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## Analysis of interdomain taxonomic patterns in urban street mats

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1                   **Analysis of interdomain taxonomic patterns in urban street mats**

2

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4

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12

13

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15

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18

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22

23 **Originality-Significance Statement**

24 This study explores at the city-scale, the microbial assemblage of the communities forming  
25 mats in the street gutters of Paris, France. High-throughput sequencing of both bacterial and  
26 eukaryotic amplicons combined with statistical analyses revealed the importance of  
27 connectivity and co-occurrences in structuring these urban microbiomes while abiotic factors  
28 such as pH and conductivity appeared to be less important drivers. Various taxa were identified  
29 including primary producers, saprotrophic, symbiotic and parasitic organisms as well as bacteria  
30 involved in nutrient cycling. The results of this study highlight the importance and the diversity  
31 of street microbes, support the need to monitor this compartment but also to bring together  
32 urban biodiversity and waste management services, in order to elaborate action plans for cities.

33

## 34 **Summary**

35 Streets are constantly crossed by billions of vehicles and pedestrians. Their gutters which  
36 convey stormwater and contribute to waste management, and are important for human health  
37 and well-being, probably play a number of ecological roles. Street surfaces may also represent  
38 an important part of city surface areas. To better characterize the ecology of this yet poorly  
39 explored compartment, we used filtration and DNA metabarcoding to address microbial  
40 community composition and assembly across the city of Paris, France. Diverse bacterial and  
41 eukaryotic taxonomic groups were identified, including members involved in key  
42 biogeochemical processes, along with a number of parasites and putative pathogens of human,  
43 animals and plants. We showed that the *beta* diversity patterns between bacterial and  
44 eukaryotic communities were correlated, suggesting interdomain associations. *Beta* diversity  
45 analyses revealed the significance of biotic factors (cohesion metrics) in shaping gutter  
46 microbial community assembly and, to a lesser extent, the contribution of abiotic factors (pH  
47 and conductivity). Co-occurrences analysis confirmed contrasting nonrandom patterns both  
48 within and between domains of life, specifically when comparing diatoms and fungi. Our results  
49 highlight microbial coexistence patterns in streets and reinforce the need to further explore  
50 biodiversity in urban ground transportation infrastructures.

## 51 **Introduction**

52 Understanding urban microbiome structure might have implications on human health and risk  
53 management (e.g., in indoor environments including hospital and mass transit systems, urban  
54 recreational water, etc.) and promote our understanding on the impacts of long-term  
55 anthropogenic pressure on environmental resources (Khreis et al., 2016; Shamarina et al.,  
56 2017). Microbial diversity and species assemblages have been shown to respond to  
57 urbanization and to be largely influenced by site characteristics, land cover or architectural  
58 design (Kembel et al., 2012). Deciphering the microbiome compositions in transportation  
59 systems remains a key issue for urban development and city sustainability. In this respect,  
60 studies have reported microbial biodiversity in train stations (Dong and Yao, 2010; Patel et al.,  
61 2018) or subway systems (Dybwad et al., 2012; Dybwad et al., 2014; Leung et al., 2014;  
62 Afshinnkoo et al., 2015; Hsu et al., 2016; Fan et al., 2017). However, the street network  
63 system, which receives and conveys oil and other engine fluids that spill from transportation,  
64 dust, and atmospheric pollutants, along with human litter and waste, has surprisingly received  
65 much less attention (Janke et al., 2017).

66

67 Studies have reported the substantial roles of streets in conveying runoff and discharge of  
68 environmental, fecal and antibiotic-resistant bacteria to various surrounding water systems,  
69 such as streams (Baral et al., 2018), lakes (Zhang et al., 2016), waterways (Calderon et al., 2017)  
70 or groundwater (Voisin et al., 2018). Analyses of soils from a motorway or highly trafficked road  
71 in Copenhagen have revealed the presence of microbial polycyclic aromatic hydrocarbon  
72 degraders (Johnsen et al., 2006; Johnsen et al., 2014). An analysis of urban soils from the street

73 green belts of the city of Chicago, USA, revealed that soils along a one-way street showed  
74 higher Shannon and phylogenetic diversities than other soils, including those along a two-way  
75 street, a feature that could be correlated with soil pH, moisture, and texture (Wang et al.,  
76 2018). We believe that the microorganisms living in the street, in street gutters and on  
77 pedestrian surfaces may present specific surface-associated diversity, such as the ones  
78 identified on concrete (Domingo et al., 2011; Li et al., 2012; Gomez-Alvarez et al., 2015; Jiang et  
79 al., 2016; Cayford et al., 2017; Cowle et al., 2017; Li et al., 2017) or buildings and monuments  
80 (Ragon et al., 2011; Chimienti et al., 2016; Gaylarde et al., 2017; Adamiak et al., 2018; Dyda et  
81 al., 2018).

82  
83 Because surfaces are important to the interaction between bacteria and microeukaryotes,  
84 addressing the biodiversity and identifying the keystone species present in the street systems,  
85 including the ones made in concrete, stone and bitumen, is essential. In a previous study, we  
86 analyzed the eukaryotic diversity from street gutters biofilms/mats and found a gutter-specific  
87 diversity (Hervé et al., 2018). Our study aimed here at: *(i)* identifying microeukaryote-associated  
88 bacteria in street gutter biofilms, *(ii)* describing bacteria-eukaryote association patterns, *(iii)*  
89 identifying the factors structuring these complex microbial communities, and *(iv)* identifying  
90 potential keystone taxa in these biofilms. Because of the physical structure of these  
91 environmental biofilms, we hypothesized that biotic factors and microbial co-occurrences will  
92 play a significant role in the street gutter assembly.

93

94 To focus on the bacterial communities that are specifically associated with  
95 microeukaryotes, we used a size fractionation approach. We expected that such procedure  
96 would give valuable information on resident bacteria along with the ecto- and endosymbiotic  
97 bacteria. Co-occurrence analysis was used to determine the intra- and interdomain (hereafter  
98 referring to domains of life) interactions and revealed different positive and negative patterns  
99 for bacteria alone, eukaryotes alone or eukaryotes and bacteria. To predict “keystone species”  
100 in gutter biofilms/mats, we used a cohesion algorithm (Herren and McMahon, 2017, 2018).  
101 Based on the biodiversity found in the street gutters, we discuss potential functional roles for  
102 these urban microbiomes.

103

104

## 105 **Results**

### 106 **Complexity of the urban street gutter microbiome**

107 Our aim was to understand the microbial community composition in street gutter biofilms, to  
108 infer putative interactions and interspecific cooperation between microeukaryotes (protists and  
109 Fungi) and bacteria as well as to identify taxa that might be involved in primary production,  
110 nutrient recycling or that might correspond to putative pathogens. Because we were interested  
111 in microeukaryote-bacterial interactions, the 60 gutter biofilms/mats sampled in Paris were  
112 filtered through a 5 µm membrane. We extracted the environmental DNA from the filter  
113 membrane and created two independent amplicon libraries for the 16S rRNA and 18S rRNA  
114 encoding genes. After amplicon sequencing, followed by sequence processing (see

115 Experimental procedures), 3,981 OTUs and 5,899 OTUs were obtained for the 18S and 16S rRNA  
116 gene library, respectively.

117  
118 The eukaryotes present in the street gutters were assigned to 9 taxonomic groups and  
119 to unclassified-eukaryotes. The highest richness was found among the Stramenopile (1,324  
120 OTUs, including 764 OTUs belonging to the Bacillariophyta clade), followed by the Opisthokonta  
121 (938 OTUs, among which we found 837 fungal OTUs), Rhizaria (461 OTUs), Alveolata (364  
122 OTUs), and Archaeplastida (360 OTUs), and then by the Amoebozoa (79 OTUs), Hacrobia (63  
123 OTUs) and Apusozoa (8 OTUs) (Fig. 1A). However, both the abundance and the diversity showed  
124 large variations depending on the sampling site, with some sites specifically rich in Fungi and  
125 others in Bacillariophyta (Supplementary Fig. S1). We also observed that Ciliophora,  
126 Chlamydomonadales, Fungi, Glissomonadida, Chrysophyceae and Oomycota were present in all  
127 samples. Within the fungal kingdom, Chytridiomycota was the most prevalent group, being  
128 present in 59 out of the 60 samples.

129  
130 The bacterial OTUs were assigned to 35 phyla and candidate phyla (Fig. 1B). The  
131 Proteobacteria phylum was the richest (2,097 OTUs, and 55.9 % of the total abundance) and  
132 was composed, in decreasing order, of Alphaproteobacteria (738 OTUs, 27.4 %),  
133 Deltaproteobacteria (509 OTUs, 1.9 %), Betaproteobacteria (411 OTUs, 13.7 %),  
134 Gammaproteobacteria (394 OTUs, 10.7 %) and Epsilonproteobacteria (8 OTUS, 0.6 %). The  
135 other dominant phyla corresponded to Bacteroidetes (1,284 OTUs, 24.7 % total abundance),  
136 Cyanobacteria (4.8 %), Actinobacteria (4.4 %), Firmicutes (1.8 %), Acidobacteria (1.3 %),

137 Deinococcus-Thermus (1.3 %) and Verrucomicrobia (1.3 %). Members of the bacterial genera  
138 *Flavobacterium*, *Saccharibacteria* and *Hymenobacter* were present in all samples, suggesting a  
139 ubiquitous distribution of these bacteria in Parisian street gutters. In contrast with the 18S  
140 rRNA gene library, the abundance profiles at the bacterial phylum-level showed low variability  
141 among the 60 samples (Supplementary Fig. S1).

142

### 143 **Diversity patterns of eukaryotic and bacterial communities**

144 In the street gutters, bacterial richness corresponded on average to 657 OTUs, ranging from  
145 210 to 1,246 OTUs. Eukaryotic richness was lower (mean value = 210 OTUs), ranging from 96 to  
146 548 OTUs. Overall, bacterial richness was significantly higher than the eukaryotic richness  
147 across the samples (Wilcoxon test,  $p$ -value  $< 2.2e^{-16}$ ) (Fig. 2A).

148

149       Regarding the *beta* diversity, dissimilarity based on Canberra distances was significantly  
150 higher in eukaryotic than in bacterial communities (Wilcoxon test,  $p$ -value  $< 2.2e^{-16}$ ), suggesting  
151 more divergence in the eukaryotic community composition between the microbial mats  
152 compared to the bacterial community composition (Fig. 2B). A strong and positive correlation  
153 between eukaryotic and bacterial *beta* diversities was observed (partial Mantel test accounting  
154 for geographic distances,  $r_M = 0.658$ ,  $p$ -value  $< 1.10^{-5}$ ), indicating that among the microbial  
155 mats, changes in eukaryotic and bacterial community composition were correlated and  
156 followed a similar trend. At the scale of the city, no significant correlation was found between  
157 the microbial community composition and the geographic distances (partial Mantel test,

158 accounting for water chemistry variables,  $r_M = 0.054$ ,  $p$ -value = 0.1 for the eukaryotes and  $r_M =$   
159 0.033,  $p$ -value = 0.4 for the bacteria) (Supplementary Fig. S2).

160

### 161 **Factors shaping the street gutter microbiome**

162 Subsequently, factors shaping the street gutter microbiome were investigated. Because of the  
163 observed correlation between *beta* diversity metrics, the contribution of biotic factors was  
164 suspected. Thus, we used a recently developed statistical method named cohesion to quantify  
165 the degree of connectivity (positive and negative cohesions) (Herren and McMahon, 2017).  
166 Additionally, we evaluated the influence of two important physico-chemical parameters,  
167 namely pH and conductivity, on the microbial community composition. Multivariate analysis of  
168 deviance of the GLMs revealed similar trends for the effects of biotic and abiotic factors on  
169 bacterial and eukaryotic community composition (Table I). The two-cohesion metrics ( $p$ -value <  
170 0.005), conductivity ( $p$ -value < 0.005) and pH ( $p$ -value < 0.05) had a significant effect on  
171 community composition. The contribution of pH (Dev = 1960 and Dev = 726 for bacterial and  
172 eukaryotic community, respectively) to community composition was always lower than the  
173 contribution of conductivity (Dev = 2635 and Dev = 1061 for bacterial and eukaryotic  
174 community, respectively). However, in the present dataset, biotic factors (positive and negative  
175 cohesions) always show a much higher explained deviance compared to the tested abiotic ones,  
176 indicating that cohesion explains more of the variation than pH and conductivity.

177

178 Regarding the cohesion metrics, we observed different patterns between bacterial and  
179 eukaryotic communities. For bacteria, positive cohesion (Dev = 7,639) appears to be more

180 important than negative cohesion (Dev = 5,410) in explaining community composition.  
181 Opposite results were observed for the eukaryote where negative cohesion (Dev = 2,027)  
182 contributed more than positive cohesion (Dev = 1,781), suggesting that connectivity patterns  
183 differ between bacteria and eukaryotes. The same analysis was performed after merging both  
184 the bacterial and eukaryotic OTU matrices. The four variables remained significant, with biotic  
185 factors (Dev = 8,040 and Dev = 5,349 for positive and negative cohesion, respectively) still  
186 showing higher explained deviance compared to abiotic factors (Dev = 2,567 and Dev = 3,420  
187 for pH and conductivity, respectively).

188

### 189 **Bacterial and eukaryotic OTU co-occurrences**

190 Because biotic factors appeared to be the highest driving forces in the street gutter  
191 microbiome, we computed co-occurrence probabilities to identify co-occurring taxa (see  
192 Experimental procedures). We found 228,695 positive and 83,749 negative significant co-  
193 occurrences, representing 16.2 % of the nonrandom co-occurrences. To gain insight into the  
194 intra- and interdomain co-occurrence patterns (eukaryote-eukaryote, eukaryote-bacteria and  
195 bacteria-bacteria), we then inspected these co-occurrences at bacterial phylum and eukaryotic  
196 clade level (Supplementary Fig. S3, S4 and S5). *Circos* visualizations revealed that in several  
197 clades, including Apusozoa, Bacillariophyta, Alveolata or Rhizaria, the patterns of positive and  
198 negative co-occurrences can be significantly different for their eukaryotic-eukaryotic  
199 interactions (Supplementary Fig. S3). Among the bacteria-bacteria interactions, we see a  
200 general pattern in the much higher number of intra-phylum positive co-occurrences compared  
201 to the negative ones, which might indicate synergistic relationships (Supplementary Fig. S5).

202

203 We then focused on the co-occurrences associated with the two eukaryotic clades that  
204 present the highest diversity and abundances. For the Bacillariophyta, the highest number of  
205 positive co-occurrences was with themselves (71.2 %), followed by the ones with  
206 Archaeplastida (7.5 %), other Stramenopiles (5.4 %) and Fungi (4.6 %), while their negative co-  
207 occurrences were with Fungi (44.0 %), other Stramenopiles (17.3 %), Rhizaria (13.8 %) and  
208 Alveolata (12.7 %) (Fig. 3A, left panel). For the Fungi, we found 2.9 % of their positive co-  
209 occurrences and 55.3 % of their negative ones with Bacillariophyta (Fig. 3A, right panel),  
210 suggesting interferences or competition for space and/or resources in street gutter  
211 biofilms/mats. Looking at the co-occurrences with bacteria, the Bacillariophyta presented  
212 17,765 positive and 4,945 negative co-occurrences, whereas the Fungi presented 11,455  
213 positive and 13,648 negative co-occurrences. For these two clades, the highest number of  
214 positive and negative co-occurrences was with the Proteobacteria (Bacillariophyta: 38.2 %  
215 positive and 39.3 % negative; Fungi: 31.0 % positive and 40.2 % negative), followed by the  
216 Bacteroidetes (Bacillariophyta: 28.0 % positive and 30.0 % negative; Fungi: 29.1 % positive and  
217 34.2 % negative) (Fig. 3B). For the other bacterial phyla, we found that most of them (except  
218 Firmicutes, Fusobacteria and Saccharibacteria) showed more positive than negative co-  
219 occurrences with Bacillariophyta. The Fungi presented more negative than positive co-  
220 occurrences with most of the phyla, except for the Actinobacteria, Chroroflexi and FBP (Fig. 3B).  
221 At the order level, the main differences in co-occurrences between the bacteria and diatoms  
222 were for the OPB35\_soil\_group (Verrucomicrobia), SC-I-84 (Betaproteobacteria) and OM190  
223 (Planctomycetes), with ratios of positives/negatives co-occurrences of 293, 164 and 134,

224 respectively. The other orders, such as Aeromonadales (Gammaproteobacteria) and  
225 Corynebacteriales (Actinobacteria), showed more negative than positives co-occurrences with  
226 ratios of 0.019 (2/103) and 0.044, respectively. Between the bacteria and Fungi, the main  
227 differences were for Frankiales (Actinobacteria) and Longimicrobiales (Gemmatimonadetes)  
228 with ratios of 235 or 63, respectively, whereas Bradymonadales (Deltaproteobacteria), OM190  
229 (Planctomycetes), and Chromatiales (Gammaproteobacteria) showed more negative co-  
230 occurrences, with ratios of 0.037, 0.041, and 0.047, respectively. Altogether, these analyses  
231 demonstrated differences in intra- and interdomain co-occurrence patterns.

232

### 233 ***Identification of highly connected taxa***

234 The cohesion algorithm mentioned above was also used to identify the highly connected OTUs  
235 within the gutter microbial mats, which can be assimilated as keystone taxa (Herren and  
236 McMahon, 2018). Because both positive and negative cohesions had a significant impact on the  
237 microbial community composition (Table I), the 15 bacterial and eukaryote OTUs presenting the  
238 highest positive or negative connectedness were further investigated. In fact, since an OTU  
239 could be ranked among the 15 most positive but also among the 15 most negative  
240 connectedness values, we identified 25 OTUs for the eukaryotes (10 positives, 10 negatives and  
241 5 OTUs that were among the most positive and negative) and 28 OTUs for the bacteria (13  
242 positives, 13 negatives and 2 OTUs that were among the positive and negative) as potential  
243 keystone taxa. When necessary, the taxonomy assignment of some of the most connected  
244 OTUs was improved by BLASTn analyses and phylogenetic trees reconstruction.

245

246 The data presented in Fig. 4A revealed that the eukaryotes presenting the highest positive  
247 and lowest negative connectedness values corresponded to Fungi (these included eight OTUs  
248 from the Ascomycota lineage and four Chytridiomycota, two Basidiomycota and one  
249 Blastocladiomycota), Bacillariophyta (three OTUs assigned to the genus *Melosira*), Cercozoa (six  
250 members of the class Sarcomonadea), and Pythiales (the single OTU corresponded to the genus  
251 *Pythium*). For the bacteria, we identified thirteen members of the Proteobacteria lineage (eight  
252 Alphaproteobacteria, two Betaproteobacteria, one Deltaproteobacteria, one  
253 Gammaproteobacteria and one unclassified with a best BLASTn corresponding to the genus  
254 *Paracoccus*), eight Bacteroidetes (four Sphingobacteriales, two Cytophagales, one  
255 Flavobacteriales and one Bacteroidetes Order II. *incertae sedis* likely to correspond to the order  
256 Rhodothermales), three Planctomycetes (two from the family Planctomycetaceae and one with  
257 best blast to uncultured bacterium), two Actinobacteria, one Acidobacteria and one  
258 Verrucomicrobia (Fig. 4B).

259

260

## 261 Discussion

262 Even if more data are needed on the total surface area covered by streets in cities, existing  
263 evaluations vary from 10 to 50 %, with about 25 % for Paris. Depending on the city size, the  
264 street transportation systems correspond to hundreds to thousands of kilometers. Therefore,  
265 the number of microorganisms living worldwide in streets and street gutters is likely to be  
266 enormous and undoubtedly these organisms might play fascinating roles in urban  
267 environments including in food webs, biogeochemical cycles, pollutants detoxification, or waste

268 and stormwater treatments. Additionally, these microorganisms should require further  
269 attention for human health and well-being concerns.

270

### 271 *Biofilm assembly in gutter mats*

272 Using a 5  $\mu\text{m}$  filtration procedure to enrich the bacterial fraction that was closely associated  
273 with the eukaryotes from sixty gutter biofilms sampled across Paris, we describe here the  
274 molecular diversity of both bacteria and eukaryotes via a metabarcoding approach (Fig. 1). A  
275 comparison of the *beta* diversity of these two domains of life revealed a positive and significant  
276 correlation. We also found that both the street water pH and conductivity had a significant  
277 effect on bacterial and eukaryotic community composition (Table I). Water chemistry is known  
278 to have an impact on aquatic biofilm community structure (Besemer, 2015). At the city scale,  
279 we did not observe any significant correlation between microbial community dissimilarity and  
280 geographical distance (Supplementary Fig. S2), indicating no spatial autocorrelation (see:  
281 (Hanson et al., 2012)). This suggests no dispersal limitation among the street gutter microbial  
282 communities of Paris. Microbial dispersal could be facilitated by rains, water transport, wind  
283 and cleaning processes (i.e., street sweeping and washing and vacuum vehicles).

284

285       Regarding the factors shaping the microbial *beta* diversity, the multivariate analysis of  
286 deviance of GLMs indicated that the biotic factors (cohesion metrics) explained more variation  
287 than the abiotic factors (pH and conductivity). Nonetheless, further studies should include  
288 measurements of more abiotic variables to confirm our results. The importance of cohesion,  
289 *i.e.*, the degree of connectivity of a microbial community, might be explained by the physical

290 structure of the microbial community being sampled. Within a biofilm, microorganisms are  
291 enclosed in a matrix containing extracellular polymeric substances (Battin et al., 2016). These  
292 microbial aggregates present enhanced intercellular communications and a higher level of  
293 organization compared to single cells (Flemming and Wuertz, 2019). Overall, supported by  
294 three different statistical approaches (Mantel test, co-occurrences and cohesion analyses), our  
295 study highlights the importance of microbial associations in street gutters.

296

### 297 *Bacteria and eukaryotes diversity in biofilms/mats from street gutters*

298 The 5,899 bacterial OTUs were assigned to 35 phyla and candidate phyla, in decreasing order  
299 beginning with the Proteobacteria (55.9 % of the total abundance), followed by the  
300 Bacteroidetes (24.7 %), Cyanobacteria (4.8 %), Actinobacteria (4.4 %), Firmicutes (1.8 %),  
301 Acidobacteria (1.3 %), Deinococcus-Thermus (1.3 %) and Verrucomicrobia (1.3 %). The  
302 Alphaproteobacteria, which corresponded to 27.4 % of the total abundance, was dominated by  
303 the order Sphingomonadales (58.8 % of the relative abundance within this class). Interestingly,  
304 among the 126 Sphingomonas-related OTUs, three of them could be defined as keystone  
305 species (see below). This bacterial order that presents interesting metabolic pathways,  
306 including carotenoid pigment synthesis (Siddaramappa et al., 2018), was found in various  
307 environments from the caves (Marques et al., 2019) to thermal springs (Pedron et al., 2019)  
308 and clouds (Amato et al., 2017), and recently, in the drinking water distribution system in Paris  
309 (Perrin et al., 2019). Microbial biofilm diversity and community composition have been  
310 addressed in several urban drinking and draining water distribution systems, and enrichments  
311 of specific communities within biofilms have been reported (Bertelli et al., 2018; Bruno et al.,

312 2018; Douterelo et al., 2018; Douterelo et al., 2019; McLellan and Roguet, 2019). Interestingly,  
313 in the street gutter biofilms/mats, we identified 21 OTUs (three *Arcobacter*, twelve  
314 *Acinetobacter*, five *Aeromonas* and one *Trichococcus*) from genera that were previously shown  
315 to be dominant in sewer pipe (McLellan and Roguet, 2019). Among these OTUs, five of them  
316 (one *Arcobacter*, two *Acinetobacter*, one *Aeromonas* and one *Trichococcus*) were among the 50  
317 most abundant OTUs in our dataset.

318  
319 We also identified several bacteria that could correspond to potential opportunistic  
320 pathogens for human, animals or plants. In particular, we found OTUs assigned to the  
321 Enterobacteriaceae family (i.e., *Escherichia-Shigella*, *Serratia*, *Pectobacterium*, *Enterobacter*,  
322 *Dickeya*, etc.), Legionellaceae (i.e., 16 OTUs of the genus *Legionella*), Pseudomonadaceae (i.e.,  
323 17 OTUs of the genus *Pseudomonas*), Aeromonadaceae (i.e., three rare and five abundant OTUs  
324 of the genus *Aeromonas*), Streptococcaceae (i.e., *Streptococcus*), Enterococcaceae (i.e.,  
325 *Enterococcus*), Mycobacteriaceae (i.e., *Mycobacterium*), Dietziaceae (i.e., *Dietzia*) and  
326 Corynebacteriaceae (i.e., *Corynebacterium*). Furthermore, we found that the Rickettsiales  
327 order, harboring parasitic and mutualistic intracellular bacteria, was prevalent in the gutter  
328 biofilms, with 97 OTUs present in 90 % of the samples. Within this order, three OTUs belonging  
329 to the genus of *Rickettsia* were identified. Many members of this latest genus are arthropod  
330 endosymbionts and include some species that are causative agents of human diseases such as  
331 typhus and spotted fever (Perlman et al., 2006). Four OTUs were assigned to *Candidatus*  
332 *Odyssella*, a bacterial genus with members that live intracellularly in Amoebozoa (Birtles et al.,  
333 2000). We are aware that to further characterize the presence of putative pathogens in the

334 street surfaces, the complete bacterial community should be analyzed (using 0.2  $\mu\text{m}$  filters),  
335 along with the virome (Fresia et al., 2019).

336  
337 Among the 3,981 eukaryotic OTUs we identified putative symbiotic eukaryotes. Among  
338 them, six OTUs were assigned to Glomeromycota, which forms arbuscular mycorrhizas with the  
339 roots of many land plants. Most likely, these sequences were amplified from fungal spores  
340 which would indicate that gutters could act as vector. A number of known plant and algae  
341 (including diatoms) parasites belong to the Oomycetes clade (Kuhn and Hofmann, 1999; Scholz  
342 et al., 2016; Garvette et al., 2018; Leonard et al., 2018). Among the Oomycetes, 14 OTUs  
343 assigned to the *Phytophthora* genus were identified in eighteen samples. This genus is  
344 composed exclusively of plant pathogens targeting a wide range of hosts. Thus, street gutters  
345 might act as a reservoir of plant pathogens. We also found a keystone species belonging to the  
346 *Pythium* genus, along with a number of other parasite-related sequences, including from  
347 *Pirsonia* and “pseudofungus,” such as *Hyphochytrium*. Finally, regarding the fungal diversity,  
348 239 out of 837 fungal OTUs were assigned to unclassified fungi, representing a potential  
349 fraction of fungal dark matter (Grossart et al., 2016) in urban environments and thus, making  
350 street gutters potential hotspots of hidden diversity.

351

352 *Keystone taxa in street gutters and co-occurrences between bacteria and eukaryotes*

353 As mentioned above, cohesion metrics were used to identify the most highly connected OTUs,  
354 which might correspond to potential keystone taxa that influence community structure (Herren  
355 and McMahon, 2018). Among the 53 keystone OTUs that we identified, three of them

356 correspond to a primary producer, and in particular to the diatom genus *Melosira*, further  
357 confirming the importance of diatoms in street gutters (Hervé et al., 2018). Indeed, among the  
358 100 most abundant OTUs, 26 corresponded to this clade with 24 related to raphid-pennate  
359 species and two to *Melosira* (abundance ranked 1<sup>st</sup> and 20<sup>th</sup>). Even if the dominance of pennate  
360 diatoms in fouling biofilm is a ubiquitous observation (see: (Richard et al., 2017; Zhang et al.,  
361 2017)), the presence of *Melosira*, a true cosmopolitan centric form capable of forming  
362 filaments, suggests that it might live and proliferate entangled within the urban biofilm  
363 matrices. Moreover, we found that diatoms present, mostly intraclade positive co-occurrences,  
364 and their negative co-occurrences were dominated by interactions with Fungi (Fig. 3A) and, in  
365 particular, with the most abundant and diverse subphylum, the Pezizomycotina. This observed  
366 pattern of negative fungi-diatoms associations could be explained by antagonistic interactions,  
367 such as allelopathic interactions between aquatic fungi and diatoms that have previously been  
368 reported and showed a negative impact of fungi on primary production (Allen et al., 2017).

369

370       Among the fifteen keystone OTUs related to Fungi, seven of them were related to  
371 Pezizomycotina (four Eurotiomycetes, two Dothideomycetes and one unclassified  
372 Pezizomycotina). BLASTn analyses revealed the proximity of these OTUs to *Bradomyces* and to  
373 *Knufia*, two fungal genera that have been found in various environments such as outdoor rocks  
374 or indoor granites surface (Reblova et al., 2016; Tesei et al., 2017). A putative member of the  
375 Cucurbitariaceae also corresponded to a keystone taxa, which suggests that members of this  
376 family usually regarded as saprotrophs or necrotrophs could play roles in street microbial  
377 community structure (Doilom et al., 2013; Jaklitsch et al., 2018).

378

379           Keystone taxa found here also belong to the Glissomonadida, an important cercozoan  
380 order of predominantly biflagellate gliding bacterivores. By recognizing the importance of  
381 micropredators in the structure of microbial communities, we can hypothesize that bacteria are  
382 used as food source in this environment and that Cercozoans play important roles in  
383 biofilms/mats formation (Cavalier-Smith and Chao, 2003). A putative phytopathogen belonging  
384 to the genus *Pythium* (best BLASTn hit) was also identified as a eukaryotic keystone taxon. The  
385 Pythiaceae that are well-known plant pathogens (Meng et al., 2009) with saprotrophic  
386 behavior, have been isolated from sediments and plant materials in decomposition in  
387 freshwater inland ecosystems (Kachour et al., 2016). Whatever their urban origin (i.e., plants  
388 covering green roofs and green walls, parking lot and sidewalk, etc.), Pythiaceae might  
389 contribute to plant organic matter decomposition in the street network system (also see  
390 below).

391

392           The 25 bacterial keystone taxa identified here belong to Proteobacteria (thirteen OTUs),  
393 Bacteroidetes (eight OTUs), Planctomycetes (three OTUs), Actinobacteria (two OTUs), one  
394 Acidobacteria, and one Verrucomicrobia (Fig. 4B). The presence of three keystone taxa  
395 belonging to Sphingomonadaceae (Proteobacteria, Alphaproteobacteria), a family that harbors  
396 aromatic hydrocarbon-degrading species (Shi et al., 2001; Marques et al., 2019; Sanchez-  
397 Gonzalez et al., 2019) suggests the need to develop studies on the mechanisms of aromatic  
398 compound biodegradation in the street gutters. Interestingly, the Sphingobacteriales (4  
399 keystone OTUs) correspond to another clade to investigate for putative roles in street gutter

400 bioremediation. For example, this order was found to be enriched in urban rivers influenced by  
401 wastewater treatment plant effluents (Drury et al., 2013).

402

403 As mentioned above, bacteria-eukaryote interactions are known to be important for the  
404 functioning of various ecosystems, and street gutters appear to be no exception. Indeed, co-  
405 occurrence analysis demonstrated preferential patterns that are phylum-specific, and in  
406 particular, the many nonrandom bacterial-fungal and bacterial-diatom co-occurrences (Fig. 3).  
407 As in freshwater or marine water environments, our data demonstrated that Proteobacteria  
408 and Bacteroidetes are the main bacterial phyla interacting with the diatoms; hence, these  
409 bacteria are expected to influence their growth, behavior and physiology in street biofilms  
410 (Amin et al., 2012; Buchan et al., 2014; Sison-Mangus et al., 2014; van Tol et al., 2017). The  
411 Cyanobacteria phylum (4.8 % of the total abundance) presented very few negative co-  
412 occurrences with the diatoms (493 positives and 29 negatives), a result which suggests a  
413 foreseeable association between diatoms and autotrophic nitrogen-fixing Cyanobacteria (Amin  
414 et al., 2012). We also found contrasting patterns in intra- and interphylum interactions when  
415 comparing diatoms and Fungi, especially for some order like the Planctomycete lineage OM190,  
416 Verrucomicrobia Subdivision 3, Aeromonadales, Corynebacteriales, Frankiales, Bradymonadales  
417 or Chromatidales. We believe that a number of these nonrandom species co-occurrence and  
418 enrichment patterns might correspond to differences in trophic level interactions between  
419 diatoms and Fungi with their associated microbial communities, for nutrient fluxes or energy  
420 production.

421

422 *Putative functional roles of microbes in street gutters*

423 While the present study is only based on small subunit rRNA gene amplicons functional  
424 inference can be proposed based on the presence of certain taxa. Regarding the carbon cycle,  
425 various phototrophic bacteria were observed, including purple sulfur bacteria (11 OTUs  
426 assigned to the genus *Rheinheimera*), purple non-sulfur bacteria (*Rhodobacter* and  
427 *Roseomonas*) and oxygenic photosynthetic Cyanobacteria (92 OTUs). Methylothrop  
428 (*Methyloversatilis* and *Methylobacterium*) and methane-oxidizing bacteria (*Candidatus*  
429 *Methylomirabilis*) were also detected. The presence of ammonia-oxidizing bacteria  
430 (*Nitrospira* and *Nitrosomonas*) and nitrite-oxidizing bacteria (*Candidatus* *Methylomirabilis*,  
431 and *Nitrospira*) suggest a role of gutter mats in nitrogen cycling. Similarly, the presence of iron-  
432 reducing (*Ferribacterium*) and iron-oxidizing bacteria (*Sideroxydans*) as well as sulfate-reducing  
433 bacteria (*Desulfomicrobium*, *Desulfobulbus*, *Desulfovibrio*, and *Desulfobacca*) are early evidence  
434 of the contribution of the gutter microbiome to urban nutrient cycling. Meanwhile, we also  
435 found OTUs related to bacteria that have been shown to be predominant in activated sludge  
436 and contribute to pollutant degradation such as members of the Nocardaceae (i.e., *Gordonia*,  
437 *Nocardia*, *Rhodococcus*, *Nocardioides*, etc.) or the Propionibacteriaceae (i.e., *Propioniciclava*).

438

439 Among the bacterial taxa present in all gutter samples, we found *Flavobacterium*,  
440 *Saccharibacteria* and *Hymenobacter*. *Saccharibacteria*, formerly known as Candidate phylum  
441 TM7, still lacks isolates from axenic culture. Thus, gutter mats could represent samples of  
442 choice for targeted isolation of this phylum. Regarding the richness of this clade in the gutter  
443 mats, 116 OTUs were assigned to this phylum, with an average of 11 OTUs per sample. This

444 phylum was also reported to be diverse in activated sludge, with some phylotypes able to  
445 utilize glucose, amino acids and N-acetylglucosamine (Kindaichi et al., 2016). The latter  
446 compound is likely to be abundant in the street gutters due to the abundant presence of fungi  
447 that possess chitin cell walls. Both *Flavobacterium* (165 OTUs identified) and *Hymenobacter* (82  
448 OTUs identified) belong to the Bacteroidetes phylum. Further research effort should focus on  
449 isolating and characterizing members of these two genera to better understand their metabolic  
450 potential and functional role in the streets. More generally, the taxonomic inventory generated  
451 in the present study could provide a foundation for investigating gutter functional ecology via  
452 both *in situ* and *in vitro* experiments to better understand the role of their microorganisms in  
453 the urban landscapes.

454

455

## 456 **Experimental procedures**

### 457 ***Field sampling strategy, DNA preparation and sequencing***

458 The sampling procedure, used herein to address the street gutter microbial communities in a  
459 metropolitan area of Paris, has been previously described (Hervé et al., 2018), except that here  
460 we focused on 60 gutter mats for which both the 16S rRNA and 18S rRNA genes could be  
461 successfully amplified. Briefly, samples were homogenized on a rotating wheel, prefiltered  
462 using a 300 µm mesh to remove putative debris, and then filtered through a 5 µm TMTP filter  
463 (Millipore). The filters were stored at -80°C until they were used. The pH and the conductivity of  
464 the samples were measured on arrival in the laboratory (within a few hours after the sampling)  
465 using a Benchtop Multiparameter Meter (Thermo Fisher Scientific).

466  
467 Total environmental DNA was extracted from the filters using a PowerBiofilm DNA Isolation kit  
468 according to the manufacturer's recommended procedure (MO BIO). To prepare the 16S and  
469 18S rRNA gene libraries, PCR amplifications were performed with primers that contained a tag  
470 preceded by 2-4 random bases at the 5' end. The V4 region of the 18S rRNA gene region was  
471 amplified using the D512for (5'-NtagATTCCAGCTCCAATAGCG-3') and D978rev (5'-  
472 NtagGACTACGATGGTATCTAATC-3') primers (Zimmermann et al., 2011). The V3-V4 region of the  
473 16S was amplified with the 341F (5'-NtagCCTACGGGNGGCWGCAG-3') and 805R (5'-  
474 NtagGACTACHVGGGTATCTAATCC-3') primers (Herlemann et al., 2011). Preparation of the DNA  
475 libraries and sequencing were performed by Fasteris SA, using the following procedure. PCR  
476 amplifications were performed using 1 µL of DNA (5-10 ng) in the following mix: 1.00 µL DNA,  
477 1.00 µL Forward Primer (10 µM), 1.00 µL Reverse Primer (10 µM), 0.75 µL DMSO, 0.25 µL BSA  
478 (10x), 8.50 µL H<sub>2</sub>O, and 12.50 µL PCR Master Mix 2x (KAPA2G Robust HotStart DNA polymerase  
479 ReadyMix, KAPA Biosystems). The following amplification program was used: 95°C 5min / 30 x  
480 (95°C 15 sec / 52°C 15 sec / 72°C 30 sec) / 72°C 3 min. The PCR products were checked on  
481 agarose gels, purified using Agencourt AMPure XP beads (Beckman Coulter), and quantified  
482 using a Qubit dsDNA HS assay kit. The PCR products were then normalized and pooled (2 pools).  
483 The libraries were prepared using 1 µg DNA from the pools and the Illumina TruSeq Genomic  
484 Nano Library Preparation Kit. The supplier's protocols were followed, with the exception of the  
485 use of a modified End-Repair mix to avoid production of chimeric constructs, and no PCR cycle  
486 was done to finalize the libraries. The resulting libraries were quantified by qPCR and  
487 sequenced using a MiSeq 2 x 300 paired-end run.

488

489 ***Sequence processing***

490 Amplicons of the 16S and 18S rRNA gene sequences were analyzed independently with *mothur*  
491 software version 1.39.5 (Schloss et al., 2009). Reads were processed largely following the  
492 Schloss standard operating procedure for Illumina MiSeq data (Kozich et al., 2013). First, contigs  
493 between the read pairs were assembled. Then, barcode, primer sequences and low-quality  
494 sequences were removed (minimum length of 400 bp and maximum length of 470 bp for 16S  
495 rRNA genes, minimum length of 370 bp and maximum length of 460 bp for 18S rRNA genes,  
496 removing any sequences with ambiguous bases and removing any sequences with  
497 homopolymers longer than 8 bp). Subsequently, sequences were aligned to the SILVA reference  
498 database release 128 (Quast et al., 2013) and preclustered (pre.cluster, diffs = 1). Singletons  
499 were excluded, and chimeras were removed with VSEARCH (Rognes et al., 2016) implemented  
500 in *mothur*. Then, sequences were classified using the k-nearest neighbor (*knn*) algorithm  
501 implemented in *mothur* and the BLASTN search method with the SILVA reference database  
502 release 128. After classification, unknown sequences along with nonbacterial, chloroplast and  
503 mitochondria and noneukaryotic were excluded from the rRNA gene datasets. To account for  
504 differences in sampling efforts, 8,631 and 4,244 sequences from the 16S and 18S rRNA gene  
505 datasets respectively, were then randomly subsampled from each sample (Weiss et al., 2017).  
506 Operational taxonomic units (OTUs) were generated using the OptiClust algorithm (Westcott  
507 and Schloss, 2017), with an OTU being defined at the 97 % and 99 % sequence similarity level  
508 for the 16S and 18S rRNA gene reads, respectively. Finally, sequences were classified using the  
509 *knn* algorithm implemented in *mothur* and the BLASTN search method (cut-off of 80 %) with the

510 SILVA reference database release 128 and the PR2 database (Guillou et al., 2013) for the 16S  
511 and 18S rRNA gene reads, respectively. The raw sequence data have been deposited in the  
512 NCBI Sequence Read Archive under the BioProjects PRJNA546091 (16S rRNA genes) and  
513 PRJNA316490 (18S rRNA genes).

514

### 515 ***Diversity and statistical analysis***

516 All statistical analyses were computed using R software version 3.4.4. *Alpha* and *beta* diversity  
517 metrics were computed using the *vegan* package (Oksanen et al., 2015). To test the relationship  
518 between the bacterial and eukaryotic community matrices and between microbial community  
519 matrices and geographical location, partial Mantel tests were performed using the *ecodist*  
520 package (Goslee and Urban, 2007)□, with Pearson correlation coefficient and 10<sup>6</sup> random  
521 permutations. Microbial community matrices were computed with Canberra distance.  
522 Geographical (latitude and longitude) and water chemistry (pH and conductivity) matrices were  
523 computed with Euclidean distances.

524

525 Cohesion (positive and negative) metrics were computed for bacterial, eukaryotic and  
526 microbial (bacterial and eukaryotic matrices merged) communities using an OTU persistence  
527 cutoff of 10 %, the “focal taxon” null model and 1,000 iterations (Herren and McMahon, 2017).  
528 After this persistence cutoff had been applied, the following were identified: 1,570 bacterial  
529 OTUs, 425 eukaryotic OTUs, and 1,995 microbial OTUs. Cohesion analysis was also used to  
530 identify the most connected OTUs, which have been shown to be potential keystone taxa of a  
531 community (Herren and McMahon, 2018). Here, we used the 15 bacterial and eukaryotic OTUs

532 with the highest positive connectedness and the lowest negative connectedness, which  
533 corresponded to 1 % and 3.5 % of the bacterial and eukaryotic richness, respectively, as  
534 recommended by Herren *et al.* (Herren and McMahon, 2018).

535

536 The impacts of the abiotic (pH, conductivity) and biotic (cohesion metrics) variables of  
537 community structure were investigated with generalized linear models (GLMs) for multivariate  
538 abundance data using the *mvabund* package (Wang et al., 2012), a method that has been  
539 shown to have more statistical power than distance-based multivariate analysis such as  
540 PERMANOVA (Warton et al., 2012). After the data had been examined, a negative binomial  
541 distribution was assumed. Subsequently, the statistical significance of the GLMs was tested  
542 with an analysis of deviance of the GLMs, performed with Monte Carlo resampling (1,000  
543 iterations) and likelihood ratio tests.

544

545 For the evaluation of the co-occurrence probability between and among bacterial and  
546 eukaryotic OTUs, the Veech probabilistic model of species co-occurrence with a hypergeometric  
547 distribution (Veech, 2013) was applied using the *cooccur* package (Griffith et al., 2016). The  
548 same OTU persistence cutoff of 10 % used for the cohesion and GLM analyses was also applied  
549 to avoid detection of spurious co-occurrences. Negative and positive co-occurrences ( $p$ -value <  
550 0.05) were then analyzed according to the taxonomic classifications of the OTUs and  
551 subsequently visualized with *Circos* v0.63-9 (Krzywinski et al., 2009).

552

553

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558

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559 **Figure Legends**

560

561 **Fig. 1. Global abundance and richness of eukaryotic and bacterial OTUs from 60 street gutter**

562 **mats. (A)** Richness (*Left*) and Abundance (*Right*) obtained from high-throughput sequencing of  
 563 18S rRNA gene amplicons. **(B)** Richness (*Left*) and Abundance (*Right*) obtained from 16S rRNA  
 564 gene amplicons assigned to the phylum level.

565

566 **Fig. 2. Alpha and beta microbial diversity in street gutter mats. (A)** OTU richness. **(B)**

567 Dissimilarity distances were calculated using Canberra distance. Statistical significance between  
 568 bacterial and eukaryotic communities were tested using Wilcoxon signed-rank test.

569

570 **Fig. 3. Circos representations of co-occurrences of the Fungi and Bacillariophyta OTUs. (A)** *Left*

571 *panel*, Bacillariophyta-eukaryote co-occurrences, which correspond to 84 diatom OTUs, co-

572 occurring positively with 259 eukaryotic OTUs and negatively with 238 eukaryotic OTUs. *Right*

573 *panel*, Fungi-eukaryote co-occurrences, which correspond to 95 Fungi OTUs, co-occurring

574 positively with 373 eukaryotic OTUs and negatively with 304 eukaryotic OTUs. **(B)** *Left panel* –

575 Bacillariophyta-bacterial co-occurrences, which correspond to 84 diatom OTUs presenting

576 positive co-occurrences with 1,194 bacterial OTUs and negative co-occurrences with 805

577 bacterial OTUs. *Right panel* - Fungi-bacterial co-occurrences. These data involved 94 fungal

578 OTUs, co-occurring positively with 1,341 bacterial OTUs and negatively with 1,099 bacterial

579 OTUs. The color code used for the different clades is presented below the figures, and black and

580 the white correspond to the positive and negative co-occurrences, respectively.

581

582 **Fig. 4. Taxonomic distribution of the most highly connected OTUs.** The data correspond to the  
583 15 bacterial and eukaryotic OTUs that show the highest positive connectedness and the lowest  
584 negative connectedness. Since some OTUs could be among both the most positively and  
585 negatively connected, 25 and 28 eukaryotic and bacterial OTUs are presented here,  
586 respectively. **(A)** Eukaryotic OTUs. **(B)** Bacterial OTUs. The presented taxonomic assignment  
587 varies among the OTUs from the kingdom, phylum, subphylum to class. The green, blue and red  
588 bars correspond to the positively, positively and negatively, or negatively connected OTUs.

589

590 **Table I.** Multivariate analysis of the deviance of generalized linear models fitting microbial  
591 communities to biotic and abiotic factors.

592 **References**

- 593 Adamiak, J., Otlewska, A., Tafer, H., Lopandic, K., Gutarowska, B., Sterflinger, K., and Pinar, G. (2018)  
594 First evaluation of the microbiome of built cultural heritage by using the Ion Torrent next  
595 generation sequencing platform. *International Biodeterioration & Biodegradation* **131**: 11-18.
- 596 Afshinnkoo, E., Meydan, C., Chowdhury, S., Jaroudi, D., Boyer, C., Bernstein, N. et al. (2015) Geospatial  
597 resolution of human and bacterial diversity with city-scale metagenomics. *Cell Systems* **1**: 72-87.
- 598 Allen, J.L., Leflaive, J., Bringuier, C., Ten-Hage, L., Chauvet, E., Cornut, J., and Danger, M. (2017)  
599 Allelopathic inhibition of primary producer growth and photosynthesis by aquatic fungi. *Fungal*  
600 *ecology* **29**: 133-138.
- 601 Amato, P., Joly, M., Besaury, L., Oudart, A., Taib, N., Mone, A.I. et al. (2017) Active microorganisms thrive  
602 among extremely diverse communities in cloud water. *PLOS One* **12**: e.0182869.
- 603 Amin, S.A., Parker, M.S., and Armbrust, E.V. (2012) Interactions between Diatoms and Bacteria.  
604 *Microbiology and Molecular Biology Reviews* **76**: 667-684.
- 605 Baral, D., Speicher, A., Dvorak, B., Admiraal, D., and Li, X. (2018) Quantifying the relative contributions of  
606 environmental sources to the microbial community in an urban stream under dry and wet weather  
607 conditions. *Applied and Environmental Microbiology* **84**: aem.00896-00818.
- 608 Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., and Packmann, A.I. (2016) The ecology and  
609 biogeochemistry of stream biofilms. *Nat Rev Microbiol* **14**: 251-263.
- 610 Bertelli, C., Courtois, S., Rosikiewicz, M., Piriou, P., Aeby, S., Robert, S. et al. (2018) Reduced chlorine in  
611 drinking water distribution systems impacts bacterial biodiversity in biofilms. *Frontiers in*  
612 *Microbiology* **9**: 2520.
- 613 Besemer, K. (2015) Biodiversity, community structure and function of biofilms in stream ecosystems. *Res*  
614 *Microbiol* **166**: 774-781.

- 615 Birtles, R.J., Rowbotham, T.J., Michel, R., Pitcher, D.G., Lascola, B., Alexiou-Daniel, S., and Raoult, D.  
616 (2000) '*Candidatus Odysseella thessalonicensis*' gen. nov., sp. nov., an obligate intracellular parasite  
617 of *Acanthamoeba* species. *Int J Syst Evol Microbiol* **50 Pt 1**: 63-72.
- 618 Bruno, A., Sandionigi, A., Bernasconi, M., Panio, A., Labra, M., and Casiraghi, M. (2018) Changes in the  
619 drinking water microbiome: effects of water treatments along the flow of two drinking water  
620 treatment plants in a urbanized area, Milan (Italy). *Front Microbiol* **9**: 2557.
- 621 Buchan, A., LeCleir, G.R., Gulvik, C.A., and Gonzalez, J.M. (2014) Master recyclers: features and functions  
622 of bacteria associated with phytoplankton blooms. *Nat Rev Microbiol* **12**: 686-698.
- 623 Calderon, O., Porter-Morgan, H., Jacob, J., and Elkins, W. (2017) Bacterial diversity impacts as a result of  
624 combined sewer overflow in a polluted waterway. *Global Journal of Environmental Science and*  
625 *Management-Gjesm* **3**: 437-446.
- 626 Cavalier-Smith, T., and Chao, E.E. (2003) Phylogeny and classification of phylum Cercozoa (Protozoa).  
627 *Protist* **154**: 341-358.
- 628 Cayford, B.I., Jiang, G.M., Keller, J., Tyson, G., and Bond, P.L. (2017) Comparison of microbial  
629 communities across sections of a corroding sewer pipe and the effects of wastewater flooding.  
630 *Biofouling* **33**: 780-792.
- 631 Chimienti, G., Piredda, R., Pepe, G., van der Werf, I.D., Sabbatini, L., Crecchio, C. et al. (2016) Profile of  
632 microbial communities on carbonate stones of the medieval church of San Leonardo di Siponto  
633 (Italy) by Illumina-based deep sequencing. *Applied Microbiology and Biotechnology* **100**: 8537-8548.
- 634 Cowle, M.W., Babatunde, A.O., and Bockelmann-Evans, B.N. (2017) The frictional resistance induced by  
635 bacterial based biofouling in drainage pipelines. *Journal of Hydraulic Research* **55**: 269-283.
- 636 Doilom, M., Liu, J.K., Jaklitsch, W.M., Ariyawansa, H., Wijayawardene, N.N., Chukeatirote, E. et al. (2013)  
637 An outline of the family Cucurbitariaceae. *Sydowia* **65**: 167-192.

- 638 Domingo, J.W.S., Revetta, R.P., Iker, B., Gomez-Alvarez, V., Garcia, J., Sullivan, J., and Weast, J. (2011)  
639 Molecular survey of concrete sewer biofilm microbial communities. *Biofouling* **27**: 993-1001.
- 640 Dong, S.F., and Yao, M.S. (2010) Exposure assessment in Beijing, China: biological agents, ultrafine  
641 particles, and lead. *Environmental Monitoring and Assessment* **170**: 331-343.
- 642 Douterelo, I., Fish, K.E., and Boxall, J.B. (2018) Succession of bacterial and fungal communities within  
643 biofilms of a chlorinated drinking water distribution system. *Water Research* **141**: 74-85.
- 644 Douterelo, I., Sharpe, R.L., Husband, S., Fish, K.E., and Boxall, J.B. (2019) Understanding microbial  
645 ecology to improve management of drinking water distribution systems. *Wiley Interdisciplinary*  
646 *Reviews-Water* **6**: e1325.
- 647 Drury, B., Rosi-Marshall, E., and Kelly, J.J. (2013) Wastewater treatment effluent reduces the abundance  
648 and diversity of benthic bacterial communities in urban and suburban rivers. *Applied and*  
649 *Environmental Microbiology* **79**: 1897-1905.
- 650 Dybwad, M., Skogan, G., and Blatny, J.M. (2014) Temporal variability of the bioaerosol background at a  
651 subway station: concentration level, size distribution, and diversity of airborne bacteria. *Applied*  
652 *and Environmental Microbiology* **80**: 257-270.
- 653 Dybwad, M., Granum, P.E., Bruheim, P., and Blatny, J.M. (2012) Characterization of airborne bacteria at  
654 an underground subway station. *Applied and Environmental Microbiology* **78**: 1917-1929.
- 655 Dyda, M., Decewicz, P., Romaniuk, K., Wojcieszak, M., Sklodowska, A., Dziewit, L. et al. (2018)  
656 Application of metagenomic methods for selection of an optimal growth medium for bacterial  
657 diversity analysis of microbiocenoses on historical stone surfaces. *International Biodeterioration &*  
658 *Biodegradation* **131**: 2-10.
- 659 Fan, H.Q., Li, X.Y., Deng, J.H., Da, G., Gehin, E., and Yao, M.S. (2017) Time-dependent size-resolved  
660 bacterial and fungal aerosols in Beijing subway. *Aerosol and Air Quality Research* **17**: 799-809.

- 661 Flemming, H.C., and Wuertz, S. (2019) Bacteria and archaea on Earth and their abundance in biofilms.  
662 *Nat Rev Microbiol* **17**: 247-260.
- 663 Fresia, P., Antelo, V., Salazar, C., Gimenez, M., D'Alessandro, B., Afshinnekoo, E. et al. (2019) Urban  
664 metagenomics uncover antibiotic resistance reservoirs in coastal beach and sewage waters.  
665 *Microbiome* **7**: 35.
- 666 Garvette, A., Nezan, E., Badis, Y., Bilien, G., Arce, P., Bresnan, E. et al. (2018) Novel widespread marine  
667 Oomycetes parasitising Diatoms, including the toxic genus *Pseudo-nitzschia*: genetic,  
668 morphological, and ecological characterisation. *Frontiers in Microbiology* **9**: 2918.
- 669 Gaylarde, C., Baptista-Neto, J.A., Ogawa, A., Kowalski, M., Celikkol-Aydin, S., and Beech, I. (2017) Epilithic  
670 and endolithic microorganisms and deterioration on stone church facades subject to urban  
671 pollution in a sub-tropical climate. *Biofouling* **33**: 113-127.
- 672 Gomez-Alvarez, V., Humrighouse, B.W., Revetta, R.P., and Domingo, J.W.S. (2015) Bacterial composition  
673 in a metropolitan drinking water distribution system utilizing different source waters. *Journal of*  
674 *Water and Health* **13**: 140-151.
- 675 Goslee, S.C., and Urban, D.L. (2007) The ecodist package for dissimilarity-based analysis of ecological  
676 data. *Journal of Statistical Software* **22**: 1-19.
- 677 Griffith, D.M., Veech, J.A., and Marsh, C.J. (2016) Cooccur: probabilistic species co-occurrence analysis in  
678 R. *J Statistical Software* **69**: 1-17.
- 679 Grossart, H.P., Wurzbacher, C., James, T.Y., and Kagami, M. (2016) Discovery of dark matter fungi in  
680 aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. *Fungal*  
681 *Ecology* **19**: 28-38.
- 682 Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L. et al. (2013) The Protist Ribosomal  
683 Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with  
684 curated taxonomy. *Nucleic Acids Research* **41**: D597-D604.

- 685 Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., and Martiny, J.B. (2012) Beyond biogeographic  
686 patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* **10**: 497-506.
- 687 Herlemann, D.P., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. (2011)  
688 Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* **5**:  
689 1571-1579.
- 690 Herren, C.M., and McMahon, K.D. (2017) Cohesion: a method for quantifying the connectivity of  
691 microbial communities. *ISME Journal* **11**: 2426-2438.
- 692 Herren, C.M., and McMahon, K.D. (2018) Keystone taxa predict compositional change in microbial  
693 communities. *Environmental Microbiology* **20**: 2207-2217.
- 694 Hervé, V., Leroy, B., Da Silva Pires, A., and Lopez, P.J. (2018) Aquatic urban ecology at the scale of a  
695 capital: community structure and interactions in street gutters. *ISME Journal* **12**: 253-266.
- 696 Hsu, T., Joice, R., Vallarino, J., Abu-Ali, G., Hartmann, E.M., Shafquat, A. et al. (2016) Urban transit  
697 system microbial communities differ by surface type and interaction with humans and the  
698 environment. *Msystems* **1**: e.00018-00016.
- 699 Jaklitsch, W.M., Checa, J., Blanco, M.N., Olariaga, I., Tello, S., and Voglmayr, H. (2018) A preliminary  
700 account of the Cucurbitariaceae. *Stud Mycol* **90**: 71-118.
- 701 Janke, B.D., Finlay, J.C., and Hobbie, S.E. (2017) Trees and streets as drivers of urban stormwater  
702 nutrient pollution. *Environmental Science & Technology* **51**: 9569-9579.
- 703 Jiang, G.M., Zhou, M., Chiu, T.H., Sun, X.Y., Keller, J., and Bond, P.L. (2016) Wastewater-enhanced  
704 microbial corrosion of concrete sewers. *Environmental Science & Technology* **50**: 8084-8092.
- 705 Johnsen, A.R., Styrishave, B., and Aamand, J. (2014) Quantification of small-scale variation in the size and  
706 composition of phenanthrene-degrader populations and PAH contaminants in traffic-impacted  
707 topsoil. *FEMS Microbiology Ecology* **88**: 84-93.

- 708 Johnsen, A.R., de Liphay, J.R., Reichenberg, F., Sorensen, S.J., Andersen, O., Christensen, P. et al. (2006)  
709 Biodegradation, bioaccessibility, and genotoxicity of diffuse polycyclic aromatic hydrocarbon (PAH)  
710 pollution at a motorway site. *Environmental Science & Technology* **40**: 3293-3298.
- 711 Kachour, L., Gacemi-Kirane, D., Loucif, L., and Alayat, H. (2016) First survey of aquatic microbial Fungi-  
712 like Pythiaceae predominantly colonizing the south-Mediterranean freshwater wetlands. *Research*  
713 *Journal of Pharmaceutical Biological and Chemical Sciences* **7**: 3067-3078.
- 714 Kembel, S.W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A.M. et al. (2012) Architectural  
715 design influences the diversity and structure of the built environment microbiome. *ISME Journal* **6**:  
716 1469-1479.
- 717 Khreis, H., Warsow, K.M., Verlinghieri, E., Guzman, A., Pellecuer, L., Ferreira, A. et al. (2016) The health  
718 impacts of traffic-related exposures in urban areas: Understanding real effects, underlying driving  
719 forces and co-producing future directions. *Journal of Transport & Health* **3**: 249-267.
- 720 Kindaichi, T., Yamaoka, S., Uehara, R., Ozaki, N., Ohashi, A., Albertsen, M. et al. (2016) Phylogenetic  
721 diversity and ecophysiology of Candidate phylum Saccharibacteria in activated sludge. *FEMS*  
722 *Microbiol Ecol* **92**: fiw078.
- 723 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013) Development of a dual-  
724 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the  
725 MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112-5120.
- 726 Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D. et al. (2009) Circos: An  
727 information aesthetic for comparative genomics. *Genome Research* **19**: 1639-1645.
- 728 Kuhn, S.F., and Hofmann, M. (1999) Infection of *Coscinodiscus granii* by the parasitoid nanoflagellate  
729 *Pirsonia diadema*: III. Effects of turbulence on the incidence of infection. *Journal of Plankton*  
730 *Research* **21**: 2323-2340.

- 731 Leonard, G., Labarre, A., Milner, D.S., Monier, A., Soanes, D., Wideman, J.G. et al. (2018) Comparative  
732 genomic analysis of the 'pseudofungus' *Hyphochytrium catenoides*. *Open Biology* **8**: 170184.
- 733 Leung, M.H., Wilkins, D., Li, E.K., Kong, F.K., and Lee, P.K. (2014) Indoor-air microbiome in an urban  
734 subway network: diversity and dynamics. *Appl Environ Microbiol* **80**: 6760-6770.
- 735 Li, H., Liu, D.F., Lian, B., Sheng, Y., and Dong, H.L. (2012) Microbial diversity and community structure on  
736 corroding concretes. *Geomicrobiology Journal* **29**: 450-458.
- 737 Li, X., Kappler, U., Jiang, G.M., and Bond, P.L. (2017) The Ecology of acidophilic microorganisms in the  
738 corroding concrete sewer environment. *Frontiers in Microbiology* **8**: 683.
- 739 Marques, E.L.S., Silva, G.S., Dias, J.C.T., Gross, E., Costa, M.S., and Rezende, R.P. (2019) Cave drip water-  
740 related samples as a natural environment for aromatic hydrocarbon-degrading bacteria.  
741 *Microorganisms* **7**: 33.
- 742 McLellan, S.L., and Roguet, A. (2019) The unexpected habitat in sewer pipes for the propagation of  
743 microbial communities and their imprint on urban waters. *Curr Opin Biotechnol* **57**: 34-41.
- 744 Meng, S.W., Torto-Alalibo, T., Chibucos, M.C., Tyler, B.M., and Dean, R.A. (2009) Common processes in  
745 pathogenesis by fungal and oomycete plant pathogens, described with Gene Ontology terms. *BMC*  
746 *Microbiology* **9**: S7.
- 747 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B. et al. (2015) vegan:  
748 community ecology package. R package version 2.3-2. In: [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)  
749 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan).
- 750 Patel, K.V., Bailey, C.L., Harding, A.H., Biggin, M., and Crook, B. (2018) Background levels of micro-  
751 organisms in the busy urban environment of transport hubs. *Journal of Applied Microbiology* **125**:  
752 1541-1551.
- 753 Pedron, R., Esposito, A., Bianconi, I., Pasolli, E., Tett, A., Asnicar, F. et al. (2019) Genomic and  
754 metagenomic insights into the microbial community of a thermal spring. *Microbiome* **7**: 8.

- 755 Perlman, S.J., Hunter, M.S., and Zchori-Fein, E. (2006) The emerging diversity of Rickettsia. *Proc Biol Sci*  
756 **273**: 2097-2106.
- 757 Perrin, Y., Bouchon, D., Delafont, V., Moulin, L., and Hechard, Y. (2019) Microbiome of drinking water: A  
758 full-scale spatio-temporal study to monitor water quality in the Paris distribution system. *Water Res*  
759 **149**: 375-385.
- 760 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2013) The SILVA ribosomal RNA  
761 gene database project: improved data processing and web-based tools. *Nucleic Acids Research* **41**:  
762 D590-D596.
- 763 Ragon, M., Restoux, G., Moreira, D., Moller, A.P., and Lopez-Garcia, P. (2011) Sunlight-exposed biofilm  
764 microbial communities are naturally resistant to chernobyl ionizing-radiation levels. *PLOS One* **6**:  
765 e21764.
- 766 Reblova, M., Hubka, V., Thureborn, O., Lundberg, J., Sallstedt, T., Wedin, M., and Ivarsson, M. (2016)  
767 From the tunnels into the treetops: new lineages of black Yeasts from biofilm in the Stockholm  
768 metro system and their relatives among Ant-associated Fungi in the Chaetothyriales. *PLOS One* **11**:  
769 e0163396.
- 770 Richard, C., Mitbavkar, S., and Landoulsi, J. (2017) Diagnosis of the Diatom community upon biofilm  
771 development on stainless steels in natural freshwater. *Scanning*: ID 5052646.
- 772 Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahe, F. (2016) VSEARCH: a versatile open source tool  
773 for metagenomics. *Peerj* **4**: e2584.
- 774 Sanchez-Gonzalez, M., Alvarez-Urbe, H., Rivera-Solis, R., Gonzalez-Burgos, A., Escalante-Rendiz, D., and  
775 Rojas-Herrera, R. (2019) Analysis of a phenol-adapted microbial community: degradation capacity,  
776 taxonomy and metabolic description. *Journal of Applied Microbiology* **126**: 771-779.

- 777 Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B. et al. (2009) Introducing  
778 mothur: open-source, platform-independent, community-supported software for describing and  
779 comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537-7541.
- 780 Scholz, B., Guillou, L., Marano, A.V., Neuhauser, S., Sullivan, B.K., Karsten, U. et al. (2016) Zoosporic  
781 parasites infecting marine diatoms - A black box that needs to be opened. *Fungal Ecology* **19**: 59-76.
- 782 Shamarina, D., Stoyantcheva, I., Mason, C.E., Bibby, K., and Elhaik, E. (2017) Communicating the  
783 promise, risks, and ethics of large-scale, open space microbiome and metagenome research.  
784 *Microbiome* **5**: 132.
- 785 Shi, T., Fredrickson, J.K., and Balkwill, D.L. (2001) Biodegradation of polycyclic aromatic hydrocarbons by  
786 *Sphingomonas* strains isolated from the terrestrial subsurface. *Journal of Industrial Microbiology &*  
787 *Biotechnology* **26**: 283-289.
- 788 Siddaramappa, S., Viswanathan, V., Thiyagarajan, S., and Narjala, A. (2018) Genomewide  
789 characterisation of the genetic diversity of carotenogenesis in bacteria of the order  
790 Sphingomonadales. *Microbial Genomics* **4**: 172.
- 791 Sison-Mangus, M.P., Jiang, S., Tran, K.N., and Kudela, R.M. (2014) Host-specific adaptation governs the  
792 interaction of the marine diatom, *Pseudo-nitzschia* and their microbiota. *ISME J* **8**: 63-76.
- 793 Tesei, D., Tafer, H., Poyntner, C., Pinar, G., Lopandic, K., and Sterflinger, K. (2017) Draft genome  
794 sequences of the black rock fungus *Knufia petricola* and its spontaneous nonmelanized mutant.  
795 *Genome Announcements* **5**: e01242-01217.
- 796 van Tol, H.M., Amin, S.A., and Armbrust, E.V. (2017) Ubiquitous marine bacterium inhibits diatom cell  
797 division. *ISME Journal* **11**: 31-42.
- 798 Veech, J.A. (2013) A probabilistic model for analysing species co-occurrence. *Global Ecology and*  
799 *Biogeography* **22**: 252-260.

- 800 Voisin, J., Cournoyer, B., Vienney, A., and Mermillod-Blondin, F. (2018) Aquifer recharge with  
801 stormwater runoff in urban areas: Influence of vadose zone thickness on nutrient and bacterial  
802 transfers from the surface of infiltration basins to groundwater. *Science of the Total Environment*  
803 **637**: 1496-1507.
- 804 Wang, H.T., Cheng, M.Y., Dsouza, M., Weisenhorn, P., Zheng, T.L., and Gilbert, J.A. (2018) Soil bacterial  
805 diversity is associated with human population density in urban greenspaces. *Environmental Science*  
806 *& Technology* **52**: 5115-5124.
- 807 Wang, Y., Naumann, U., Wright, S.T., and Warton, D.I. (2012) mvabund- an R package for model-based  
808 analysis of multivariate abundance data. *Methods in Ecology and Evolution* **3**: 471-474.
- 809 Warton, D.I., Wright, S.T., and Wang, Y. (2012) Distance-based multivariate analyses confound location  
810 and dispersion effects. *Methods in Ecology and Evolution* **3**: 89-101.
- 811 Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A. et al. (2017) Normalization and  
812 microbial differential abundance strategies depend upon data characteristics. *Microbiome* **5**: 27.
- 813 Westcott, S.L., and Schloss, P.D. (2017) OptiClust, an improved method for assigning amplicon-based  
814 sequence data to operational taxonomic units. *MSphere* **2**: e00073-00017.
- 815 Zhang, J., Wei, Z., Jia, H.F., and Huang, X. (2017) Factors influencing water quality indices in a typical  
816 urban river originated with reclaimed water. *Frontiers of Environmental Science & Engineering* **11**:  
817 8.
- 818 Zhang, S.H., Pang, S., Wang, P.F., Wang, C., Han, N.N., Liu, B. et al. (2016) Antibiotic concentration and  
819 antibiotic-resistant bacteria in two shallow urban lakes after stormwater event. *Environmental*  
820 *Science and Pollution Research* **23**: 9984-9992.
- 821 Zimmermann, J., Jahn, R., and Gemeinholzer, B. (2011) Barcoding diatoms: evaluation of the V4  
822 subregion on the 18S rRNA gene, including new primers and protocols. *Organisms Diversity and*  
823 *Evolution* **11**: 173-192.

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## 825 **Supplementary Information**

826 Additional Supporting Information may be found in the online version of this article at the  
827 publisher's web-site:

828

### 829 **Fig. S1. Relative abundance and richness of bacterial and eukaryotic OTUs in street gutter**

830 **mats. (A)** Abundance (top panel) and diversity (bottom panel) obtained from 18S rRNA gene  
831 amplicons. **(B)** Abundance (top panel) and richness (bottom panel) obtained from high-  
832 throughput sequencing of 16S rRNA gene amplicons assigned to the phylum level. The 60  
833 samples were sorted according to the abundance of the Bacillariophyta clade.

834

### 835 **Fig. S2. Relationship between geographical distance and (A) eukaryote and (B) bacterial** 836 **community dissimilarity.** Community dissimilarity was computed based on Canberra distances.

837 The significance of the relationship was tested using partial Mantel tests, to control for the  
838 effect of water chemistry (pH and conductivity).

839

840 **Fig. S3. Eukaryote-Eukaryote co-occurrence patterns.** Circos visualization of the eukaryote-  
841 eukaryote positive (black) and negative (white) co-occurrences. The total set corresponds to  
842 12,128 positive co-occurrences involving 425 eukaryotic OTUs and 3,908 negative co-  
843 occurrences involving 408 eukaryotic OTUs.

844

845 **Fig. S4. Eukaryote-Bacteria co-occurrence patterns.** Circos visualization of the eukaryotic-  
846 bacterial positive (black) and negative (white) co-occurrences. The set corresponds to 60,776

847 positive co-occurrences involving 425 eukaryotic OTUs and 1,546 bacterial OTUs and 38,122  
848 negative co-occurrences involving 425 eukaryotic OTUs and 1,515 bacterial OTUs.

849

850 **Fig. S5. Bacteria-Bacteria co-occurrence patterns.** Circos visualization of the bacteria-bacteria  
851 positive (black) and negative (white) co-occurrences. The total set corresponds to 155,790  
852 positive co-occurrences involving 1,557 bacterial OTUs, and 41,719 negative co-occurrences  
853 involving 1,553 bacterial OTUs.

854

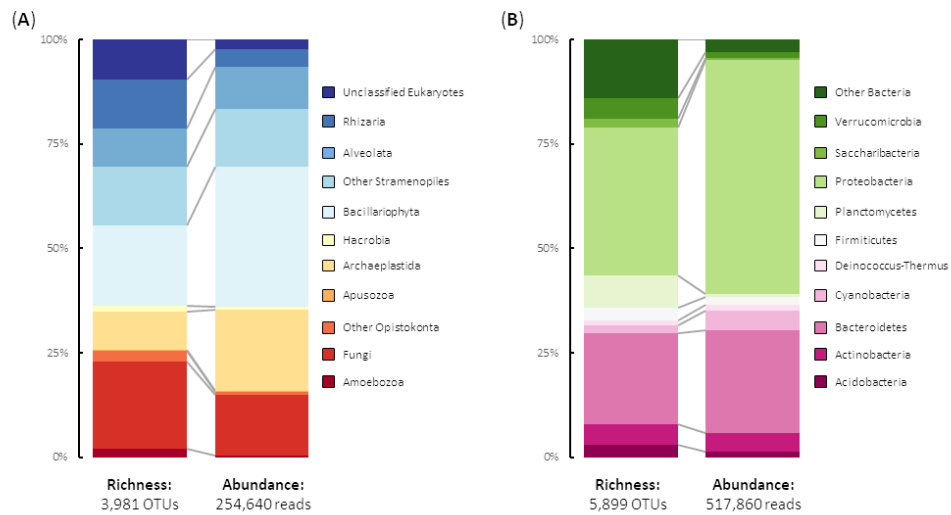


Fig. 1. Global abundance and richness of eukaryotic and bacterial OTUs from 60 street gutter mats. (A) Richness (left) and Abundance (right) obtained from high-throughput sequencing of 18S rRNA gene amplicons. (B) Richness (left) and Abundance (right) obtained from 16S rRNA gene amplicons assigned to the phylum level.

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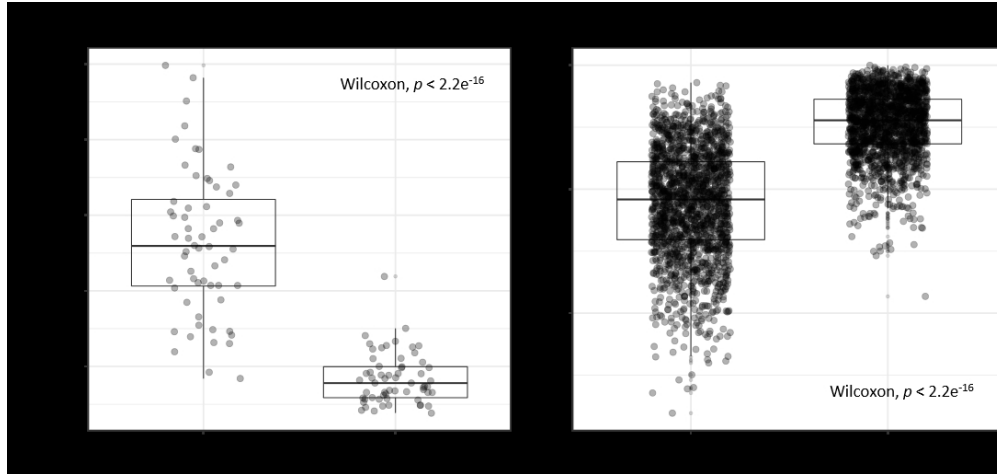


Fig. 2. Alpha and beta microbial diversity in street gutter mats. (A) OTU richness. (B) Dissimilarity distances were calculated using Canberra distance. Statistical significance between bacterial and eukaryotic communities were tested using Wilcoxon signed-rank test.

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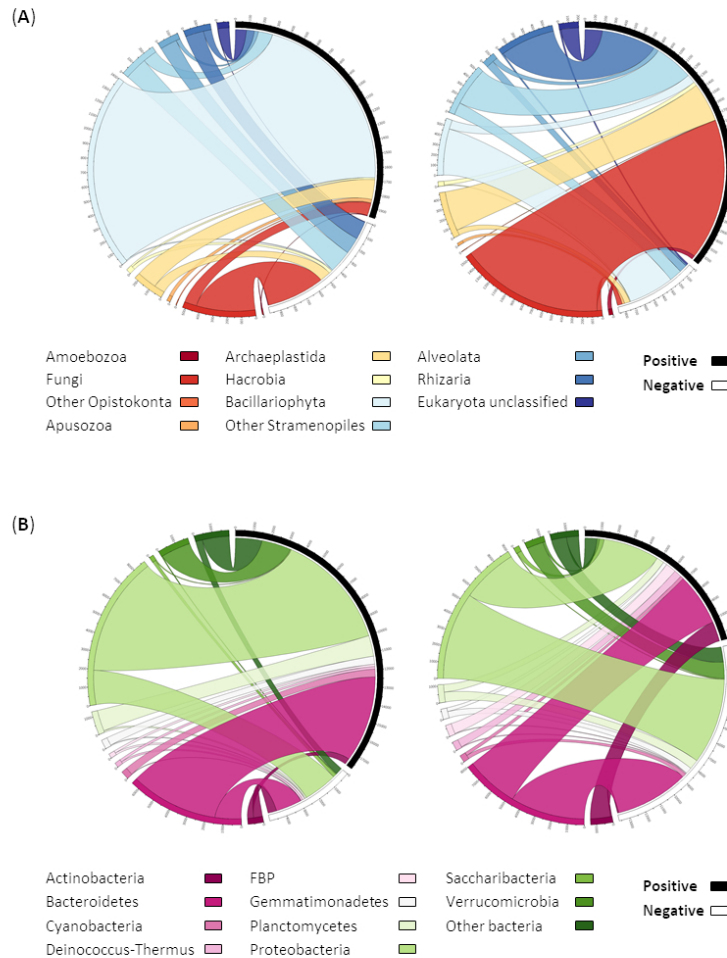


Fig. 3. Circos representations of co-occurrences of the Fungi and Bacillariophyta (diatoms) OTUs. (A) Left panel, Bacillariophyta-eukaryote co-occurrences, which correspond to 84 diatom OTUs, co-occurring positively with 259 eukaryotic OTUs and negatively with 238 eukaryotic OTUs. Right panel, Fungi-eukaryote co-occurrences, which correspond to 95 Fungi OTUs, co-occurring positively with 373 eukaryotic OTUs and negatively with 304 eukaryotic OTUs. (B) Left panel - Bacillariophyta-bacterial co-occurrences, which correspond to 84 diatom OTUs presenting positive co-occurrences with 1,194 bacterial OTUs and negative co-occurrences with 805 bacterial OTUs. Right panel - Fungi-bacterial co-occurrences. These data involved 94 fungal OTUs, co-occurring positively with 1,341 bacterial OTUs and negatively with 1,099 bacterial OTUs. The color code used for the different clades is presented below the figures, and black and the white correspond to the positive and negative co-occurrences, respectively.

86x97mm (300 x 300 DPI)

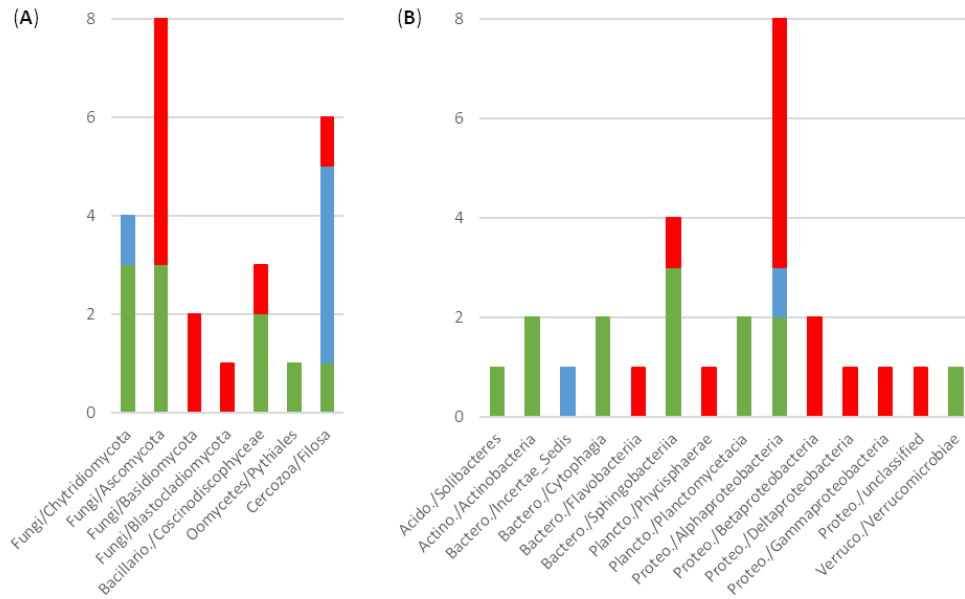


Fig. 4. Taxonomic distribution of the most highly connected OTUs. The data correspond to the 15 bacterial and eukaryotic OTUs that show the highest positive connectedness and the lowest negative connectedness. Since some OTUs could be among both the most positively and negatively connected, 25 and 28 eukaryotic and bacterial OTUs are presented here, respectively. (A) Eukaryotic OTUs. (B) Bacterial OTUs. The presented taxonomic assignment varies among the OTUs from the kingdom, phylum, subphylum to class. The green, blue and red bars correspond to the positively, positively and negatively, or negatively connected OTUs.

80x51mm (300 x 300 DPI)

**Table I.** Multivariate analysis of the deviance of generalized linear models fitting microbial communities to biotic and abiotic factors.

Factor	Bacteria		Eukaryotes		Bacteria and Eukaryotes	
	Deviance	<i>p</i> value	Deviance	<i>p</i> value	Deviance	<i>p</i> value
Positive Cohesion	7,639	< 1x10 <sup>-3</sup>	1,781	< 1x10 <sup>-3</sup>	8,040	< 1x10 <sup>-3</sup>
Negative Cohesion	5,410	< 1x10 <sup>-3</sup>	2,027	< 2x10 <sup>-3</sup>	5,349	< 1x10 <sup>-3</sup>
pH	1,960	0.018	726	0.018	2,567	0.041
Conductivity	2,635	< 1x10 <sup>-3</sup>	1,061	< 2x10 <sup>-3</sup>	3,420	< 2x10 <sup>-3</sup>