (Beckett et al., 1998; Dzierzanowski et al., 2011; McPherson et al., 1998; Nowak and Crane, 2002; Nowak et al., 2006; Paoletti, 2009; Redford et al., 2010; Yang et al., 2005; Zhao et al., 2010). In this context, the regulation of ecosystem services (the direct and indirect contributions of ecosystems to human well-being (TEEB, 2011)) provided by vegetation in urban areas is of great importance (Baró et al., 2014). Many studies, in fact, indicated that the management of urban forests to enhance ecosystem service supply can be a cost-effective strategy to meet specific environmental standards or policy targets (Escobedo et al., 2010, 2011). Furthermore, it has been recognized that also plant-associated bacteria can play a crucial role in air bioremediation processes (Glick, 2015; Weyens et al., 2015). Particularly, the aerial parts of terrestrial plants, mainly leaves (i.e. the phyllosphere) host huge amounts of bacteria. In fact, although phyllospheric microorganisms comprise also fungi, yeasts, algae, protozoa and nematodes, bacteria are by far the most abundant inhabitants of leaf surfaces (Lindow and Brandl, 2003). Since phyllospheric bacteria are often found at an average of 10^6 - 10^7 cells cm^{-2} of leaf surface (Lindow and Brandl, 2003), the planetary phyllospheric bacterial population has been estimated to be as large as 10^{26} cells (Morris and Kinkel, 2015). Among them, epiphytic bacteria, which primarily live on leaf surfaces, are directly positioned to the

66 interface with the atmosphere. Thus, they are exposed to several detrimental factors such as
67 UV radiation, desiccation, severe temperature changes and, especially in urban areas,
68 atmospheric pollutants (Lindow and Brandl, 2003). For this reason, they are expected to have
69 developed metabolic abilities towards atmospheric hydrocarbons and therefore to play a
70 potential role in air bioremediation processes. Indeed, several papers have already reported
71 the ability of phyllospheric bacteria to degrade aliphatic (Al-Awadhi et al., 2012) and
72 aromatic hydrocarbons, namely phenolic compounds, toluene, xylene and phenanthrene (De
73 Kempeneer et al., 2004; Sandhu et al., 2007; Sangthong et al., 2016; Scheublin et al., 2014;
74 Waight et al., 2007; Yutthammo et al., 2010).

75 Despite their continuous exchange with airborne populations (Lighthart, 2006;
76 Lympelopoulou et al., 2016; Whipps et al., 2008), phyllospheric bacteria are not random
77 assemblages but they rather form actual communities. In fact, some bacterial taxa are
78 recurrently retrieved from leaf-associated habitats, leading to the hypothesis that, after
79 recruitment, they undergo some selection processes (Delmotte et al., 2009; Rastogi et al.,
80 2013; Vorholt, 2012; Yang et al., 2001). The relative abundance of a specific bacterial taxon
81 in phyllospheric communities, however, can vary considerably. The main drivers that were
82 suggested to shape community structure include host plant species, leaf age and physiology,
83 season, geographical location, and environmental factors, such as solar radiation, humidity
84 and nutrient availability (Laforest-Lapointe et al., 2016; Müller and Ruppel, 2014; Peñuelas
85 et al., 2012; Rastogi et al., 2012; Redford et al., 2010; Vokou et al., 2012). Interactions
86 between these factors can also affect bacterial communities. For example, Wagner and
87 colleagues (2016) suggested that the plant genotype-by-sampling site interaction was a
88 stronger driver than plant genotype only. Moreover, the occurrence of a contribution from
89 stochastic processes was also observed (Maignien et al., 2014). Therefore, due to the high
90 variability of phyllospheric community structure, a more profound knowledge about bacterial

communities hosted by different plant species in different environments is needed to assess their potential contribution to air bioremediation. Among plant species that can be typically found in urban areas, *Platanus* trees are widespread in most cities of central and southern Europe. They are frequently planted along high traffic roads, since they are known to be considerably resistant to stresses caused by urban pollution (Yang et al., 2015). To the best of our knowledge, bacterial communities associated to *Platanus* leaves were characterized only by Zhang et al. (2015), who however limited their research to the assessment of functional diversity of the culturable fraction.

The aims of this work were: (i) a deep phylogenetic characterization of bacterial communities hosted by urban *Platanus x acerifolia* leaves using high-throughput sequencing (HTS) methods; (ii) an evaluation of the diversity of alkane hydroxylase (*alkB*) phylotypes harboured by phyllospheric bacteria associated to urban *P. x acerifolia* trees; (iii) the assessment of temporal and spatial variability of bacterial phyllospheric communities associated to *P. x acerifolia* trees located in different areas of the city of Milan (Italy) and sampled in different months.

Materials and Methods

Sampling

Leaves were collected from eight different *Platanus x acerifolia* trees in the city of Milan (Italy). Four of them were located in an urban park (Parco Nord), next to a low-traffic secondary road, and the other four were planted along a high-traffic road (Viale Fulvio Testi), which is one of the major arterial roads in the northern part of the city (Fig. S1). Meteorological conditions and atmospheric pollutant concentrations for this area are reported in Fig. S2. Sampling was performed at the beginning (April 17, 2014) and in the middle of the growing season (July 11, 2014). For each tree, samples were collected in triplicates, for a

total of 48 samples. Each sample was composed by three young leaves in April and by two mature leaves in July, collected at a height ranging approximately between 1.50 and 2.00 m. Leaves were handled with metal scissors and tweezers rinsed with ethanol and immediately put in sterile 120 mm Petri dishes to prevent DNA contamination from external sources.

DNA extraction

Total DNA of epiphytic bacteria was extracted with FastDNA Spin for Soil kit (MP Biomedicals, Solon, OH, USA). Leaves were thoroughly rinsed in sterile Petri dishes with approximately 4 mL of Sodium Phosphate Buffer supplied with the kit under a laminar flow hood. After rinsing, it was possible to recover approximately 2 mL of the used buffer. It was collected from the Petri dish with a micropipette and placed in the kit Lysing Matrix E Tube. Further steps were performed according to manufacturer's instructions.

Illumina sequencing

The V5-V6 hypervariable regions of the bacterial 16S rRNA gene were PCR-amplified using 783F and 1046R primers (Huber et al., 2007; Wang and Qian, 2009). For the characterization of *alkB* diversity, three different primer pairs were preliminarily tested on our samples (pairs (d), (e) and (f) of Jurelevicius et al. (2013)); detectable amplification was obtained with primer pair (f) only, which was therefore chosen for subsequent analyses. At the 5' end of each primer, a 6-bp barcode was included to allow sample pooling and sequence sorting. All amplicons were sequenced by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) with a 250 bp \times 2 paired-end protocol. For each sample, 2 \times 75 μ L volume PCR reactions were performed with GoTaq® G2 Green Master Mix (Promega Corporation, Madison, WI, USA) and 1 μ M of each primer. The cycling conditions for the amplification of the 16S rRNA gene fragment were: initial denaturation at 94 °C for 4 min; 28 cycles at 94 °C for 50 s, 47 °C for

30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min. The cycling conditions for the amplification of the *alkB* fragment were: initial denaturation at 96 °C for 4 min; 40 cycles at 96 °C for 45 s, 47 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 5 min. The amplicons were purified with the Wizard® SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI, USA) and purified DNA was quantified using Qubit® (Life Technologies, Carlsbad, CA, USA). Groups of 9/12 amplicons bearing different barcode pairs were pooled together to build a single library. Further library preparation with the addition of standard Nextera indexes (Illumina, Inc., San Diego, CA, USA) and sequencing were carried out at Parco Tecnologico Padano (Lodi, Italy).

Sequence analysis

Reads from both 16S rRNA and *alkB* genes sequencing were demultiplexed according to the indexes. Uparse pipeline was used for the following elaborations (Edgar, 2013). In case of 16S rRNA genes, forward and reverse reads were merged with perfect overlapping and quality filtered with default parameters. Conversely, since *alkB* reads were not overlapping, only one read was analysed. Suspected chimeras and singleton sequences (i.e. sequences appearing only once in the whole data set) were removed. Phylotypes were defined on the whole data set clustering the sequences at a 97% of similarity and defining a representative sequence for each cluster. Representative sequences of 16S rRNA gene phylotypes (Operational Taxonomic Units – OTUs) were classified using SINA with SILVA database (Pruesse et al., 2012) and sequences not classified as belonging to Bacteria domain (i.e. Archaea, chloroplasts and mitochondria) were discarded. Abundance of each OTU was estimated by mapping the sequences of each sample against the remaining OTU representative sequences at 97% of similarity. Representative sequences of *alkB* gene phylotypes were translated into aminoacid sequences considering the proper frame and annotated with Blastp (Altschul et al., 1990).

Sequences not annotated as *alkB* were discarded; sequences of each sample were then mapped against the remaining representative phylotype sequences at 97% of similarity. To assess the spatial and temporal variability both of the structure of phyllospheric bacterial communities hosted by *Platanus* leaves and of *alkB* phylotypes, samples were grouped according to their sampling location (urban park or high-traffic road) and to sampling month (April or July). Non-metric Multidimensional Scaling (NMDS) analyses based on Hellinger distances were performed using R (Vegan package) (Oksanen et al., 2009). Differences in abundance of the most abundant genera ($\geq 2\%$) between months (April and July) or locations (park and road) were tested by t-tests. P-values were corrected for multiple testing according to the False Discovery Rate (FDR) procedure (Benjamini and Hochberg, 1995) using the MULTTEST package in R.

Results and Discussion

Phylogenetic diversity

From NMDS analysis, two main groups could be identified, corresponding to the April and July samples respectively (Fig. 1). Within each of the two sampling months, samples from the urban park and from the high-traffic road were close but clearly distinguishable. Therefore, it can be hypothesized that temporal changes, which are in turn related to seasonality, acted as a stronger driver on the *Platanus* phyllospheric community structure than sampling location. This is in agreement with the observations of several authors, e.g. Copeland et al. (2015), Rastogi et al. (2012) and Peñuelas et al. (2012), which identified seasonal changes as a major factor shaping bacterial phyllospheric communities associated to different plant species. Furthermore, environmental conditions and atmospheric pollutant concentrations are known to be substantially homogeneous in the Po Valley, where Milan is located; therefore, this area as a whole is generally considered as a pollutant hot-spot

(Marcazzan et al, 2002; Maurizi et al., 2013; Vecchi and Valli, 1999). For this reason, it can be hypothesized that environmental variables may have been not sufficiently different at the two sites, which are approximately 2 km from each other, to cause appreciable dissimilarities in bacterial community composition.

The relative abundance of the main bacterial phyllospheric populations at the taxonomic levels of Class and Genus is shown in Fig. 2 (a and b, respectively). Overall, the most abundant classes were *Gammaproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Bacilli* and *Actinobacteria*. They have already been described by several authors as common classes in phyllospheric bacterial communities associated with different plant species (Dees et al., 2015; Rastogi et al., 2012; Vorholt, 2012; Whipps et al., 2008), although in some cases they were reported with very different relative abundances (Redford et al., 2010). In April, the *Platanus* phyllospheric communities were not clearly dominated by any class or genus. On the contrary, July communities exhibited a large prevalence of *Gammaproteobacteria*, with a relative abundance of approximately 50%. Within this class, most sequences belonged to the genus *Stenotrophomonas* (approximately 42% of total bacteria). This genus has already been reported to be one of the major genera commonly detected in phyllospheric communities, although at much lower percentages (Vorholt, 2012). Particularly, *Stenotrophomonas* has been generally described as a member of endophytic, rather than epiphytic, bacterial communities of different plant species (Ferrando and Fernández Scavino, 2015; Kgomotso et al., 2015; Mastretta et al., 2009; Romero et al., 2014; Taghavi et al., 2009). Several isolates belonging to this genus were demonstrated to possess plant-growth promoting properties (Calciolari and Silva, 2013; Islam et al., 2015). The same abilities were observed for a rhizospheric *S. maltophilia* strain and confirmed through genome sequencing (Wu et al., 2015). Furthermore, some plant-associated *Stenotrophomonas* strains were reported as able to degrade oil hydrocarbons (Ali et al., 2012) and phenanthrene (Muratova et

al., 2015). In a culture-independent study on endophytic communities of *Cucurbita pepo*, members of genera *Stenotrophomonas* and *Sphingomonas* showed a significantly higher abundance in the presence of DDE, the most common and persistent degradation product of the pesticide DDT, than in the absence of the molecule (Eevers et al., 2016). Thus, it can be hypothesized that the genus *Stenotrophomonas* may play a key role also in the ecology of phyllospheric communities associated to urban *Platanus* leaves. Table S1 reports the results of multiple t-tests on abundant genera that significantly varied between months. The genus *Hymenobacter* was identified as significantly more abundant in July phyllospheric communities, with average relative abundances of 11.1% and 4.3% in park and road samples, respectively. Some members of this genus have been described as radiation tolerant (Kim et al., 2016; Lee et al., 2014; Su et al., 2014) and psychrophilic or psychrotolerant (Klassen and Foght, 2011; Mi et al., 2014). Due to these features, it can be hypothesized that these bacteria may undergo a selection process, throughout the growing season, by the harsh conditions of the phyllospheric environment. Given the continuous exchange of bacterial populations between leaf surface and air, and the shared characteristics of high UV radiation and low temperature of the two environments, it is not surprising that the genus *Hymenobacter* was also reported in outdoor airborne communities (Fahlgren et al., 2011; Yooseph et al., 2013). The other genus identified as significantly more abundant in July samples was *Massilia* (Table S1). Members of this genus have already been described as commonly retrieved in phyllospheric epiphytic communities (Rastogi et al., 2013, 2012), as well as endophytes (Croes et al., 2015; Thijs et al., 2014). Therefore, it may have been enriched over time due to the selective conditions of the phyllospheric environment, which could favour it over other genera.

The genus *Buttiauxella* was the only one to be recognized as significantly more abundant in park samples, with average abundances of 8.3% and 2.0% in April and July samples,

respectively (Table S2). It is not reported to be one of the most common genera among phyllospheric bacteria (Bulgarelli et al., 2013; Vorholt, 2012). However, some *Buttiauxella* sp. strains were previously cultivated from atmospheric particulate matter (Fang et al., 2007; Gandolfi et al., 2011). On the contrary, the only genus identified as significantly more abundant in road communities was *Aeribacillus* (Table S2). Members of this genus have been often described as thermophilic bacteria, isolated from hot springs, geothermal reservoirs and different environments of sub-tropical areas (Aanniz et al., 2015; Filippidou et al., 2015; Yanmis and Adiguzel, 2014). Moreover, some strains can produce exo-polysaccharides as a way to survive high temperatures (Radchenkova et al., 2013; Zheng et al., 2012). These features can possibly be also useful to deal with locally very high temperatures on leaf surfaces exposed to solar radiation.

Among the other most abundant genera, as reported in Fig. 2b, *Sphingomonas*, *Arthrobacter*, *Methylobacterium*, *Pseudomonas*, *Pantoea*, *Rhodococcus* and *Flavobacterium* have already been retrieved in phyllospheric environments (Delmotte et al., 2009; Maignien et al., 2014; Rastogi et al., 2013, 2012; Vorholt, 2012). Thus, a “core” of phyllospheric bacterial communities appears to exist (Laforest-Lapointe et al., 2016), although the relative abundance of each genus can show high variability both in different plant species and in different individuals of the same plant species (Bulgarelli et al., 2013).

The average number of OTUs detected in April samples was significantly higher than that in July samples (Fig. S3). Moreover, genera that were less abundant than 2% in all the four sample groups, indicated as “Others” in Fig. 2b, together constituted approximately 61% and 26% of April and July communities, respectively. Thus, the diversity of bacterial communities of young leaves appeared to be higher than that of the communities hosted by older leaves, as already observed by several authors (Copeland et al., 2015; Dees et al., 2015; Lindow and Brandl, 2003). This phenomenon is generally explained by a selection effect on

biodiversity, which is due both to harsh environmental conditions typical of the phyllospheric habitat and to the plant characteristics determined by its genotype (Whipps et al., 2008). It has also been suggested that seasonality and/or leaf maturation may determine a progressive decrease of nutrient availability (Dees et al., 2015), thus decreasing the number of bacterial populations that can be sustained. Nevertheless, this trend, although widespread, can not be considered to be the general rule, since in some cases phyllospheric communities remained stable over time (Delmotte et al., 2009), or even an increase in the richness of epiphytic bacteria was observed with increasing time of colonization (Peñuelas et al., 2012). Moreover, Laforest-Lapointe and colleagues (2016) observed that phyllospheric communities of five tree species in Canada underwent a succession during the growing season, although plant species was a stronger driver on bacterial diversity than sampling time. Therefore, more research is needed in order to better describe time-dependent shifts in phyllospheric community structures of an extensive range of plant species. This is particularly important for perennial plants, which can undergo a wide variability of climatic conditions throughout the year, especially in temperate areas.

*Diversity of *alkB* phylotypes*

In addition to the phylogenetic-based community structure, knowledge about potential metabolic abilities of phyllospheric bacteria and their functional diversity are of critical importance to assess their possible contribution to air remediation. Zhang et al. (2015) evaluated the carbon substrate utilization pattern through the BIOLOG method, in order to estimate the functional diversity of bacteria associated to leaves of urban trees in China, including a species of *Platanus* (*P. orientalis*). They found that phyllospheric communities associated with different trees significantly differed in their metabolic abilities. However, this method relies on laboratory cultivation. Thus, results are limited to the culturable fraction of

291 bacterial communities. For this reason, it would be also necessary to explore a range of
292 suitable marker genes in phyllospheric metagenomes. Up to now, only *chiA*, encoding a
293 chitinase, was extensively studied through amplicon HTS (Cretoiu et al., 2012). More
294 comprehensive approaches were chosen instead, in order to identify the main metabolic
295 adaptations to phyllospheric life: shotgun metagenomic sequencing was applied to bacterial
296 communities hosted by *Tamarix aphylla* leaves (Finkel et al., 2016) while metaproteomics
297 was used on soybean, clover and *Arabidopsis thaliana* communities (Delmotte et al., 2009).
298 In this work, alkane hydroxylase (*alkB*) was selected as reference gene to roughly estimate
299 the diversity of *Platanus* phyllospheric bacteria possessing the potential ability to degrade
300 alkanes. Diversity of alkane hydroxylases has already been studied in the rhizosphere of
301 different tree and grass species, both in isolates (Fatima et al., 2015; Tesar et al., 2002;
302 Yousaf et al., 2010) and in whole bacterial communities through culture-independent
303 methods (Mukherjee et al., 2015; Tsuboi et al., 2015). However, a characterization of the
304 diversity of *alkB* phylotypes in phyllospheric communities is still lacking. From NMDS
305 analysis, April and July samples were clearly distinguishable (Fig. 3).
306 Moreover, while samples from the two sampling locations formed two separate groups in
307 April, in July they showed a high overlapping. Therefore, although *alkB* phylotypes were
308 different in the two sampling locations at the beginning of the growing season, they became
309 highly similar over time. As already observed for phylogenetic diversity, it can be
310 hypothesized that both environmental conditions such as temperature, humidity and solar
311 exposure, and pollution levels were probably similar at the two locations. Thus, not only
312 bacterial communities considered as a whole, but also hydrocarbon-degrading populations
313 could have been subjected to the same selection drivers regardless the sampling location.
314 However, in contrast with what observed for phylogenetic biodiversity, the number of *alkB*
315 phylotypes was significantly higher in July (Fig. S4). This led to put forward the hypothesis

that atmospheric hydrocarbons might play a key role in the selection of phyllospheric populations in urban areas. In fact, the selective pressure they exert would cause a decrease in phylogenetic diversity while increasing the diversity of hydrocarbon-degrading populations. The overall number of detected *alkB* phylotypes was 3036. A phylogenetic tree was built with the 51 phylotypes with a total abundance $\geq 0.3\%$ (Fig. 4). Most of these phylotypes clustered together, and showed high similarities with *alkB* from different species of the genus *Rhodococcus*, particularly with *R. aetherivorans*, and with *Mycobacterium smegmatis*. Other 18 out of 51 phylotypes, which formed a separate cluster, revealed their best similarity with uncultured bacteria from various molecular studies. Although there are no indications on the taxonomy of these uncultured bacteria, the cluster to which they belong appears to be nearer to that including *Rhodococcus* sequences than to other reference strains. However, when comparing this cluster with sequences reported in a comprehensive *alkB* tree that was recently published, it was not possible to clearly identify its position on it (Nie et al., 2014). Conversely, only one of the considered phylotypes was highly similar to *alkB* belonging to a Gammaproteobacteria genus, i.e. *Shewanella*. The high prevalence of sequences from Actinobacteria suggests the mainly terrestrial origin of potential alkane-degrading bacteria (Nie et al., 2014). However, the overall diversity of *alkB* phylotypes in bacterial communities hosted by *Platanus* leaves, although increased over time as observed above, appears to be still lower than that reported for other habitats (Nie et al., 2014). This may be possibly due to the harsher conditions in the phyllospheric environment than in other environments, which limit biodiversity.

Conclusions

A proper management of vegetation has been suggested to be a promising strategy to decrease air pollution in urban areas. However, our understanding of the potential

effectiveness of urban plants in air quality improvement is still affected by several uncertainties. Therefore, we need at least to be able to estimate the actual involvement of plants, and of plant-phyllospheric bacteria associations, in air pollutant removal. On *Platanus x acerifolia* leaves, biodiversity of bacterial communities decreased along the growing season, while the diversity of hydrocarbon-degrading populations increased. This phenomenon might indicate that, in the phyllosphere of urban plants, selection effects on bacteria are driven more strongly by atmospheric hydrocarbons than by other environmental factors, such as temperature, humidity or solar radiation. However, the actual ability of phyllospheric bacterial communities to degrade hydrocarbons *in situ* still needs to be confirmed. Therefore, future research should be aimed at the quantification of the actual contribution of bacteria in air pollutant removal per unit of leaf weight or leaf area under different environmental conditions, and at the evaluation of the efficiency of different plant-bacteria systems in air quality improvement.

Acknowledgements

This work was partially funded by INAIL within the project BRIC-SINERGIA.

References

- Aanniz, T., Ouadghiri, M., Melloul, M., Swings, J., Elfahime, E., Ibijbijen, J., Ismaili, M., Amar, M., 2015. Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Braz. J. Microbiol.* 46, 443–53. doi:10.1590/S1517-838246220140219
- Al-Awadhi, H., Al-Mailem, D., Dashti, N., Hakam, L., Elias, M., Radwan, S., 2012. The abundant occurrence of hydrocarbon-utilizing bacteria in the phyllospheres of cultivated and wild plants in Kuwait. *Int. Biodeterior. Biodegrad.* 73, 73–79. doi:10.1016/j.ibiod.2012.05.016

366 Ali, N., Sorkhoh, N., Salamah, S., Eliyas, M., Radwan, S., 2012. The potential of epiphytic
 367 hydrocarbon-utilizing bacteria on legume leaves for attenuation of atmospheric
 368 hydrocarbon pollutants. *J. Environ. Manage.* 93, 113–120.
 369 doi:10.1016/j.jenvman.2011.08.014

370 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment
 371 search tool. *J. Mol. Biol.* 215, 403–10. doi:10.1016/S0022-2836(05)80360-2

372 Baró, F., Chaparro, L., Gómez-Baggethun, E., Langemeyer, J., Nowak, D.J., Terradas, J.,
 373 2014. Contribution of ecosystem services to air quality and climate change mitigation
 374 policies: the case of urban forests in Barcelona, Spain. *Ambio* 43, 466–79.
 375 doi:10.1007/s13280-014-0507-x

376 Beckett, K.P., Freer-Smith, P.H., Taylor, G., 1998. Urban woodlands: their role in reducing
 377 the effects of particulate pollution. *Environ. Pollut.* 99, 347–360. doi:10.1016/S0269-
 378 7491(98)00016-5

379 Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate : A Practical and
 380 Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* 57, 289–300. doi:
 381 10.2307/2346101

382 Bolund, P., Hunhammer, S., 1999. Ecosystem services in urban areas. *Ecol. Econ.* 29, 293–
 383 301. doi:10.1016/S0921-8009(99)00013-0

384 Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P., 2013.
 385 Structure and Functions of the Bacterial Microbiota of Plants. *Annu. Rev. Plant Biol.* 64,
 386 807–838. doi:10.1146/annurev-arplant-050312-120106

387 Calciolari, M., Silva, P., 2013. Plant growth promoting bacteria in *Brachiaria brizantha*.
 388 *World J. Microbiol. Biotechnol.* 29, 163–171. doi:10.1007/s11274-012-1169-0

389 Copeland, J.K., Yuan, L., Layeghifard, M., Wang, P.W., Guttman, D.S., 2015. Seasonal
 390 community succession of the phyllosphere microbiome. *Mol. Plant. Microbe. Interact.*

391 28, 274–85. doi:10.1094/MPMI-10-14-0331-FI

392 Cretoiu, M.S., Kielak, A.M., Abu Al-Soud, W., Sørensen, S.J., van Elsas, J.D., 2012. Mining
 393 of unexplored habitats for novel chitinases--chiA as a helper gene proxy in
 394 metagenomics. *Appl. Microbiol. Biotechnol.* 94, 1347–58. doi:10.1007/s00253-012-
 395 4057-5

396 Croes, S., Weyens, N., Colpaert, J., Vangronsveld, J., 2015. Characterization of the cultivable
 397 bacterial populations associated with field grown *Brassica napus* L.: An evaluation of
 398 sampling and isolation protocols. *Environ. Microbiol.* 17, 2379–2392.
 399 doi:10.1111/1462-2920.12701

400 De Kempeneer, L., Sercu, B., Vanbrabant, W., Van Langenhove, H., Verstraete, W., 2004.
 401 Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Appl.*
 402 *Microbiol. Biotechnol.* 64, 284–8. doi:10.1007/s00253-003-1415-3

403 Dees, M.W., Lysøe, E., Nordskog, B., Brurberg, M.B., 2015. Bacterial Communities
 404 Associated with Surfaces of Leafy Greens: Shift in Composition and Decrease in
 405 Richness over Time. *Appl. Environ. Microbiol.* 81, 1530–1539.
 406 doi:10.1128/AEM.03470-14

407 Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von
 408 Mering, C., Vorholt, J. a, 2009. Community proteogenomics reveals insights into the
 409 physiology of phyllosphere bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 106, 16428–16433.
 410 doi:10.1073/pnas.0905240106

411 Dzierzanowski, K., Popek, R., Gawrońska, H., Saebø, A., Gawroński, S.W., 2011. Deposition
 412 of particulate matter of different size fractions on leaf surfaces and in waxes of urban
 413 forest species. *Int. J. Phytoremediation* 13, 1037–46.
 414 doi:10.1080/15226514.2011.552929

415 Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon

416 reads. *Nat. Methods* 10, 996–8. doi:10.1038/nmeth.2604

417 Eevers, N., Hawthorne, J.R., White, J.C., Vangronsveld, J., Weyens, N., 2016. Exposure of
 418 *Cucurbita pepo* to DDE-contamination alters the endophytic community : A cultivation
 419 dependent vs a cultivation independent approach. *Environ. Pollut.* 209, 147–154.
 420 doi:10.1016/j.envpol.2015.11.038

421 Escobedo, F., Varela, S., Zhao, M., Wagner, J.E., Zipperer, W., 2010. Analyzing the efficacy
 422 of subtropical urban forests in offsetting carbon emissions from cities. *Environ. Sci.*
 423 *Policy* 13, 362–372. doi:10.1016/j.envsci.2010.03.009

424 Escobedo, F.J., Kroeger, T., Wagner, J.E., 2011. Urban forests and pollution mitigation:
 425 analyzing ecosystem services and disservices. *Environ. Pollut.* 159, 2078–87.
 426 doi:10.1016/j.envpol.2011.01.010

427 Fahlgren, C., Bratbak, G., Sandaa, R.-A.R.-A., Thyraug, R., Zweifel, U.L., 2011. Diversity
 428 of airborne bacteria in samples collected using different devices for aerosol collection.
 429 *Aerobiologia (Bologna)*. 27, 107–120. doi:10.1007/s10453-010-9181-z

430 Fang, Z., Ouyang, Z., Zheng, H., Wang, X., Hu, L., 2007. Culturable airborne bacteria in
 431 outdoor environments in Beijing, China. *Microb. Ecol.* 54, 487–496.
 432 doi:10.1007/s00248-007-9216-3

433 Fatima, K., Afzal, M., Imran, A., Khan, Q.M., 2015. Bacterial rhizosphere and endosphere
 434 populations associated with grasses and trees to be used for phytoremediation of crude
 435 oil contaminated soil. *Bull. Environ. Contam. Toxicol.* 94, 314–320.
 436 doi:10.1007/s00128-015-1489-5

437 Ferrando, L., Fernández Scavino, A., 2015. Strong shift in the diazotrophic endophytic
 438 bacterial community inhabiting rice (*Oryza sativa*) plants after flooding. *FEMS*
 439 *Microbiol. Ecol.* 91, 1–12. doi:10.1093/femsec/fiv104

440 Filippidou, S., Jaussi, M., Junier, T., Wunderlin, T., Jeanneret, N., Regenspurg, S., Li, P.-E.,

- Lo, C.-C., Johnson, S., McMurry, K., Gleasner, C.D., Vuyisich, M., Chain, P.S., Junier, P., 2015. Genome Sequence of *Aeribacillus pallidus* Strain GS3372, an Endospore-Forming Bacterium Isolated in a Deep Geothermal Reservoir. *Genome Announc.* 3, e00981-15. doi:10.1128/genomeA.00981-15
- Finkel, O.M., Delmont, T.O., Post, A.F., Belkin, S., 2016. Metagenomic Signatures of Bacterial Adaptation to Life in the Phyllosphere of a Salt-Secreting Desert Tree. *Appl. Environ. Microbiol.* 82, 2854–2861. doi:10.1128/AEM.00483-16
- Gandolfi, I., Franzetti, A., Bertolini, V., Gaspari, E., Bestetti, G., 2011. Antibiotic resistance in bacteria associated with coarse atmospheric particulate matter in an urban area. *J. Appl. Microbiol.* 110, 1612–1620. doi:10.1111/j.1365-2672.2011.05018.x
- Glick, B.R., 2015. *Beneficial Plant-Bacterial Interactions*. Springer International Publishing, Cham. doi:10.1007/978-3-319-13921-0
- Huber, J.A., Mark Welch, D.B., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., Sogin, M.L., 2007. Microbial population structures in the deep marine biosphere. *Science* 318, 97–100. doi:10.1126/science.1146689
- Islam, S., Akanda, A.M., Prova, A., Sultana, F., Sheikh, B., Rahman, M., 2015. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbiol.* 6, 1–12. doi:10.3389/fmicb.2015.01360
- Jurelevicius, D., Alvarez, V.M., Peixoto, R., Rosado, A.S., Seldin, L., 2013. The Use of a Combination of *alkB* Primers to Better Characterize the Distribution of Alkane-Degrading Bacteria. *PLoS One* 8, 1–10. doi:10.1371/journal.pone.0066565
- Kgomotso, M., Maropola, A., Ramond, J., Trindade, M., 2015. Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum bicolor* (L. Moench). *J. Microbiol. Methods* 112, 104–117.

doi:10.1016/j.mimet.2015.03.012

Kim, M.K., Joo, E.S., Lee, S.Y., Lee, D.S., Srinivasan, S., Jung, H.Y., 2016. Complete

genome sequence of *Hymenobacter* sp. DG25B, a novel bacterium with gamma-

radiation resistance isolated from soil in South Korea. *J. Biotechnol.* 217, 98–99.

doi:10.1016/j.jbiotec.2015.11.015

Klassen, J.L., Foght, J.M., 2011. Characterization of *Hymenobacter* isolates from Victoria

Upper Glacier, Antarctica reveals five new species and substantial non-vertical

evolution within this genus. *Extremophiles* 15, 45–57. doi:10.1007/s00792-010-0336-1

Laforest-Lapointe, I., Messier, C., Kembel, S.W., 2016. Host species identity, site and time

drive temperate tree phyllosphere bacterial community structure. *Microbiome* 4, 27.

doi:10.1186/s40168-016-0174-1

Lee, J.J., Srinivasan, S., Lim, S., Joe, M., Lee, S.H., Kwon, S.A., Kwon, Y.J., Lee, J., Choi,

J.J., Lee, H.M., Auh, Y.K., Kim, M.K., 2014. *Hymenobacter swuensis* sp. nov., a

gamma-radiation-resistant bacteria isolated from mountain soil. *Curr. Microbiol.* 68,

305–310. doi:10.1007/s00284-013-0478-3

Lighthart, B., 2006. The ecology of bacteria in the alfresco atmosphere. *FEMS Microbiol.*

Ecol. 23, 263–274. doi:10.1111/j.1574-6941.1997.tb00408.x

Lindow, S.E., Brandl, M.T., 2003. Microbiology of the Phyllosphere. *Appl. Environ.*

Microbiol. 69, 1875–1883. doi:10.1128/AEM.69.4.1875

Lymperopoulou, D.S., Adams, R.I., Lindow, S.E., 2016. Contribution of vegetation to the

microbial composition of nearby outdoor air. *Appl. Environ. Microbiol.* 82, 3822–3833.

doi:10.1128/AEM.00610-16

Maignien, L., DeForce, E.A., Chafee, M.E., Eren, A.M., Simmons, S.L., 2014. Ecological

succession and stochastic variation in the assembly of *Arabidopsis thaliana* phyllosphere

communities. *MBio* 5, e00682–13. doi:10.1128/mBio.00682-13

491 Marcazzan, G.M., Valli, G., Vecchi, R., 2002. Factors influencing mass concentration and
 492 chemical composition of fine aerosols during a PM high pollution episode. *Sci. Total*
 493 *Environ.* 298, 65-79. doi: 10.1016/S0048-9697(02)00171-7
 494 Mastretta, C., Taghavi, S., van der Lelie, D., Mengoni, A., Galardi, F., Gonnelli, C., Barac,
 495 T., Boulet, J., Weyens, N., Vangronsveld, J., 2009. Endophytic bacteria from seeds of
 496 *Nicotiana tabacum* can reduce cadmium phytotoxicity. *Int. J. Phytoremediation* 11, 251–
 497 267. doi:10.1080/15226510802432678
 498 Maurizi, A., Russo, F., Tampieri, F., 2013. Local vs. external contribution to the budget of
 499 pollutants in the Po Valley (Italy) hot spot. *Sci. Total Environ.* 458, 459-465. doi:
 500 10.1016/j.scitotenv.2013.04.026
 501 McPherson, E.G., Scott, K.I., Simpson, J.R., 1998. Estimating cost effectiveness of
 502 residential yard trees for improving air quality in Sacramento, California, using existing
 503 models. *Atmos. Environ.* 32, 75–84. doi:10.1016/S1352-2310(97)00180-5
 504 Mi, Y., Eun, L., Kim, H., Kum, H., 2014. Biodiversity and physiological characteristics of
 505 Antarctic and Arctic lichens-associated bacteria 2711–2721. doi:10.1007/s11274-014-
 506 1695-z
 507 Morris, C.E., Kinkel, L.L., 2015. Fifty years of phyllosphere microbiology: significant
 508 contributions to research in related fields, in: *Phyllosphere Microbiology*. pp. 365–375.
 509 Mukherjee, S., Sipilä, T., Pulkkinen, P., Yrjälä, K., 2015. Secondary successional trajectories
 510 of structural and catabolic bacterial communities in oil-polluted soil planted with hybrid
 511 poplar. *Mol. Ecol.* 24, 628–642. doi:10.1111/mec.13053
 512 Müller, T., Ruppel, S., 2014. Progress in cultivation-independent phyllosphere microbiology.
 513 *FEMS Microbiol. Ecol.* 87, 2–17. doi:10.1111/1574-6941.12198
 514 Muratova, A., Dubrovskaya, E., Golubev, S., Grinev, V., Chernyshova, M., Turkovskaya, O.,
 515 2015. The coupling of the plant and microbial catabolisms of phenanthrene in the

rhizosphere of *Medicago sativa*. *J. Plant Physiol.* 188, 1–8.
doi:10.1016/j.jplph.2015.07.014

Nie, Y., Chi, C.-Q., Fang, H., Liang, J.-L., Lu, S.-L., Lai, G.-L., Tang, Y.-Q., Wu, X.-L.,
2014. Diverse alkane hydroxylase genes in microorganisms and environments. *Sci. Rep.*
4, 4968. doi:10.1038/srep04968

Nowak, D.J., Crane, D.E., 2002. Carbon storage and sequestration by urban trees in the USA.
Environ. Pollut. 116, 381–389. doi:10.1016/S0269-7491(01)00214-7

Nowak, D.J., Crane, D.E., Stevens, J.C., 2006. Air pollution removal by urban trees and
shrubs in the United States. *Urban For. Urban Green.* 4, 115–123.
doi:10.1016/j.ufug.2006.01.007

Oksanen, J., Kindt, R., Legendre, P., O’Hara, B., Simpson, G.L., Solymos, P., Stevens,
M.H.H., Wagner, H., 2009. *Vegan: Community Ecology Package*. R package version
1.15-3.

Paoletti, E., 2009. Ozone and urban forests in Italy. *Environ. Pollut.* 157, 1506–12.
doi:10.1016/j.envpol.2008.09.019

Peñuelas, J., Rico, L., Ogaya, R., Jump, A.S., Terradas, J., 2012. Summer season and long-
term drought increase the richness of bacteria and fungi in the foliar phyllosphere of
Quercus ilex in a mixed Mediterranean forest. *Plant Biol. (Stuttg.)* 14, 565–75.
doi:10.1111/j.1438-8677.2011.00532.x

Pruesse, E., Peplies, J., Glöckner, F.O., 2012. SINA: Accurate high-throughput multiple
sequence alignment of ribosomal RNA genes. *Bioinformatics* 28, 1823–1829.
doi:10.1093/bioinformatics/bts252

Radchenkova, N., Vassilev, S., Panchev, I., Anzelmo, G., Tomova, I., Nicolaus, B.,
Kuncheva, M., Petrov, K., Kambourova, M., 2013. Production and properties of two
novel exopolysaccharides synthesized by a thermophilic bacterium *Aeribacillus pallidus*

541 418. Appl. Biochem. Biotechnol. 171, 31–43. doi:10.1007/s12010-013-0348-2

542 Rastogi, G., Coaker, G.L., Leveau, J.H.J., 2013. New insights into the structure and function
543 of phyllosphere microbiota through high-throughput molecular approaches. FEMS
544 Microbiol. Lett. 348, 1–10. doi:10.1111/1574-6968.12225

545 Rastogi, G., Sbodio, A., Tech, J.J., Suslow, T. V, Coaker, G.L., Leveau, J.H.J., 2012. Leaf
546 microbiota in an agroecosystem: spatiotemporal variation in bacterial community
547 composition on field-grown lettuce. ISME J. 6, 1812–1822. doi:10.1038/ismej.2012.32

548 Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y., Fierer, N., 2010. The ecology of the
549 phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on
550 tree leaves. Environ. Microbiol. 12, 2885–93. doi:10.1111/j.1462-2920.2010.02258.x

551 Romero, F.M., Marina, M., Pieckenstain, F.L., 2014. The communities of tomato (*Solanum*
552 *lycopersicum* L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene
553 pyrosequencing. FEMS Microbiol. Lett. 351, 187–194. doi:10.1111/1574-6968.12377

554 Sandhu, A., Halverson, L.J., Beattie, G.A., 2007. Bacterial degradation of airborne phenol in
555 the phyllosphere. Environ. Microbiol. 9, 383–92. doi:10.1111/j.1462-2920.2006.01149.x

556 Sangthong, S., Suksabye, P., Thiravetyan, P., 2016. Air-borne xylene degradation by
557 *Bougainvillea buttiana* and the role of epiphytic bacteria in the degradation. Ecotox.
558 Environ. Safe. 126, 273-280. doi:10.1016/j.ecoenv.2015.12.017

559 Scheublin, T.R., Deusch, S., Moreno-Forero, S.K., Müller, J. a., van der Meer, J.R., Leveau,
560 J.H.J., 2014. Transcriptional profiling of Gram-positive *Arthrobacter* in the
561 phyllosphere: Induction of pollutant degradation genes by natural plant phenolic
562 compounds. Environ. Microbiol. 16, 2212–2225. doi:10.1111/1462-2920.12375

563 Su, S., Chen, M., Teng, C., Jiang, S., Zhang, C., Lin, M., Zhang, W., 2014. *Hymenobacter*
564 *kanuolensis* sp. nov., a novel radiation-resistant bacterium. Int. J. Syst. Evol. Microbiol.
565 64, 2108–2112. doi:10.1099/ijs.0.051680-0

566 Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T.,
 567 Vangronsveld, J., Van Der Lelie, D.D., 2009. Genome survey and characterization of
 568 endophytic bacteria exhibiting a beneficial effect on growth and development of poplar
 569 trees. *Appl. Environ. Microbiol.* 75, 748–757. doi:10.1128/AEM.02239-08
 570 TEEB, 2011. TEEB manual for cities: Ecosystem services in urban management. *Econ.*
 571 *Ecosyst. Biodivers.* 48.
 572 Tesar, M., Reichenauer, T.G., Sessitsch, A., 2002. Bacterial rhizosphere populations of black
 573 poplar and herbal plants to be used for phytoremediation of diesel fuel. *Soil Biol.*
 574 *Biochem.* 34, 1883–1892. doi:10.1016/S0038-0717(02)00202-X
 575 Thijs, S., Van Dillewijn, P., Sillen, W., Truyens, S., Holtappels, M., D’Haen, J., Carleer, R.,
 576 Weyens, N., Ameloot, M., Ramos, J.L., Vangronsveld, J., 2014. Exploring the
 577 rhizospheric and endophytic bacterial communities of *Acer pseudoplatanus* growing on
 578 a TNT-contaminated soil: towards the development of a rhizocompetent TNT-
 579 detoxifying plant growth promoting consortium. *Plant Soil* 385, 15–36.
 580 doi:10.1007/s11104-014-2260-0
 581 Tsuboi, S., Yamamura, S., Nakajima-Kambe, T., Iwasaki, K., 2015. Diversity of alkane
 582 hydroxylase genes on the rhizoplane of grasses planted in petroleum-contaminated soils.
 583 *Springerplus* 4, 526. doi:10.1186/s40064-015-1312-0
 584 Vecchi, R., Valli, G., 1999. Ozone assessment in the southern part of the Alps. *Atmos.*
 585 *Environ.* 33, 97-109. doi: 10.1016/S1352-2310(98)00133-2
 586 Vokou, D., Vareli, K., Zarali, E., Karamanoli, K., Constantinidou, H.I.A., Monokrousos, N.,
 587 Halley, J.M., Sainis, I., 2012. Exploring Biodiversity in the Bacterial Community of the
 588 Mediterranean Phyllosphere and its Relationship with Airborne Bacteria. *Microb. Ecol.*
 589 64, 714–724. doi:10.1007/s00248-012-0053-7
 590 Vorholt, J.A., 2012. Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* 10, 828–40.

doi:10.1038/nrmicro2910

Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., Mitchell-Olds, T.,
2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial
plant. *Nat. Commun.* 7, 12151. doi:10.1038/ncomms12151

Waight, K., Pinyakong, O., Luepromchai, E., 2007. Degradation of phenanthrene on plant
leaves by phyllosphere bacteria. *J. Gen. Appl. Microbiol.* 53, 265–72.
doi:10.2323/jgam.53.265

Wang, Y., Qian, P., 2009. Conservative fragments in bacterial 16S rRNA genes and primer
design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One* 4, e7401.
doi:10.1371/journal.pone.0007401

Weyens, N., Thijs, S., Popek, R., Witters, N., Przybysz, A., Espenshade, J., Gawronska, H.,
Vangronsveld, J., Gawronski, S.W., 2015. The Role of Plant-Microbe Interactions and
Their Exploitation for Phytoremediation of Air Pollutants. *Int. J. Mol. Sci.* 16, 25576–
604. doi:10.3390/ijms161025576

Whipps, J.M., Hand, P., Pink, D., Bending, G.D., 2008. Phyllosphere microbiology with
special reference to diversity and plant genotype. *J. Appl. Microbiol.* 105, 1744–1755.
doi:10.1111/j.1365-2672.2008.03906.x

Wu, Y., Wang, Y., Li, J., Hu, J., Chen, K., Wei, Y., Bazhanov, D.P., Bazhanova, A.A., Yang,
H., 2015. Draft Genome Sequence of *Stenotrophomonas maltophilia* Strain B418 , a
Promising Agent for Biocontrol of Plant Pathogens and Root-Knot Nematode 3, 1998–
1999. doi:10.1128/genomeA.00015-15

Yang, C.H., Crowley, D.E., Borneman, J., Keen, N.T., 2001. Microbial phyllosphere
populations are more complex than previously realized. *Proc. Natl. Acad. Sci. U. S. A.*
98, 3889–3894. doi:10.1073/pnas.051633898

Yang, J., Chang, Y.M., Yan, P.B., 2015. Ranking the suitability of common urban tree

species for controlling PM_{2.5} pollution. *Atmos. Pollut. Res.* 6, 267–277.
doi:10.5094/apr.2015.031

Yang, J., McBride, J., Zhou, J., Sun, Z., 2005. The urban forest in Beijing and its role in air pollution reduction. *Urban For. Urban Green.* 3, 65–78. doi:10.1016/j.ufug.2004.09.001

Yanmis, D., Adiguzel, A., 2014. Molecular Typing of Thermophilic Bacilli isolated from Different Hot Springs of Turkey. *Res. J. Biotechnol.* 9, 83–88.

Yooseph, S., Andrews-Pfannkoch, C., Tenney, A., McQuaid, J., Williamson, S., Thiagarajan, M., Bami, D., Zeigler-Allen, L., Hoffman, J., Goll, J.B., Fadrosch, D., Glass, J., Adams, M.D., Friedman, R., Venter, J.C., 2013. A metagenomic framework for the study of airborne microbial communities. *PLoS One* 8. doi:10.1371/journal.pone.0081862

Yousaf, S., Andria, V., Reichenauer, T.G., Smalla, K., Sessitsch, A., 2010. Phylogenetic and functional diversity of alkane degrading bacteria associated with Italian ryegrass (*Lolium multiflorum*) and Birdsfoot trefoil (*Lotus corniculatus*) in a petroleum oil-contaminated environment. *J. Hazard. Mater.* 184, 523–532.
doi:10.1016/j.jhazmat.2010.08.067

Yutthammo, C., Thongthammachai, N., Pinphanichakarn, P., Luepromchai, E., 2010. Diversity and activity of PAH-degrading bacteria in the phyllosphere of ornamental plants. *Microb. Ecol.* 59, 357–368. doi:10.1007/s00248-009-9631-8

Zhang, H., Chen, S., Huang, T., 2015. Structure and Functional Metabolism of Bacterial Communities on Leaves of Typical Urban Greening Tree Species. *Polish J. Environ. Stud.* 24, 823–828. doi:10.15244/pjoes/30592

Zhao, M., Kong, Z., Escobedo, F.J., Gao, J., 2010. Impacts of urban forests on offsetting carbon emissions from industrial energy use in Hangzhou, China. *J. Environ. Manage.* 91, 807–13. doi:10.1016/j.jenvman.2009.10.010

Zheng, C., Li, Z., Su, J., Zhang, R., Liu, C., Zhao, M., 2012. Characterization and

641 emulsifying property of a novel bioemulsifier by *Aeribacillus pallidus* YM-1. J. Appl.
642 Microbiol. 113, 44–51. doi:10.1111/j.1365-2672.2012.05313.x
643

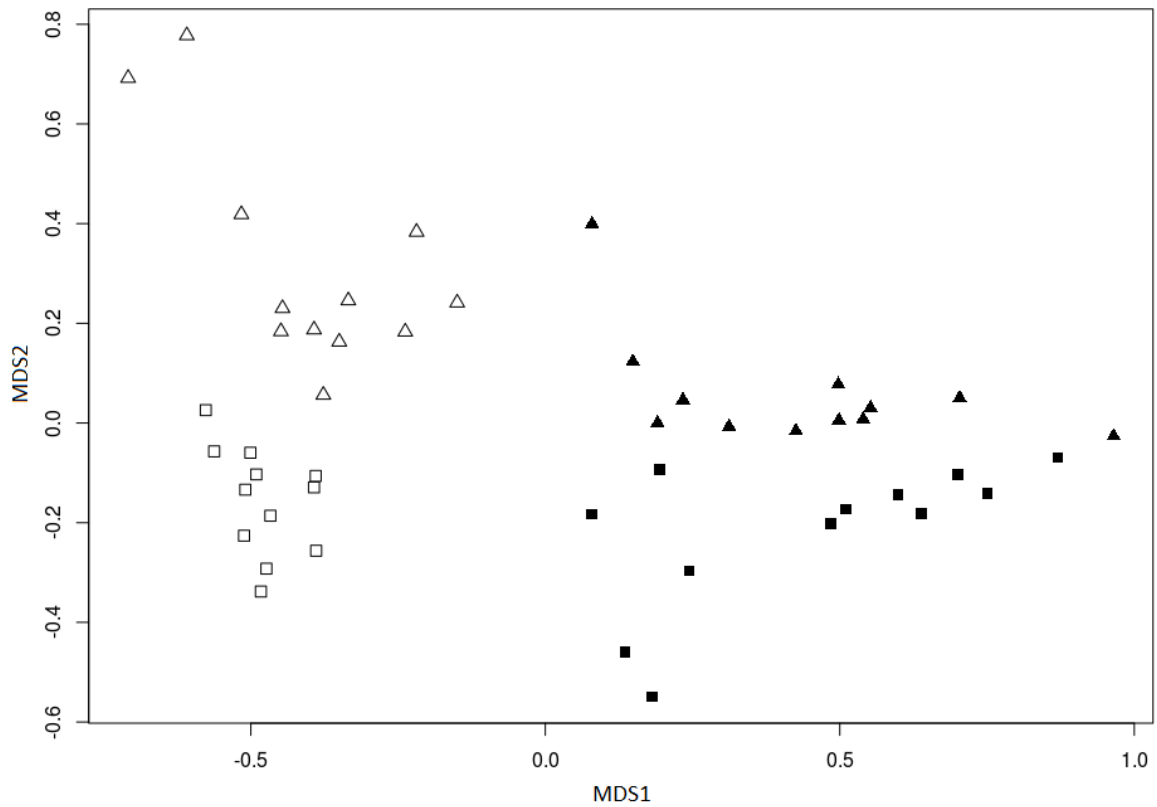
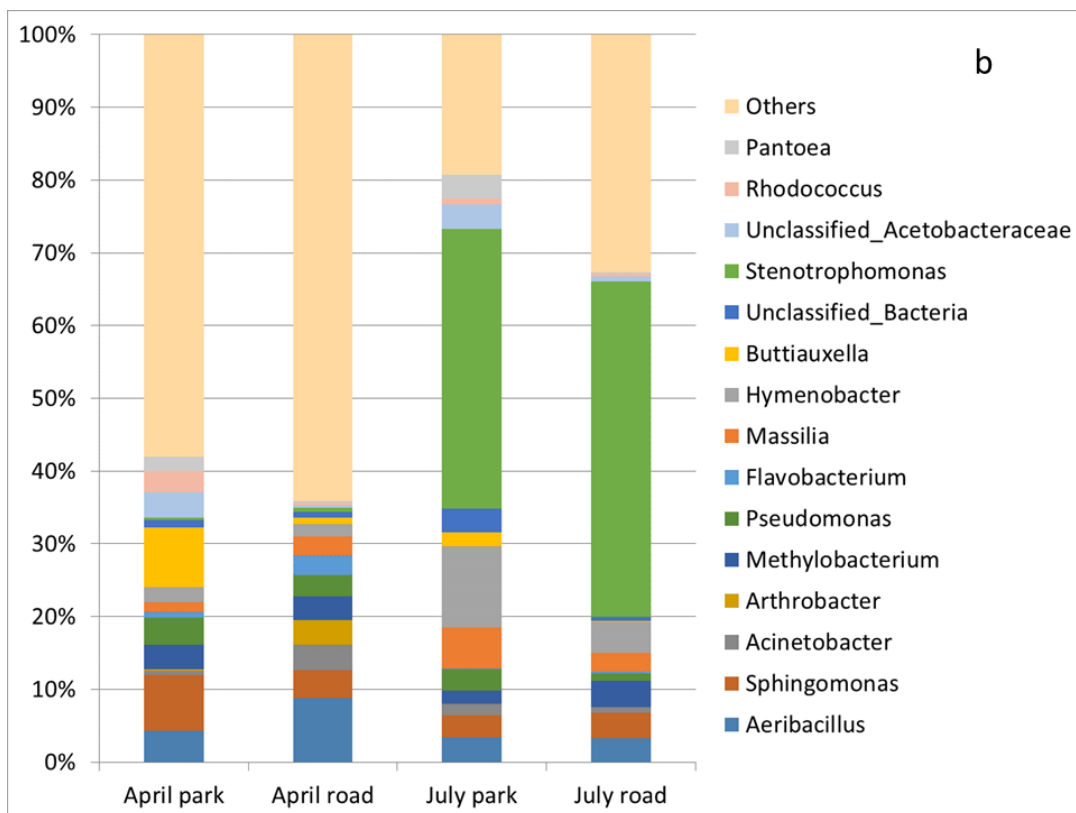
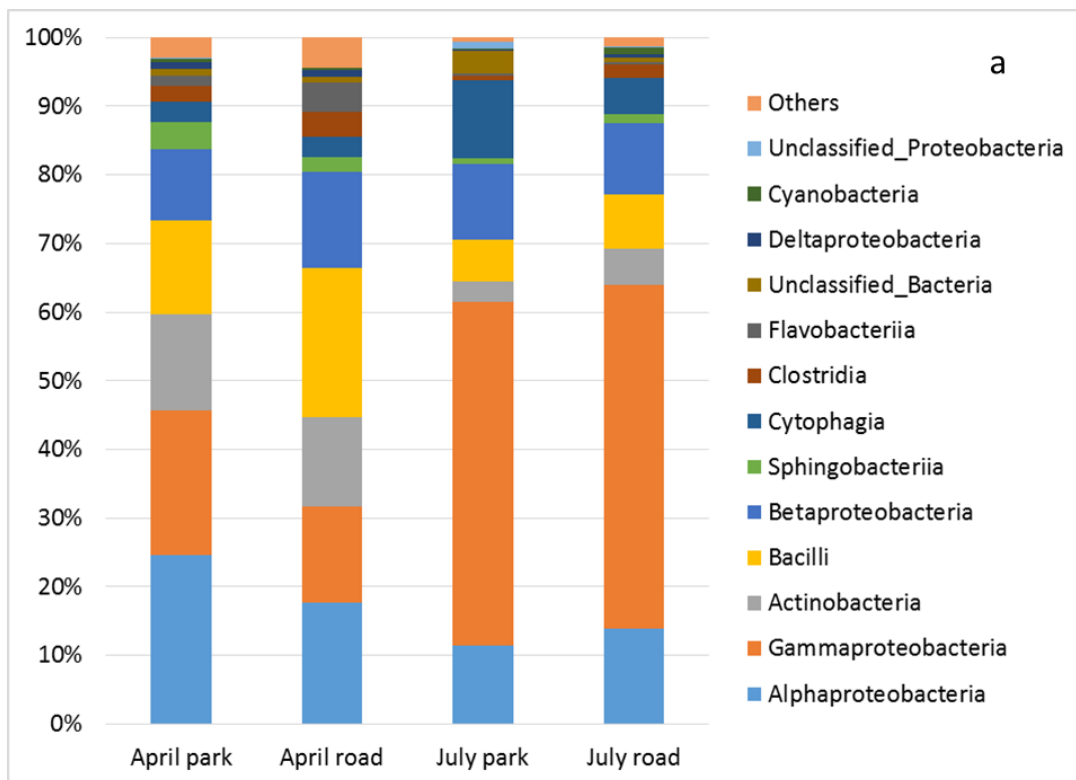


Fig. 1 – NMDS analysis of bacterial phylogenetic diversity. Hellinger distances among samples were calculated on the basis of presence and abundance of OTUs. Empty symbols: April; filled symbols: July; squares: park; triangles: road.



652 Fig. 2 – Relative abundance of phyllospheric bacterial taxa at Class (a) and Genus (b) level.
653 Only taxa with an abundance $\geq 1\%$ (Class) or $\geq 2\%$ (Genus) in at least one of the four groups
654 of samples are shown. Samples are grouped according to month and sampling location.
655

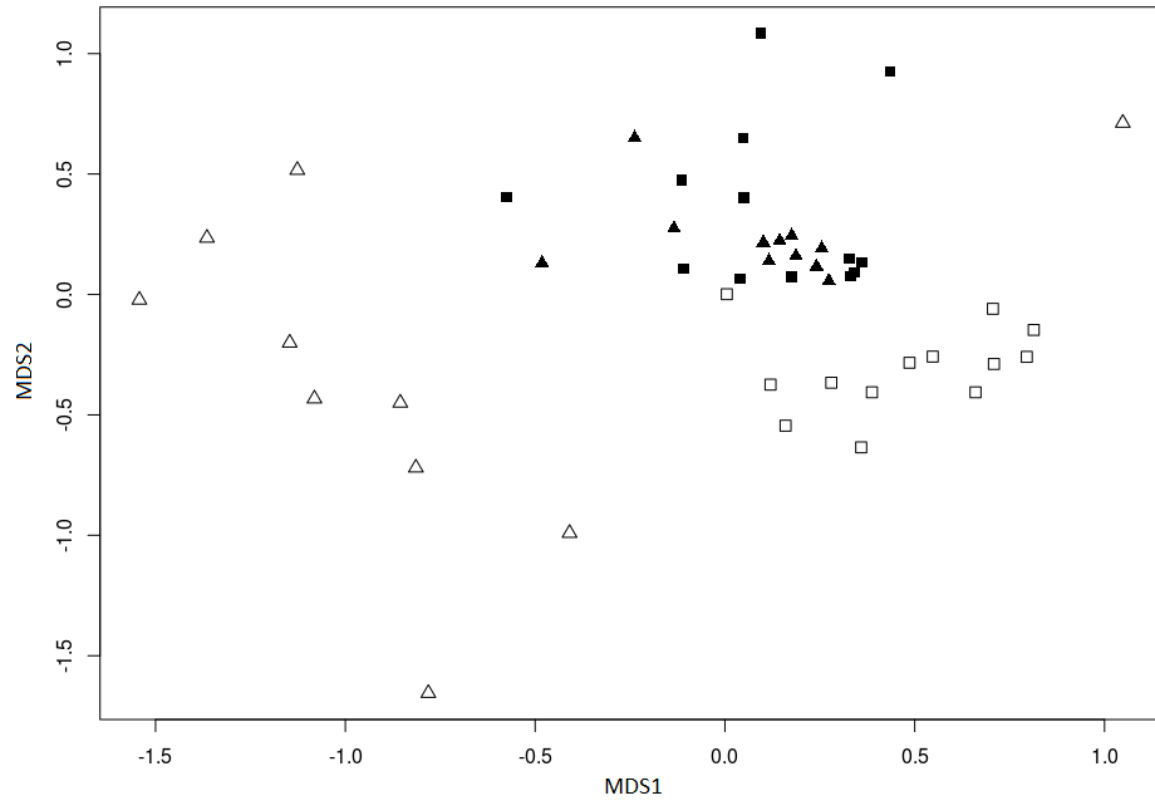
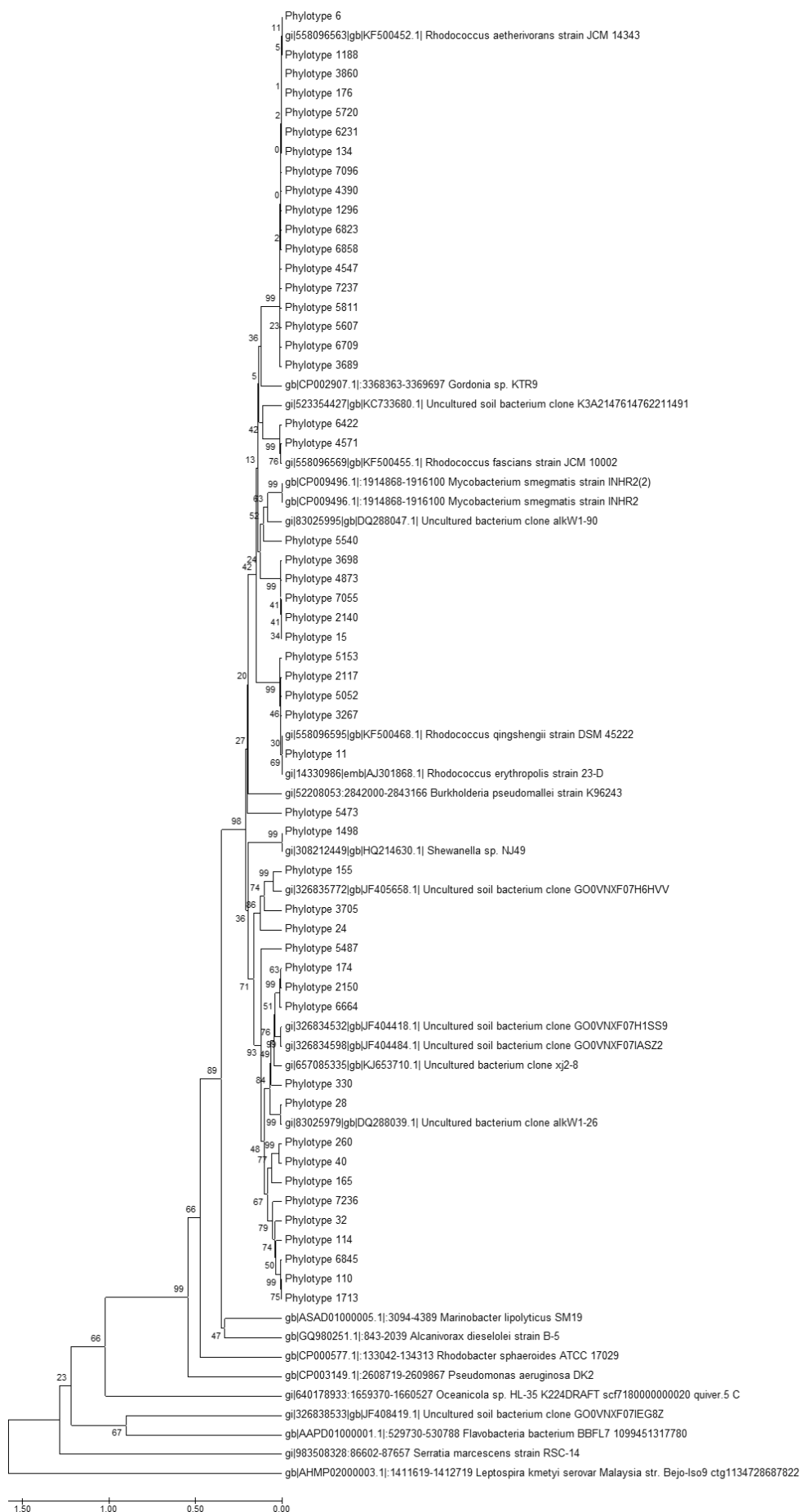


Fig. 3 – NMDS analysis of *alkB* diversity. Hellinger distances among samples were calculated on the basis of presence and abundance of OTUs. Empty symbols: April; filled symbols: July; squares: park; triangles: road.



662 Fig. 4 – Phylogenetic tree of *alkB* phylotypes based on nucleotide sequence. Only phylotypes
663 with a total abundance $\geq 0.3\%$ were included. Sequences of *alkB* from some reference strains
664 and from uncultured bacteria having a high similarity with phylotypes of this work were also
665 included for comparison. The tree was built with the UPGMA method using MEGA7. The
666 percentage of replicate trees in which the associated taxa clustered together in the bootstrap
667 test (1000 replicates) are shown next to the branches. The evolutionary distances were
668 computed using the Maximum Composite Likelihood method and are in the units of the
669 number of base substitutions per site.