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

3

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16 **Abstract**

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

34

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

38

39 **Keywords:** phyllosphere, phylloremediation, hydrocarbons, alkane hydroxylase, air
40 purification

41 **Introduction**

42 Air pollution is a matter of global concern, especially in urban areas, due to the harmful
43 effects of atmospheric pollutants on human health and on the environment. Current emission
44 reduction methods and mitigation strategies are not adequate to fully meet the World Health
45 Organization (WHO) guidelines for air pollutants (Ali et al., 2012; Weyens et al., 2015).
46 Since most of the environmental problems in urban areas are generated at local level, often
47 one of the most effective ways to deal with them is through local solutions (Bolund and
48 Hunhammer, 1999). Plants have been suggested to effectively contribute to reduce air
49 pollution levels and offsetting greenhouse gas emissions in cities (Beckett et al., 1998;
50 Dzierzanowski et al., 2011; McPherson et al., 1998; Nowak and Crane, 2002; Nowak et al.,
51 2006; Paoletti, 2009; Redford et al., 2010; Yang et al., 2005; Zhao et al., 2010). In this
52 context, the regulation of ecosystem services (the direct and indirect contributions of
53 ecosystems to human well-being (TEEB, 2011)) provided by vegetation in urban areas is of
54 great importance (Baró et al., 2014). Many studies, in fact, indicated that the management of
55 urban forests to enhance ecosystem service supply can be a cost-effective strategy to meet
56 specific environmental standards or policy targets (Escobedo et al., 2010, 2011).
57 Furthermore, it has been recognized that also plant-associated bacteria can play a crucial role
58 in air bioremediation processes (Glick, 2015; Weyens et al., 2015). Particularly, the aerial
59 parts of terrestrial plants, mainly leaves (i.e. the phyllosphere) host huge amounts of bacteria.
60 In fact, although phyllospheric microorganisms comprise also fungi, yeasts, algae, protozoa
61 and nematodes, bacteria are by far the most abundant inhabitants of leaf surfaces (Lindow
62 and Brandl, 2003). Since phyllospheric bacteria are often found at an average of 10^6 - 10^7 cells
63 cm^{-2} of leaf surface (Lindow and Brandl, 2003), the planetary phyllospheric bacterial
64 population has been estimated to be as large as 10^{26} cells (Morris and Kinkel, 2015). Among
65 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

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

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

106

107 **Materials and Methods**

108 *Sampling*

109 Leaves were collected from eight different *Platanus x acerifolia* trees in the city of Milan
110 (Italy). Four of them were located in an urban park (Parco Nord), next to a low-traffic
111 secondary road, and the other four were planted along a high-traffic road (Viale Fulvio Testi),
112 which is one of the major arterial roads in the northern part of the city (Fig. S1).

113 Meteorological conditions and atmospheric pollutant concentrations for this area are reported
114 in Fig. S2. Sampling was performed at the beginning (April 17, 2014) and in the middle of
115 the growing season (July 11, 2014). For each tree, samples were collected in triplicates, for a

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

120

121 *DNA extraction*

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

128

129 *Illumina sequencing*

130 The V5-V6 hypervariable regions of the bacterial 16S rRNA gene were PCR-amplified using
131 783F and 1046R primers (Huber et al., 2007; Wang and Qian, 2009). For the characterization
132 of *alkB* diversity, three different primer pairs were preliminarily tested on our samples (pairs
133 (d), (e) and (f) of Jurelevicius et al. (2013)); detectable amplification was obtained with
134 primer pair (f) only, which was therefore chosen for subsequent analyses. At the 5' end of
135 each primer, a 6-bp barcode was included to allow sample pooling and sequence sorting. All
136 amplicons were sequenced by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) with a
137 250 bp \times 2 paired-end protocol. For each sample, 2 \times 75 μ L volume PCR reactions were
138 performed with GoTaq® G2 Green Master Mix (Promega Corporation, Madison, WI, USA)
139 and 1 μ M of each primer. The cycling conditions for the amplification of the 16S rRNA gene
140 fragment were: initial denaturation at 94 °C for 4 min; 28 cycles at 94 °C for 50 s, 47 °C for

141 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min. The cycling conditions for
142 the amplification of the *alkB* fragment were: initial denaturation at 96 °C for 4 min; 40 cycles
143 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.
144 The amplicons were purified with the Wizard® SV Gel and PCR Clean-up System (Promega
145 Corporation, Madison, WI, USA) and purified DNA was quantified using Qubit® (Life
146 Technologies, Carlsbad, CA, USA). Groups of 9/12 amplicons bearing different barcode
147 pairs were pooled together to build a single library. Further library preparation with the
148 addition of standard Nextera indexes (Illumina, Inc., San Diego, CA, USA) and sequencing
149 were carried out at Parco Tecnologico Padano (Lodi, Italy).

150

151 *Sequence analysis*

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

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

177

178 **Results and Discussion**

179 *Phylogenetic diversity*

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

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

195 The relative abundance of the main bacterial phyllospheric populations at the taxonomic
196 levels of Class and Genus is shown in Fig. 2 (a and b, respectively). Overall, the most
197 abundant classes were *Gammaproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*,
198 *Bacilli* and *Actinobacteria*. They have already been described by several authors as common
199 classes in phyllospheric bacterial communities associated with different plant species (Dees
200 et al., 2015; Rastogi et al., 2012; Vorholt, 2012; Whipps et al., 2008), although in some cases
201 they were reported with very different relative abundances (Redford et al., 2010). In April,
202 the *Platanus* phyllospheric communities were not clearly dominated by any class or genus.
203 On the contrary, July communities exhibited a large prevalence of *Gammaproteobacteria*,
204 with a relative abundance of approximately 50%. Within this class, most sequences belonged
205 to the genus *Stenotrophomonas* (approximately 42% of total bacteria). This genus has already
206 been reported to be one of the major genera commonly detected in phyllospheric
207 communities, although at much lower percentages (Vorholt, 2012). Particularly,
208 *Stenotrophomonas* has been generally described as a member of endophytic, rather than
209 epiphytic, bacterial communities of different plant species (Ferrando and Fernández Scavino,
210 2015; Kgomotso et al., 2015; Mastretta et al., 2009; Romero et al., 2014; Taghavi et al.,
211 2009). Several isolates belonging to this genus were demonstrated to possess plant-growth
212 promoting properties (Calciolari and Silva, 2013; Islam et al., 2015). The same abilities were
213 observed for a rhizospheric *S. maltophilia* strain and confirmed through genome sequencing
214 (Wu et al., 2015). Furthermore, some plant-associated *Stenotrophomonas* strains were
215 reported as able to degrade oil hydrocarbons (Ali et al., 2012) and phenanthrene (Muratova et

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

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

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

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

259 The average number of OTUs detected in April samples was significantly higher than that in
260 July samples (Fig. S3). Moreover, genera that were less abundant than 2% in all the four
261 sample groups, indicated as “Others” in Fig. 2b, together constituted approximately 61% and
262 26% of April and July communities, respectively. Thus, the diversity of bacterial
263 communities of young leaves appeared to be higher than that of the communities hosted by
264 older leaves, as already observed by several authors (Copeland et al., 2015; Dees et al., 2015;
265 Lindow and Brandl, 2003). This phenomenon is generally explained by a selection effect on

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

281

282 *Diversity of alkB phylotypes*

283 In addition to the phylogenetic-based community structure, knowledge about potential
284 metabolic abilities of phyllospheric bacteria and their functional diversity are of critical
285 importance to assess their possible contribution to air remediation. Zhang et al. (2015)
286 evaluated the carbon substrate utilization pattern through the BIOLOG method, in order to
287 estimate the functional diversity of bacteria associated to leaves of urban trees in China,
288 including a species of *Platanus* (*P. orientalis*). They found that phyllospheric communities
289 associated with different trees significantly differed in their metabolic abilities. However, this
290 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

316 that atmospheric hydrocarbons might play a key role in the selection of phyllospheric
317 populations in urban areas. In fact, the selective pressure they exert would cause a decrease in
318 phylogenetic diversity while increasing the diversity of hydrocarbon-degrading populations.
319 The overall number of detected *alkB* phylotypes was 3036. A phylogenetic tree was built
320 with the 51 phylotypes with a total abundance $\geq 0.3\%$ (Fig. 4). Most of these phylotypes
321 clustered together, and showed high similarities with *alkB* from different species of the genus
322 *Rhodococcus*, particularly with *R. aetherivorans*, and with *Mycobacterium smegmatis*. Other
323 18 out of 51 phylotypes, which formed a separate cluster, revealed their best similarity with
324 uncultured bacteria from various molecular studies. Although there are no indications on the
325 taxonomy of these uncultured bacteria, the cluster to which they belong appears to be nearer
326 to that including *Rhodococcus* sequences than to other reference strains. However, when
327 comparing this cluster with sequences reported in a comprehensive *alkB* tree that was
328 recently published, it was not possible to clearly identify its position on it (Nie et al., 2014).
329 Conversely, only one of the considered phylotypes was highly similar to *alkB* belonging to a
330 Gammaproteobacteria genus, i.e. *Shewanella*. The high prevalence of sequences from
331 Actinobacteria suggests the mainly terrestrial origin of potential alkane-degrading bacteria
332 (Nie et al., 2014). However, the overall diversity of *alkB* phylotypes in bacterial communities
333 hosted by *Platanus* leaves, although increased over time as observed above, appears to be still
334 lower than that reported for other habitats (Nie et al., 2014). This may be possibly due to the
335 harsher conditions in the phyllospheric environment than in other environments, which limit
336 biodiversity.

337

338 **Conclusions**

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

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

354

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357

358 **References**

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

441 Lo, C.-C., Johnson, S., McMurry, K., Gleasner, C.D., Vuyisich, M., Chain, P.S., Junier,
442 P., 2015. Genome Sequence of *Aeribacillus pallidus* Strain GS3372, an Endospore-
443 Forming Bacterium Isolated in a Deep Geothermal Reservoir. *Genome Announc.* 3,
444 e00981-15. doi:10.1128/genomeA.00981-15

445 Finkel, O.M., Delmont, T.O., Post, A.F., Belkin, S., 2016. Metagenomic Signatures of
446 Bacterial Adaptation to Life in the Phyllosphere of a Salt-Secreting Desert Tree. *Appl.*
447 *Environ. Microbiol.* 82, 2854–2861. doi:10.1128/AEM.00483-16

448 Gandolfi, I., Franzetti, A., Bertolini, V., Gaspari, E., Bestetti, G., 2011. Antibiotic resistance
449 in bacteria associated with coarse atmospheric particulate matter in an urban area. *J.*
450 *Appl. Microbiol.* 110, 1612–1620. doi:10.1111/j.1365-2672.2011.05018.x

451 Glick, B.R., 2015. *Beneficial Plant-Bacterial Interactions*. Springer International Publishing,
452 Cham. doi:10.1007/978-3-319-13921-0

453 Huber, J.A., Mark Welch, D.B., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A.,
454 Sogin, M.L., 2007. Microbial population structures in the deep marine biosphere.
455 *Science* 318, 97–100. doi:10.1126/science.1146689

456 Islam, S., Akanda, A.M., Prova, A., Sultana, F., Sheikh, B., Rahman, M., 2015. Isolation and
457 identification of plant growth promoting rhizobacteria from cucumber rhizosphere and
458 their effect on plant growth promotion and disease suppression. *Front. Microbiol.* 6, 1–
459 12. doi:10.3389/fmicb.2015.01360

460 Jurelevicius, D., Alvarez, V.M., Peixoto, R., Rosado, A.S., Seldin, L., 2013. The Use of a
461 Combination of *alkB* Primers to Better Characterize the Distribution of Alkane-
462 Degrading Bacteria. *PLoS One* 8, 1–10. doi:10.1371/journal.pone.0066565

463 Kgomotso, M., Maropola, A., Ramond, J., Trindade, M., 2015. Impact of metagenomic DNA
464 extraction procedures on the identifiable endophytic bacterial diversity in *Sorghum*
465 *bicolor* (L. Moench). *J. Microbiol. Methods* 112, 104–117.

466 doi:10.1016/j.mimet.2015.03.012

467 Kim, M.K., Joo, E.S., Lee, S.Y., Lee, D.S., Srinivasan, S., Jung, H.Y., 2016. Complete
468 genome sequence of *Hymenobacter* sp. DG25B, a novel bacterium with gamma-
469 radiation resistance isolated from soil in South Korea. *J. Biotechnol.* 217, 98–99.
470 doi:10.1016/j.jbiotec.2015.11.015

471 Klassen, J.L., Foght, J.M., 2011. Characterization of *Hymenobacter* isolates from Victoria
472 Upper Glacier, Antarctica reveals five new species and substantial non-vertical
473 evolution within this genus. *Extremophiles* 15, 45–57. doi:10.1007/s00792-010-0336-1

474 Laforest-Lapointe, I., Messier, C., Kembel, S.W., 2016. Host species identity, site and time
475 drive temperate tree phyllosphere bacterial community structure. *Microbiome* 4, 27.
476 doi:10.1186/s40168-016-0174-1

477 Lee, J.J., Srinivasan, S., Lim, S., Joe, M., Lee, S.H., Kwon, S.A., Kwon, Y.J., Lee, J., Choi,
478 J.J., Lee, H.M., Auh, Y.K., Kim, M.K., 2014. *Hymenobacter swuensis* sp. nov., a
479 gamma-radiation-resistant bacteria isolated from mountain soil. *Curr. Microbiol.* 68,
480 305–310. doi:10.1007/s00284-013-0478-3

481 Lighthart, B., 2006. The ecology of bacteria in the alfresco atmosphere. *FEMS Microbiol.*
482 *Ecol.* 23, 263–274. doi:10.1111/j.1574-6941.1997.tb00408.x

483 Lindow, S.E., Brandl, M.T., 2003. Microbiology of the Phyllosphere. *Appl. Environ.*
484 *Microbiol.* 69, 1875–1883. doi:10.1128/AEM.69.4.1875

485 Lympelopoulou, D.S., Adams, R.I., Lindow, S.E., 2016. Contribution of vegetation to the
486 microbial composition of nearby outdoor air. *Appl. Environ. Microbiol.* 82, 3822–3833.
487 doi:10.1128/AEM.00610-16

488 Maignien, L., DeForce, E.A., Chafee, M.E., Eren, A.M., Simmons, S.L., 2014. Ecological
489 succession and stochastic variation in the assembly of *Arabidopsis thaliana* phyllosphere
490 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 0.1016/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

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

518 Nie, Y., Chi, C.-Q., Fang, H., Liang, J.-L., Lu, S.-L., Lai, G.-L., Tang, Y.-Q., Wu, X.-L.,
519 2014. Diverse alkane hydroxylase genes in microorganisms and environments. *Sci. Rep.*
520 4, 4968. doi:10.1038/srep04968

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

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

526 Oksanen, J., Kindt, R., Legendre, P., O’Hara, B., Simpson, G.L., Solymos, P., Stevens,
527 M.H.H., Wagner, H., 2009. *Vegan: Community Ecology Package*. R package version
528 1.15-3.

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

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

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

538 Radchenkova, N., Vassilev, S., Panchev, I., Anzelmo, G., Tomova, I., Nicolaus, B.,
539 Kuncheva, M., Petrov, K., Kambourova, M., 2013. Production and properties of two
540 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 *kauolensis* 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.

591 doi:10.1038/nrmicro2910

592 Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., Mitchell-Olds, T.,
593 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial
594 plant. *Nat. Commun.* 7, 12151. doi:10.1038/ncomms12151

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

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

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

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

608 Wu, Y., Wang, Y., Li, J., Hu, J., Chen, K., Wei, Y., Bazhanov, D.P., Bazhanova, A.A., Yang,
609 H., 2015. Draft Genome Sequence of *Stenotrophomonas maltophilia* Strain B418 , a
610 Promising Agent for Biocontrol of Plant Pathogens and Root-Knot Nematode 3, 1998–
611 1999. doi:10.1128/genomeA.00015-15

612 Yang, C.H., Crowley, D.E., Borneman, J., Keen, N.T., 2001. Microbial phyllosphere
613 populations are more complex than previously realized. *Proc. Natl. Acad. Sci. U. S. A.*
614 98, 3889–3894. doi:10.1073/pnas.051633898

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

616 species for controlling PM2.5 pollution. *Atmos. Pollut. Res.* 6, 267–277.
617 doi:10.5094/apr.2015.031

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

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

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

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

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

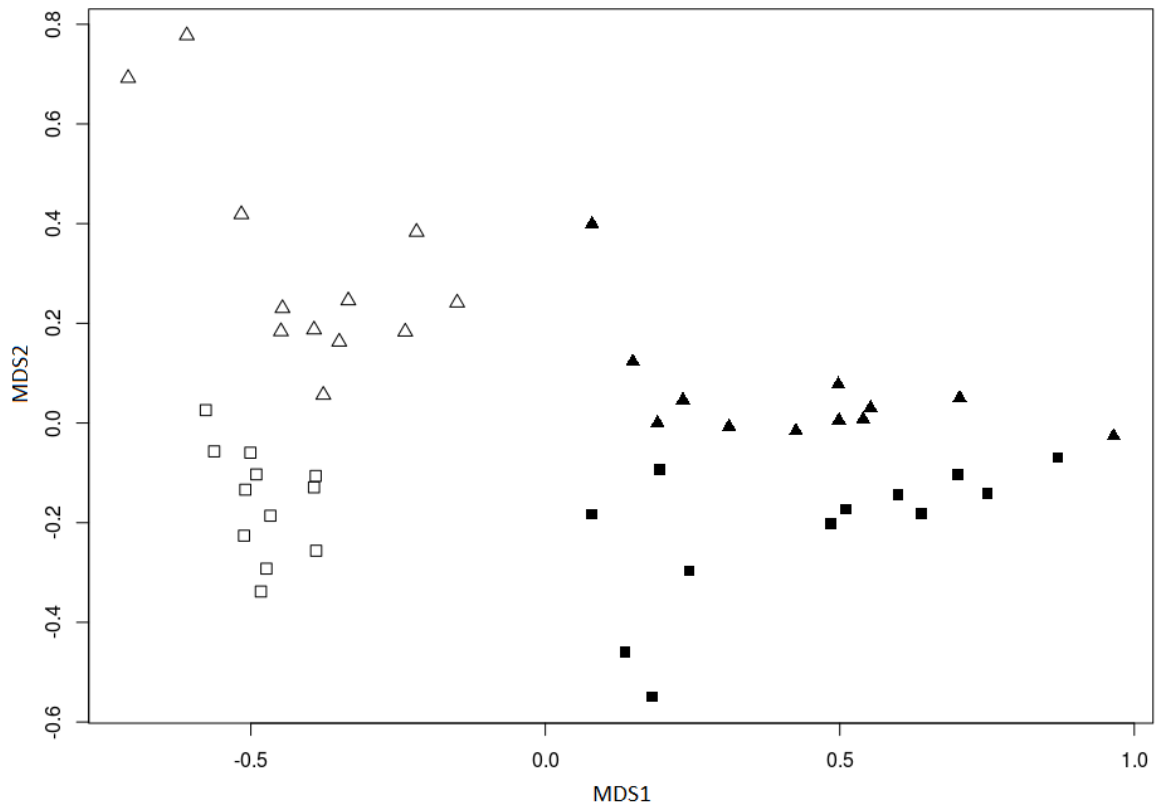
634 Zhang, H., Chen, S., Huang, T., 2015. Structure and Functional Metabolism of Bacterial
635 Communities on Leaves of Typical Urban Greening Tree Species. *Polish J. Environ.*
636 *Stud.* 24, 823–828. doi:10.15244/pjoes/30592

637 Zhao, M., Kong, Z., Escobedo, F.J., Gao, J., 2010. Impacts of urban forests on offsetting
638 carbon emissions from industrial energy use in Hangzhou, China. *J. Environ. Manage.*
639 91, 807–13. doi:10.1016/j.jenvman.2009.10.010

640 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

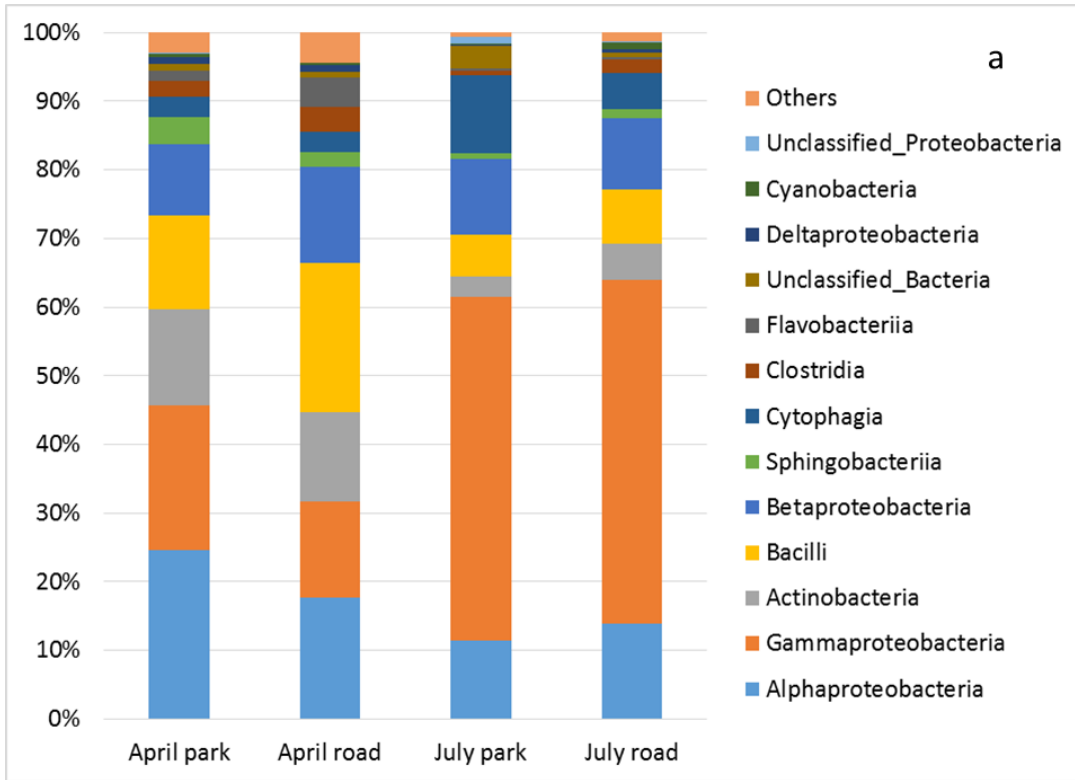
643



644

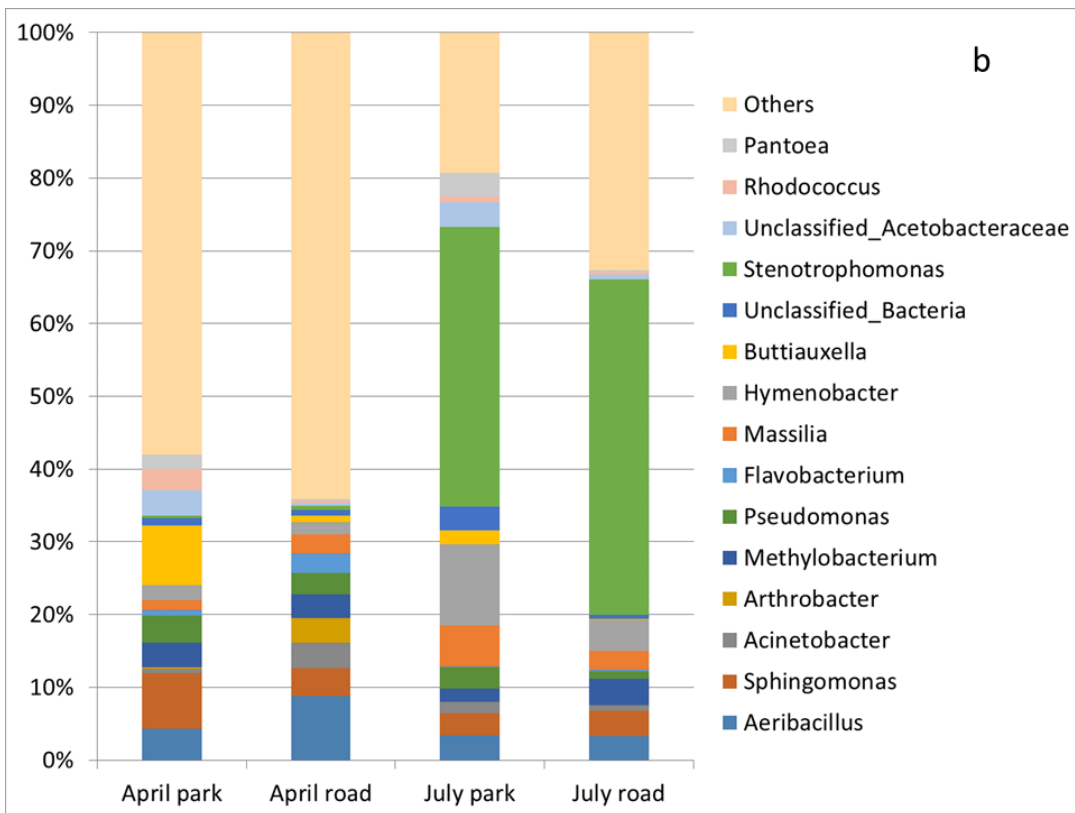
645 Fig. 1 – NMDS analysis of bacterial phylogenetic diversity. Hellinger distances among
 646 samples were calculated on the basis of presence and abundance of OTUs. Empty symbols:
 647 April; filled symbols: July; squares: park; triangles: road.

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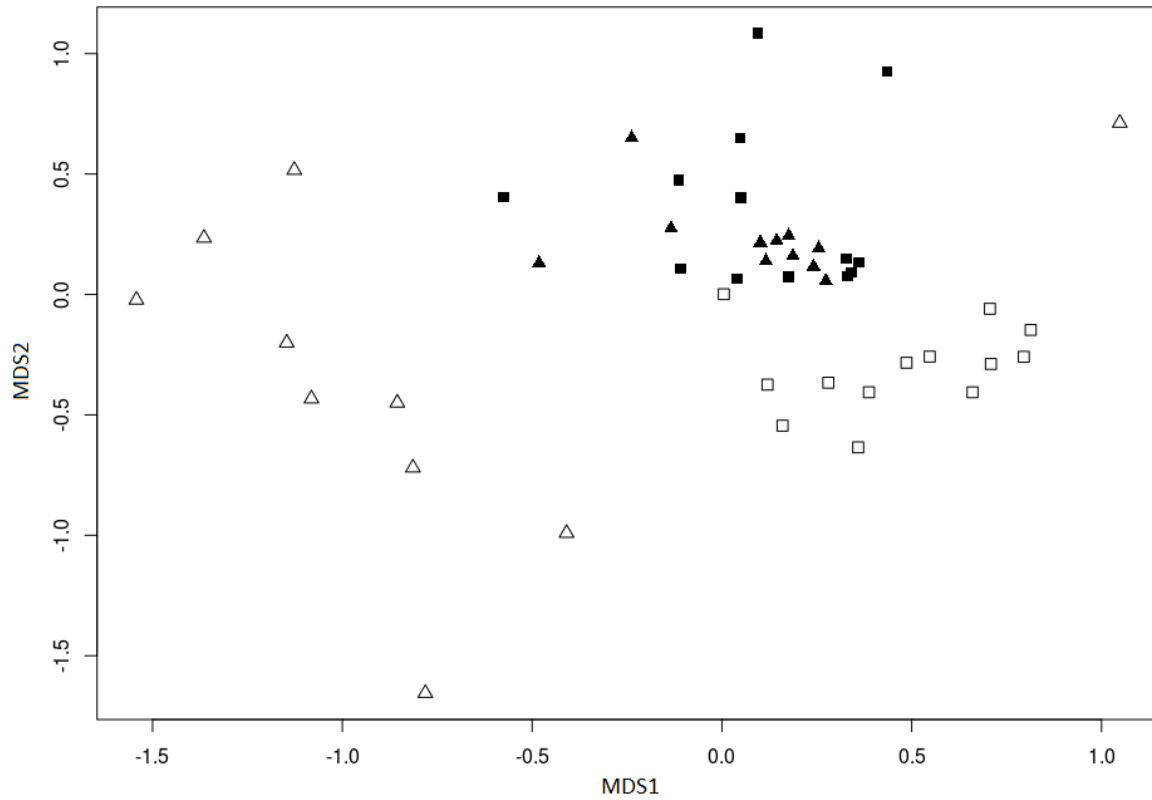
649

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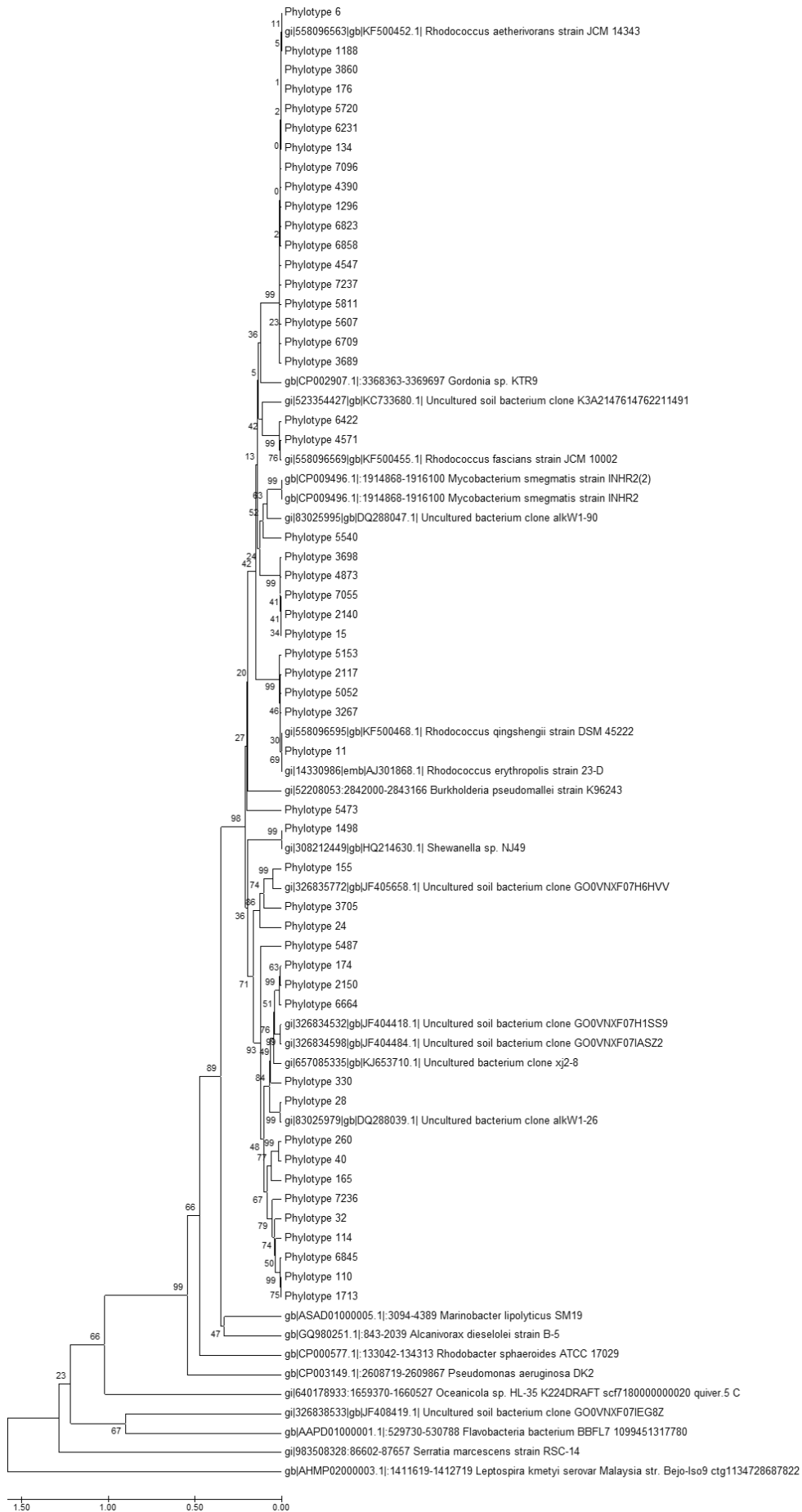
652 Fig. 2 – Relative abundance of phyllospheric bacterial taxa at Class (a) and Genus (b) level.
653 Only taxa with an abundance $\geq 1\%$ (Class) or $\geq 2\%$ (Genus) in at least one of the four groups
654 of samples are shown. Samples are grouped according to month and sampling location.
655



656

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

660



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.