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Bacterial assemblages of urban microbiomes mobilized by runoff waters match land use typologies and harbor core species involved in pollutant degradation and opportunistic human infections

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1 **1. Introduction**

2 Humans concentrate in urban settings, with half of the world population now living in
3 cities. This proportion is likely to rise up to 70% in 2050, according to the World Health
4 Organization (2015), representing a population of six billions. Cities can be divided according
5 to their main functional categories (hereafter termed morphotypes) as defined by their
6 commercial, residential and industrial activities (e. g. Revitt et al., 2014). These morphotypes
7 can then be subdivided according to their typologies which are defined according to variables
8 such as: (i) land cover composition including green (e. g. parks) and blue (streams, lake) belts,
9 (ii) land use intensity, (iii) connectivity between impervious surfaces such as car parks and the
10 presence of urban drainage systems, roads and sidewalks, and (iv) other factors including
11 industrial and commercial activities (Göbel et al., 2007; Revitt et al., 2014). These typologies
12 can generate shelters for, among others, several birds, rats, spiders, and favor the development
13 of particular microbiota (e. g. Marti et al., 2017; Aigle et al., 2021).

14 Ecological trends among microbial communities of urban biomes (conceptualized under
15 the term urban microbiomes) have been investigated for some typologies (see Gilbert and
16 Stephens (2018) for review). To illustrate, the bacterial taxa recovered from surface swabs or
17 air samples from transit systems (e. g. Danko et al., 2021), soils of urban parks (Ramirez et
18 al., 2014), deposits and sediments from streets (Marti et al., 2017; Aigle et al., 2021) and
19 urban waters (McLellan et al., 2015; Voisin et al., 2018) were investigated through 16S rRNA
20 (ribosomal RNA) and *tpm* (thiopurine methyltransferase) meta-barcoding (meaning DNA
21 sequence analysis of PCR products) approaches or metagenomics (meaning an exhaustive
22 sequencing of total bacterial DNA extracts). The diversity observed on city surfaces was
23 found to be significantly different from the ones of agricultural soils and meadows (Ibekwe et
24 al., 2013). Skin-associated bacterial genera were significantly found among air samples of
25 transit systems during peak commuting hours, and relative humidity and air temperature were

26 inversely linked to the ecological richness of these samples as inferred from 16S rRNA meta-
27 barcoding profilings (Leung et al., 2014). Interestingly, an equilibrium between indoor and
28 outdoor air-borne microbial communities was observed in these systems e. g. Leung et al.
29 (2014); Robertson et al. (2013). Furthermore, the microbiome of urban deposits was found to
30 greatly evolve over time while pollutants were accumulating (Aigle et al., 2021; Marti et al.,
31 2017).

32 Urban microbiomes are expected to be structured around core and versatile opportunistic
33 microbial groups. These would be representative of the main sources of microbial taxa
34 seeding a particular biotope, and being organized in efficient functional units by the
35 prevailing selective forces. Even though stochastic microbial distribution patterns likely led to
36 some of the observed DNA imprints among these communities (e. g. Hao et al., 2016), one
37 can hypothesize that the principles of the r/K ecological theory (e. g. Song et al., 2017) can
38 apply to these biomes, and gradually lead to a selection of K-like specialists (best fit). In fact,
39 a core urban microbiome for transit systems was recently reported, supporting this hypothesis
40 (Danko et al., 2021). Synurbic (defined as “living in the city”) microbial species thus appear
41 to have been selected over time among urban biomes. However, on the short run, these urban
42 infrastructures are likely to represent transient opportunities for r-ecological strategists (at the
43 other end of this r/K gradient) which can grow rapidly, and efficiently colonize empty or
44 disturbed habitats. These r-strategists are considered better fit for uncrowded microbiome
45 because of their fast growth rate (e. g. Song et al., 2017) and greater metabolic versatility.
46 These r-strategists have often been associated to human- and animal-opportunistic pathogens
47 (Vadstein et al., 2018). However, recent investigations have shown that the numbers of
48 virulence and antibiotic resistance genes can be more abundant among bacterial communities
49 growing under oligotrophic growth conditions than copiotrophic ones (Song et al., 2017).
50 These species could have selected virulence or defense genes to increase their competitiveness

51 when resource supply rates are low. A better knowledge of these r/K gradients among urban
52 biomes could identify risky behaviors (e. g. chemical spills) or practices that can favor
53 undesirable opportunistic bacteria such as the human pathogenic ones.

54 Here, the incidence of land use specificities and human activities on core (K-strategists)
55 and versatile bacterial taxa (r-strategists) colonizing urban surfaces was investigated. To
56 address these issues, a long term experimental site, the Mi-plaine catchment of the Field
57 Observatory for Urban Water Management (OTHU) located in Lyon (France), was used. This
58 catchment is an impervious area (impervious rate of 75%) drained by a separated stormwater
59 system made of detention and infiltration basins (SIS) located at the outlet, in order to avoid
60 flooding and allow a recharge of the connected aquifer (the one of Lyon) (e. g. Sébastien et
61 al., 2014).

62 Main objectives of this study were to:

- 63 (1) evaluate similarities between sub-catchments based on their socio-urbanistic
64 patterns, relative runoff volumes per rain event, and contents in classical bacterial
65 indicators
- 66 (2) investigate relationships between sub-catchments' specificities and the numbers of
67 core bacterial K- and versatile r-strategists recovered over urban surfaces (using
68 runoff waters) through a 16S rRNA gene meta-barcoding approach allowing
69 differentiation at the genus level
- 70 (3) investigate relationships in the numbers of core bacterial K- and versatile r-
71 strategists recovered over surfaces (using runoff waters) through a *tpm* gene meta-
72 barcoding approach allowing differentiation at the species level
- 73 (4) resolve co-occurrence networks between bacterial taxa inferred from the meta-
74 barcoding profilings, and identify keystone bacterial taxa indicative of sorting
75 processes triggered by socio-urbanistic variables

76 2. Materials and Methods

77 2.1. Experimental site and its main socio-urbanistic features

78 The Mi-plaine urban catchment of the Lyon area
79 (http://www.chassieu.fr/la_zone_industrielle_mi_plaine.html) was investigated in this study.
80 The selected watershed is part of the town of Chassieu, and covers a 210 ha which is
81 impervious at 75% (**Fig. 1 and Fig. 2**). Roads are cleaned every week by a mechanical
82 sweeper. The watershed of Chassieu is divided into sub-catchments (**Fig. S1**) transferring
83 their runoffs into the main rain water network and SIS of the catchment. These sub-
84 catchments were used as references for the socio-urbanistic surveys and definition of
85 typologies, and for inferring relationships with the genetic structures of bacterial communities
86 inferred from 16S rRNA and *tpm* DNA profilings (see section 2.3).

87 Socio-urbanistic and industrial surveys were performed over three periods (spring,
88 summer, and fall). Technical objects, traces and human activities over the study site that could
89 impact the urban microbiomes were recorded. The first series of surveys (n=3) allowed
90 defining runoff sampling points (n=21) per sub-catchment according to the observed activities
91 and organizations shown in **Table S1**. Urbanistic features and objects separating private
92 properties (gardens and parking lots, buildings) were considered to define the area leading to
93 the observed runoffs at each sampling point (**Table S1, and Fig. 2**). Low concrete walls often
94 separated the private and public areas but sometimes porous delimitations (portal, shrubs,
95 lawn) were observed (**Table S1**). Economical and industrial activities at the sampling points
96 were identified and divided into categories for further analyses (**Table S2a, b, c**). Twelve
97 field surveys of the technical objects, traces and human activities completed these socio-
98 industrial profilings of the watershed (**Table S2**). These surveys were performed over a
99 surface covering a 50 m diameter per sampling zone. These surveys were performed in the
100 morning, afternoon and at night, over 30-minute long sessions per point on working days and

101 considering peak hours (noon and evening, and n=3 were performed at night). This allowed
102 the identification of 54 variables representing objects and traces indicative of particular
103 behaviors that have impacted the selected sampling sites (**Table S2**).

104 **2.2. Runoff water samplings, and their main physico-chemical and microbiological** 105 **features**

106 Three rain events (October 16 2014, March 25 2015, and September 17 2015) were
107 considered in this study. Meteorological parameters for these events are shown in **Fig. S1**.
108 These rain events came from different geographical locations. The Canoe urban hydrology
109 computational platform (Chocat, 2013) was used to estimate runoff flow values and volumes
110 per sub-catchment during these events (**Fig. S1**). Runoff waters were collected just before the
111 grate inlet of each sampling site except for the outlet of the separated rain stormwater
112 drainage system (sampling point C23). Waters at the outlet were recovered from the inlet of
113 the detention basin. One liter was collected per sampling point using a single-use manual
114 pump. Electrical conductivity, turbidity, pH, oxygen, and temperature were measured on site
115 using a multi-parametric probe (Horiba, Piscataway, USA) (Table S3). Samples were kept at
116 4°C until performing the microbiological analyses described below. Numbers of total
117 heterotrophic bacteria were estimated by plating serial dilutions of the runoff waters onto 1/10
118 diluted TSB amended with 1.5 % agar. Numbers of intestinal *Enterococci* and *Escherichia*
119 *coli* were estimated using the IDEXX most probable number methods named Colilert and
120 Enterolert (IDEXX, Westbrook, USA).

121 Microbial DNA extracts were produced from filtered water samples through 0.2 µm pore
122 size polycarbonate filters (Merckmillipore, Burlington, USA) by using the FastDNA SPIN®
123 Kit for Soil (MP Biomedicals, Carlsbad, France). Depending on the amount of suspended
124 matters, between 23 and 100 mL of runoff waters could be filtered. DNA concentrations were
125 measured using a Nanodrop ND1000 (Thermofisher, Waltham, USA). Triplicated qPCR

126 assays were applied on these DNA extracts using a Bio-Rad CFX96 qPCR device. These
127 assays used the Brilliant II SYBR Green low ROX qPCR mix (Agilent, Vénissieux, France).
128 The CFX Manager 3.0 software (Bio-Rad, Marnes-la-Coquette, France) was used to estimate
129 the numbers of copies of targeted genes per ng DNA (or equivalent runoff volume). The
130 human-specific HF183 *Bacteroides* qPCR assay was performed according to Seurinck et al.
131 (2005), and the assay for the 16S rRNA gene copies of Bacteria was performed according to
132 Park and Crowley (2006) using primers 338F and 518R. Melting T° was 60°C for all assays.
133 Linearized plasmid DNA containing 16S rRNA genes from the targeted DNAs were run as
134 standards using 10-fold dilutions of the plasmids. These plasmids were obtained from Marti et
135 al. (2017). Class 1 integrons (*int1*) were quantified using primers targeting the integrase gene.
136 Presence of inhibitors in the DNA extracts was checked according to Marti et al. (2017).
137 When inhibition was detected, extracts were diluted 10 to 100 times using sterile ultra-pure
138 water until a positive PCR product could be obtained.

139 **2.3. Meta-barcoding analyses of V5-V7 16S rRNA and *tpm* gene PCR products**

140 **2.3.1. PCR products and their MiSeq Illumina sequencing**

141 The V5-V7 16S rRNA gene PCR products were generated using DNA primers 799F
142 (barcode + ACCMGGATTAGATACCCKG) and 1193R (CRTCCMCACCTTCCTC)
143 reported by Beckers et al. (2016). PCR amplifications were performed using the HotStarTaq
144 Plus Master Mix Kit (Qiagen, USA) using the following temperature cycles: 94 °C for 3 min,
145 followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, with a final
146 elongation step at 72 °C for 5 min. PCR products and blank control samples were verified
147 using a 2% agarose gel and the electrophoretic procedures described in Colin et al. (2020).
148 PCR products obtained from field samples showed sizes around 430 bp but blanks did not
149 show detectable and quantifiable PCR products. Dual-index adapters were ligated to the PCR
150 fragments using the TruSeq® DNA Library Prep Kit which also involved quality controls of

151 the ligation step (Illumina, Paris, France). Illumina V3 Miseq DNA sequencings were
152 performed by MrDNA services (Shallowater, Texas, USA). DNA sequences were paired-end,
153 and set up to obtain around 40K reads per sample.

154 The *tpm* DNA libraries were also sequenced by the Illumina MiSeq V3 technology but by
155 the Biofidal DNA sequencing services (Vaulx-en-Velin, France). PCR products were
156 generated using the following mix of degenerated PCR primers as reported in Aigle et al.
157 (2021): ILMN-PTCF2 (5'- P5 adapter tag + universal primer +
158 GTGCCGYTRTGYGGCAAGA-3'), ILMN-PTCF2m (5'- P5 adapter tag + universal primer
159 + GTGCCCYTRTGYGGCAAGT-3'), ILMN-PTCR2 (5'- P7 adapter tag + universal primer
160 + ATCAKYGCGGCGCGGTCRTA-3'), and ILMN-PTCR2m (5'- P7 adapter tag + universal
161 primer + ATGAGBGCTGCCCTGTCRTA-3'). The universal primer was 5'-
162 AGATGTGTATAAGAGACAG-3'. The P5 adapter tag was 5'-TCGTCGGCAGCGTC-3'.
163 The P7 adapter tag was: 5'- GTCTCGTGGGCTCGG-3'. PCR reactions were performed and
164 verified as described in Aigle et al. (2021). PCR products obtained from field samples showed
165 sizes around 320 bp but blanks did not show detectable PCR products. Still, *tpm* harboring
166 bacteria being in low number among a bacterial community (about 2-3%), blank samples
167 were run during the Miseq DNA sequencing of the PCR products. Illumina Miseq DNA
168 sequencings of the *tpm* PCR products were paired-end, and set up to obtain around 40K reads
169 per sample. Blank samples generated low numbers of *tpm* reads that were retrieved from the
170 dataset by the decontam procedure. The 16S rRNA and *tpm* gene sequences reported in this
171 work are available at the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under the
172 accession #PRJEB33507.

173 **2.3.2. Processing of meta-barcoding V5-V7 16S rRNA and *tpm* gene DNA sequences, and**
174 **computation of diversity indices and taxonomic allocations**

175 In order to select the bioinformatic processing packages for the construction of the 16S
176 rRNA gene and *tpm* contingency tables, raw reads were treated by both the Mothur (Schloss
177 et al., 2009) and dada2 packages (Callahan et al., 2016). Regarding dada2 manipulations,
178 linker, barcode and primers were removed from the DNA reads by using the Trimgalore
179 v0.6.5 software (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The
180 Standard Operating Procedure (SOP) for merged reads was used for the processing of the 16S
181 rRNA raw reads (https://benjjneb.github.io/dada2/bigdata_1_2.html) while the SOP for the
182 paired-end reads was used for the *tpm* raw reads
183 (https://benjjneb.github.io/dada2/bigdata_paired.html). Regarding Mothur, sequences were
184 treated according to the pipeline defined by Kozich et al. (2013). In both cases, raw sequences
185 were discarded when they were: (1) shorter than 350 bp and longer than 400 bp for V5-V7
186 16S rRNA gene, and than 250 bp and 270 bp for *tpm*; (2) had few identities with reference
187 sequences of the database, (3) contained homopolymers longer than 8 bp, (4) contained
188 ambiguous bases. Chimeric DNA sequences were detected and discarded using the
189 chimera.uchime command (Edgar et al., 2011). For Mothur, the number of sequences was
190 normalized between samples by performing a random resampling at $n = 10783$ for the V5-V7
191 16S rRNA gene reads and $n = 8072$ for the *tpm* reads. Operational Taxonomic Units (OTUs)
192 were defined at 97 % identity for the V5-V7 16S rRNA gene reads or 100 % (exact sequence
193 variants) identity for *tpm* reads. Contaminant OTUs which may come from the extraction kit,
194 or the 0.2 μm pore size polycarbonate filters were detected using the Decontam v1.8.0
195 package (Davis et al., 2018). The Decontam package was used on the OTU tables using the
196 DNA concentration method for the 16S rRNA gene datasets (**Table S4**). The *tpm* OTU
197 contaminants of the blank samples were identified by Decontam (**Table S5**). The R Decontam
198 package detected only 370 16S rRNA contaminant gene reads (representing 0.059 % of the
199 total reads) distributed into 50 OTUs among the dataset (**Table S5**). These low numbers of

200 contaminant reads did not have any effect on the statistical analyses. The validated OTU
201 contingency tables are given in **Tables S6** and **S7**. Diversity indices were computed from
202 OTU and ASV (amplicon sequence variants), and by grouping the OTU (**Table S8**) and ASV
203 (**Table S9**) per genus. High goodness of fit (R^2) and significant correlations were observed by
204 Spearman correlation tests using both types (OTU against ASV) of contingency tables (**Fig.**
205 **S2**). Dada2 16S rRNA gene ASV diversity indices were significantly higher than those
206 computed from the Mothur OTU dataset (Wilcoxon-test: p-value < 0.001). For the *tpm*
207 datasets, diversity indices were similar between the ASV/OTU datasets (Wilcoxon test, p-
208 value > 0.05). Spearman correlation tests confirmed the high positive correlations between
209 both datasets (**Fig. S2**). OTUs being found in higher numbers in both the 16S rRNA gene and
210 *tpm* datasets, the following analyses were limited to the OTU contingency Tables. OTUs
211 taxonomic allocations were performed by comparison with the Silva database (16S rRNA
212 genes) or the “BD_TPM_Mar18_v1.unique_770seq” database (Aigle et al., 2021) and using
213 the “Wang” text-based Bayesian classifier (Wang et al., 2007). Only classifications resolved
214 with a bootstrap above 80% were considered. Some functional inferences were made from the
215 taxonomic allocations using the Faprotax software version 1.2.1 (Louca et al., 2016).

216 **2.4. Statistical analyses**

217 Alpha diversity indices (Shannon, Simpson and Evenness) were computed using the Vegan
218 package v2.5-6 for Rv3.5.3 as well as Hellinger transformation. Spearman correlations were
219 computed using the R package Hmisc v4.4.0 and the correlation networks were computed
220 using the Igraph package v1.2.6. The adjusted Wallace coefficient analyses were performed at
221 the UMMI web site of Lisboa University (<http://www.comparingpartitions.info/>). Chord
222 diagrams showing the relative abundance of 16S rRNA or *tpm* – based taxonomic allocations
223 were made using the “circlize” v0.4.6 package for R. Repartition biases were investigated
224 using the DESeq2 package v1.28.1 for R (Love et al., 2014). Co-occurrence networks and

225 keystone taxa were resolved using the fast greedy modularity optimization method of the
226 Molecular Ecological Network Analyzes Pipeline (MENAp) and the Cytoscape software
227 v3.8.0 (Deng et al., 2012). Simple linear regressions were computed using the R package
228 ggpubr v0.4.0 and illustrated with ggplot2 v3.3.2. Confrontation between microbial datasets
229 and the explanatory variables were also computed using a multilinear regression model
230 described in **Fig. S8a**.

231 **3. Results**

232 **3.1. Similarities in land-use and human behaviors between sub-catchments**

233 Socio-urbanistic indicators were recorded over the Mi-plaine Chassieu catchment, and
234 were used to compare urban typologies and activities between sub-catchments (**Table S2b**;
235 **Table S2c**). Overall, 559 industries were recorded over the Chassieu Mi-plaine catchment
236 (**Fig. 1**). Most significant activities concerned retailing of specialized industrial materials (n =
237 63 companies), construction (n = 53), and metal transformation (n = 33) (e. g. **Fig. 2**). Hotels
238 offering up to 170 rooms, and restaurants welcoming up to 300 customers during service
239 hours, were found in this area. 139 companies distributed into 44 commercial sectors
240 impacted this catchment, and were involved in wholesale trading (n = 15), specialized
241 construction works (n = 10), financial services (n = 10), metal product manufacturing (n = 8),
242 rental and leasing (n = 7) and real estate (n = 5) (**Table S2**).

243 The “land use” dataset was converted into a frequency table (**Table S2c**). Spearman
244 correlations were computed from this dataset to analyze co-occurrence patterns (**Fig. 3**). This
245 led to the observation of recurrent significant associations such as those of (i) the chemical
246 and plastic industries, (ii) the entertainment – related ones, and (iii) the building and metal-
247 work industries. Similarities between these partitions per sampling point were tested by
248 computing adjusted Wallace indices. These indices of relatedness between sites are indicated
249 in **Fig. 1**, and led to the identification of two large groups termed I (C01, C02, C05, C06, C09,

250 C13, C14, C17) and II (C22, C07, C19, C21, C15, C16), but five sub-watersheds showed
251 specific urbanistic organizations as observed in **Table S2c**. Group I sampling points showed
252 more industries involved in specialized construction works; these points were mainly found
253 among quiet streets and dead ends. Group II sampling points were found distributed along a
254 large avenue showing several rental and leasing services.

255 In order to go deeper in the understanding of variables impacting the urban surface
256 microbiomes, technical objects and social behaviors over the catchment were investigated,
257 and led to a dataset of 26 descriptors (**Table S2b**). Number of employees (total = 3421) and
258 overall frequentations per sub-catchment were compared. Most significant movements over
259 the catchment were associated with gasoline engines with a total number of 3880 events
260 involving these engines per day (n=3 surveys; total = 11640 events) (**Table S2b**). Soft
261 traveling modes such as walking (n = 308 events per day survey period) and cycling (n = 36
262 events per day survey period) were also frequent. Other technical objects mobilized at
263 daytime and behaviors were observed such as high uses of portals (n=106 mobilizations per
264 day survey period), construction engines (n=63), lorry for horses (n=46), bus shelters (n=27)
265 or cigarettes (n=18). Wastes were also frequently detected, and concerned feces (12
266 observations per day survey period), food-derivatives (n=9) or hygienic products (n=5)
267 (**Table S2b**). Chemical spills were observed including petrol and oil stains (n=58), clear fluid
268 spills (n=6) or white spills (n=3). Spearman correlations were computed from these traces to
269 highlight relationships between all socio-urbanistic parameters including the economic and
270 industrial activities (**Fig. 3**). Interestingly, sampling points on streets with the highest numbers
271 of gasoline engines (“trucks”, “cars”, “2 wheels”, “commercial vehicles” and “bus”) were
272 correlated with restaurants and rental activities of group II (Spearman correlation tests;
273 $p < 0.05$) (**Fig. 3**). Areas with the highest numbers of pedestrians and employees were

274 associated with higher numbers of wastes. Furthermore, quiet streets and dead ends showed
275 more fecal matters, and night activities such as prostitution (Wilcoxon test: p-value <0.05).

276 To avoid collinearity effects while performing statistical analyses between the socio-
277 urbanistic and microbiological datasets, the following variables were selected (**Fig. 3**): (a1)
278 number of cars; also representing numbers of buses, commercial vehicles, motorized two
279 wheels and trucks, (b1) number of employees; also representing counts of cigarettes,
280 pedestrians and domestic wastes, (c1) number of freight lifts; also representing counts of
281 construction materials, liquid spills and bus shelters (**Fig. 3**). Analysis of these social traces
282 led to the identification of two sectors with high gasoline engines circulation (North-West and
283 South-East) (**Fig. 1 and 2**) which were termed “group III”, and were composed of sampling
284 points C02, C03, C05, C06, C07, C08, C15, C16, C17, C19 and C22. These sectors were
285 associated with significantly higher numbers of trucks, cars, two-wheels, commercial
286 vehicles, non-motorized scooters, and buses, but also cigarettes, food and other wastes,
287 employees and pedestrians, and restaurants (Wilcoxon test: p-value <0.05). Dead end (C01,
288 C14, C18 (no industrial or commercial activities)) and quiet streets (C09, C10, C11, C13, C20
289 or C21) were defined as group IV (**Fig. 1 and 2**) and were characterized by significantly
290 higher numbers of hygienic wastes, prostitution and fecal matters (Wilcoxon test: p-value
291 <0.05).

292 **3.2. Variations in runoff volumes, physico-chemical monitoring and classical bacterial** 293 **indicators between sub-catchments**

294 Geographical origin of the clouds and rain events which led to the three runoff sampling
295 campaigns investigated in this study are indicated in **Fig. S1**. Runoff waters were subjected to
296 physico-chemical monitorings (**Table S3**). A PCA of these datasets was performed (**Fig. S3**).
297 pH values of the runoff waters of campaign 2 were higher, and their turbidity was lower, in
298 most instances. Campaign 2 had the largest differences between the measured min-max values

299 for pH (pH 6.3 – 9.1). Lowest value was obtained from runoff waters recovered at site C14,
300 and appeared related to an industrial chemical spill. This pH shift was associated with a drop
301 in turbidity (lowest value recorded in this study) but an increase in conductivity (highest value
302 recorded), explaining the peculiar PCA ordination of this sampling point (**Fig. S3**). A negative
303 correlation between pH and the presence of chemical industries was observed (C14, C2)
304 (Spearman correlation tests; $p < 0.05$). High pH values were correlated to rental and leasing
305 activities such as those of group II sampling points (**Fig. 3**) (Spearman correlation tests;
306 $p < 0.05$) (**Fig. S4**). High conductivity values were correlated to food catering activities
307 (Spearman correlation tests; $p < 0.05$) (**Fig. S4**), which were positively correlated to the
308 number of food wastes on the streets (**Fig. 3**). Conductivity and turbidity values were
309 positively correlated with the numbers of cars, trucks, commercial vehicles and pedestrians
310 observed on the catchment (**Fig. S5**). Samples from campaign 2 had the lowest temperatures,
311 with an average of 8.5°C, while the runoffs had temperatures between 16-18°C for samples of
312 the other campaigns (**Table S3**). Runoff flow values and total volumes were similar between
313 sampling campaigns 1 and 3 but campaign 2 had lower values (**Table S3**).

314 Concerning the bacterial indicators, runoffs from the watershed showed high level of
315 heterotrophic bacterial plate counts ranging from 4.0×10^5 to 1.8×10^8 CFU (colony forming
316 units) per 100 mL (**Table S3**). These plate counts were positively correlated with sub-group
317 b1 variables (employees, cigarettes, pedestrians, wastes, **Fig. 3**), and merchandise storage
318 (**Fig. S6**; Spearman correlation tests; $p < 0.05$). *E. coli* and intestinal enterococci numbers were
319 high all over the catchment (**Fig. 1**; **Table S3**), and found to be correlated (Spearman
320 correlation test; $p < 0.05$). Intestinal *enterococci* cell numbers were negatively correlated with
321 group b1 variables but also merchandise storage and non-motorized scooters (**Fig. S6**,
322 Spearman correlation tests; $p < 0.05$). *E. coli* and intestinal enterococci numbers were highest
323 among sites of group IV (dead ends, hygienic wastes, and fecal matters) (Wilcoxon test;

324 $p < 0.05$), and were associated with a higher occurrence of HF183 DNA marker confirming
325 significant human fecal contaminations at these sites (Spearman correlation test; $p < 0.05$) (**Fig.**
326 **1, Table S3**). Twenty-two samples over the three sampling campaigns were found positive to
327 HF183 by qPCR (**Table S3**). Relative ratios of HF183 marker (of human fecal contamination)
328 over numbers of total bacterial 16S rRNA gene targets ranged from 3.1×10^{-6} to 3.7×10^{-3} (at
329 sampling point C23 which is the outlet of the watershed). The relative numbers of HF183
330 DNA targets were positively correlated with the mobilization of portals. This technical object
331 was mainly used among quiet places or secondary roads of group IV sites (Spearman
332 correlation tests; $p < 0.05$). Presence of integron 1 (*int1*) was detected among 24 runoff
333 samples (**Fig. 1**). Relative *int1* counts over total 16S rRNA gene copies ranged from 6.2×10^{-7}
334 to 3.5×10^{-3} (at sampling point C23). Integron 1 was mainly detected among group III sites
335 associated with high counts of gasoline engines (Wilcoxon test; $p < 0.05$).

336 **3.3. Core K- and opportunistic r-strategists observed over urban surfaces (using runoff** 337 **waters) through 16S rRNA gene meta-barcoding analyses** 338 **- general features**

339 Removal of chimeric sequences, short reads and a random resampling based on the lowest
340 number of V5-V7 16S rRNA gene reads ($n=10783$) recovered for a runoff water sample were
341 performed. This led to a dataset of 4 048 699 16S rRNA gene reads. These reads were divided
342 into 9155 OTUs. Rarefaction curves showed a plateau phase in the relation between the
343 number of reads analyzed and the number of OTUs observed per sample (**Fig. S7a**). Most
344 samples had between 700 to 1000 OTUs (**Table S8**). Shannon, Simpson and Evenness indices
345 showed the samples from the 1st campaign to have a significantly higher diversity than those
346 of the 2nd and 3rd campaigns (Wilcoxon test; $p < 0.05$), and their content in 16S rRNA gene
347 copies per 100 mL were slightly lower (**Table S3**). These indices showed that samples from
348 group I (construction industries) (**Fig. 1**) had significantly more diversity than those of group

349 II (large avenue) (Wilcoxon test; $p < 0.05$) but not of groups III (high motor engines) and IV
350 (quiet streets). NMDS analysis confirmed the significant relationships between the socio-
351 urbanistic groupings (defined in section 3.1) and the 16S rRNA gene meta-barcoding
352 profilings. All four socio-urbanistic groups defined above were associated with significantly
353 different profiles as computed from Adonis analyses using the Bray-Curtis dissimilarities
354 between samples (**Fig. 4**).

355 - *standard partition analyses*

356 Runoff OTUs were affiliated to bacterial taxa using the Silva database in order to identify
357 the core K- and versatile r-like bacterial taxa thriving in the investigated urban system. Thirty-
358 five phyla were inferred from the dataset (for more than 99% of the reads; **Table S10**).
359 Sample C14_2 (impacted by a chemical spill) harbored the smallest number of phyla (n=11).
360 Sample C16_1 (group III, **Figs. 1 & 4**) showed the greatest number of phyla (n= 24). Nine
361 core phyla were found over the industrial catchment: *Proteobacteria*, *Actinobacteria*,
362 *Bacteroidetes*, *Gemmatimonadetes*, *Planctomycetes*, *Acidobacteria*, *Firmicutes*,
363 *Saccharibacteria* and *Armatimonadetes*. Reads allocated to the *Proteobacteria*,
364 *Actinobacteria* and *Bacteroidetes* represented in average 93.6 % of the dataset and varied
365 respectively from 39 % to 81 %, 0.70 % to 54 % and 2% to 49 % per campaign (**Table S10**).
366 *Proteobacteria* was the dominant phylum (showing an equivalent of 2×10^9 to 2×10^5 16S
367 rRNA gene copies per 100 mL; median value = $2,4 \times 10^8$) in all samples except C07_3 and
368 C08_3 which were dominated by *Actinobacteria*. It is to be noted that C07 and C08 are part
369 of the group III sites (**Figs. 1 & 4**) associated with high traffic of gasoline engines. In average,
370 *Actinobacteria* were the second most dominant group over the catchment with 19.75 % of
371 relative abundance followed by the *Bacteroidetes* (15.48 %). However, *Bacteroidetes* were
372 more abundant than the *Actinobacteria* in 29 samples over 60 (**Table S10**). This dataset was
373 characterized by a very low number of *Firmicutes* with scores ranging from 0.02 to 3.5% per

374 sample representing an equivalent of about 4×10^3 to 2.5×10^7 16S rRNA gene copies per
375 100 mL (median= 1×10^6). Sample C14_2 (showing a toxic chemical spill) was again
376 characterized by unusual values with a very low relative number of reads allocated to the
377 *Actinobacteria* (0.7 %; highly sensitive to the chemical spill) but very high (more than 81%;
378 representing an equivalent of about 10^8 16S rRNA gene copies per 100 mL) for reads
379 allocated to *Proteobacteria* (likely more resistant to the chemical spill). Similar trends were
380 observed for sample C14_3 but this sample also showed the highest score for
381 *Saccharibacteria* (5.40 %) of the TM7 phylum which are related to obligate epibiont of
382 *Actinomyces* spp. (He et al., 2015).

383 Deeper analysis of these phyla showed a differentiation into 717 genera (**Table S11**).
384 Twenty-eight of these genera including the *Pseudomonas* (representing an equivalent of about
385 3×10^3 to 3.8×10^7 16S rRNA gene copies per 100 mL; median= 7×10^5) were conserved
386 among all samples, representing almost 90 % of the total 16S rRNA gene reads, and were thus
387 considered to be related to K-strategists. **Fig. 5** shows the genera with a relative abundance
388 over 0.5 % over the dataset, and highlights those which were classified as core bacterial taxa
389 (in all samples) likely grouping K-strategists. The C14 sampling site was confirmed to be
390 peculiar with high numbers of reads allocated to *Novosphingobium* (α -proteobacteria) (**Table**
391 **S6; Table S11**). C14 also showed high numbers of DNA reads allocated to the
392 *Sulfurospirillum* (ϵ -proteobacteria) (5.61 %) and *Paludibacter* (*Bacteroidetes*) (11.6 %).
393 These latter enriched genera were considered r-like opportunistic strategists.

394 Distribution biases of the above genera against the behavioral (groups III or IV; **Fig. 2**) and
395 industrial/commercial activities (groups I to II; **Fig. 1**) were investigated by DESeq2 (**Table**
396 **S12**). Reads from *Novosphingobium* were found in significantly higher proportions in the
397 runoff water samples from group I (construction industries) and IV (quiet areas) (DESeq2;
398 $p < 0.05$). Reads from *Actinobacteria* such as *Geodermatophilus*, *Marmoricola*, and *Dietzia*,

399 and from *Methylobacterium* (α -proteobacteria) were found in higher proportions in samples
400 from group II (large avenues) (DESeq2; $p < 0.05$). Reads affiliated to *Bdellovibrio* were found
401 in significantly higher proportions in the samples from group I (DESeq2; $p < 0.05$), and those
402 from *Roseomonas* and *Arthrobacter* in group III (DESeq2; $p < 0.05$). Reads allocated to
403 *Pseudomonas* and *Gemmatimonas* were in higher proportion among group IV samples
404 (DESeq2; $p < 0.05$). These strong relationships with particular socio-urbanistic patterns are
405 indicative of the occurrences of K-like strategists over the catchment.

406 The sources of the above taxa were explored by comparing their relatedness with taxa in
407 higher numbers (i) among old urban sediments (*Acidibacter*, *Haliangium*) that had
408 accumulated metallic trace elements and naphthalene, or (ii) among recent urban sediments of
409 a detention basin which had high chrysene contents (*Aquabacterium*, *Paludibacter*) according
410 to Marti et al. (2017). 16S rRNA gene reads allocated to taxa known to be enriched in old
411 urban sediments were in very low relative abundances among the sampled runoff waters
412 (**Table S11**). However, reads affiliated to taxa in higher numbers in recent sediments/deposits
413 found in a detention basin (*Peredibacter*, *Aquabacterium*, *Paludibacter* and *Cloacibacterium*)
414 were recorded among the sampled runoffs, with relative abundances going from 0.20 to 3.4 %
415 (**Table S11**). These taxa did not show distribution biases matching the socio-urbanistic
416 parameters of the sub-catchments, and were likely to contain K-strategists.

417 - *correlation network analyses*

418 Co-occurrence patterns between numbers of 16S rRNA gene reads and their allocation to
419 bacterial genera were further explored through multiple Spearman correlation tests (**Fig. 6a**),
420 and were considered indicative of the occurrence of K-strategists. Statistics of the most
421 significant networks are presented in **Table S14**, and the modules are shown in **Fig. 6a**. The
422 largest module of correlated taxa was made of 17 genera mainly grouping *Actinobacteria*
423 involved in hydrocarbon degradation (based on Faprotax and literature searches). Only two

424 genera from another phylum were recorded in this module and belonged to the *Roseomonas*
425 and *Methylobacterium* (α -*proteobacteria*). This module was in line with the above DESeq2
426 analyses which had previously shown positive relationships between some of these genera
427 and the group II urban typologies (**Fig. 1 and Fig. 3**; large avenue with high numbers of
428 gasoline engines). The second largest module (Mod2) showed correlations between three
429 *Bacteroidetes* (*Cytophaga*, *Flavobacterium* and *Pedobacter*) and a connection between
430 *Flavobacterium* read numbers and those allocated to *Pseudospirillum* (hydrocarbon degrader)
431 of the γ -*proteobacteria* which were further connected to *Perlucidibaca*, and *Arenimonas* 16S
432 rRNA gene reads distribution patterns (**Fig. 6a**). These last three genera are known to harbor
433 bacterial species involved in oil / hydrocarbon degradation processes (Morais et al., 2016).
434 Module-EigenGene analyses highlighted several significant (p-value < 0.001) Spearman
435 correlations between the selected explanatory variables and the above modules (**Fig. 6a**). For
436 example, module 1 (Mod1) showed positive correlations with sub-group a1 (gasoline engines)
437 variables represented by car numbers (ratio=0.45), and which contributed to the definition of
438 group III socio-urbanistic patterns (**Fig. 2**). Genera from Mod1 showed negative relationships
439 with hygienic wastes (-0.47) associated with group IV sampling sites located in more quiet
440 streets (**Fig. 2 and Fig. 6a**). Mod2 genera were positively correlated to higher uses of
441 construction engines (0.69) and freight_lift – like variables (0.49) of sub-group c1, and, as
442 Mod1, were negatively associated with hygienic wastes (-0.46).

443 Further computations using a multilinear regression model (described in **Fig. S8a**) were
444 performed to confirm these relationships between the meta-barcoding, behavioral and
445 organizational explanatory variables (**Fig. S8a**). Interestingly, this analysis showed that core
446 genera likely harboring K-strategists had lower numbers of significant R² correlation values
447 than those of the flexible r-like bacterial taxa (Wilcoxon-test: p-value < 0.001). Reads from
448 seven r-like genera including *Thauera*, *Sulfurospirillum*, *Noviherbaspirillum*, hgcl_clade,

449 *Novosphingobium*, *Microcella*, and *Phenylobacterium* showed significant correlations with
450 the physico-chemical variables ($R^2 > 0.1$). Number of reads of *Sulfurospirillum* were
451 negatively correlated with the pH values ($R^2 = -0.14$), and positively correlated with the
452 conductivity ones ($R^2 = 0.08$) (**Fig. S8a**), confirming an opportunistic development matching
453 with the occurrence of a chemical spill at C14. Read numbers affiliated to *Novosphingobium*
454 also showed a negative relationship with the pH values. *Thaurea* read numbers were
455 negatively correlated to temperature and positively with turbidity. Several other significant
456 relationships were detected but with low R^2 (**Fig. S8c**).

457 **3.4. Core K- and opportunistic r-strategists observed over urban surfaces (using runoff** 458 **waters) through *tpm* gene meta-barcoding analyses**

459 **- general features**

460 A high quality dataset of 910 048 *tpm* sequences was generated, re-sampled (n=8087), and
461 used to infer the effects of urban land use typologies and human behaviors on *tpm*-harboring
462 bacterial species. These *tpm* sequences were divided into 9 641 OTUs (exact sequence
463 variants) (**Tables S7 and S8**). Rarefaction curves showed a plateau phase (**Fig. S7b**). Most
464 samples had between 150 and 300 *tpm* OTUs. Diversity indices have been computed and are
465 shown in **Table S8**. These diversity indices were compared with those computed from the 16S
466 rRNA gene meta-barcoding dataset through linear regression analyses. No correlations were
467 observed for the Shannon, Simpson and Evenness indices. In the same way, no significant
468 differentiation was observed between diversity indices computed from the three sampling
469 campaigns and the four socio-urbanistic groups of sampling points defined in section 3.1.

470 NMDS and RDA analyses of the Bray-Curtis dissimilarities between the *tpm* gene read
471 patterns were performed. Adonis analyses did not differentiate the *tpm* profilings according to
472 the groups of samples defined from the analyzed urban typologies and activities (groups I to
473 IV). However, RDA indicated a significant relationship between the ordinations and the

474 activities of group III sampling points such as high traffic of gasoline engines. Anova.cca and
475 ordistep tests confirmed the significance of the computed RDA model and relationships with
476 traffic ($p < 0.01$). To illustrate, the position of *tpm* Otu00135 affiliated to *Pseudomonas*
477 *extremaustralis* in the RDA plot showed higher read numbers among the runoff waters of
478 group III samples. This OTU showed positive relationships with cars (and correlated variables
479 shown in **Fig. 3**) and the quantity of food wastes observed over the watershed. Similar
480 positive relationships on the RDA were recorded for *tpm* OTU00154 (a non-classified
481 *Pseudomonas* species). The goodness of fit test confirmed the significance of these
482 relationships.

483 - *standard partition analyses*

484 Taxonomic allocations at the genus level allowed a classification of 62 % of the *tpm* reads
485 into 20 well-defined genera (**Table S15**). The genera inferred by the *tpm* meta-barcoding
486 approach represented on average an equivalence of 6.28 % (0.35 to 21.53 % per sample) of
487 the 16S rRNA gene dataset (computed from the shared genera between both datasets). Only
488 *Pseudomonas tpm* reads were recorded in all the runoff samples. They represented in average
489 35.79 % of the total *tpm* reads (from 0.21 to 75.93 %) (**Table S15**). Other *tpm*-harboring
490 genera were found among at least half of the runoff samples such as *Herbaspirillum*,
491 *Xanthomonas* and *Aeromonas* representing respectively 11, 5 and 0.8 % of the total *tpm* reads.
492 These four genera were considered core *tpm*-harboring taxa circulating over the investigated
493 urban catchment likely harboring K-strategists (**Table S15**).

494 Relative abundances inferred from the *tpm* gene dataset showed the *Pseudomonas* to be
495 fairly homogenous across the sampling points except for C16_S2 (0.21 %; group III variables
496 associated with traffic of gasoline engines), C10_S3 (5.41 %; group IV variables associated
497 with quiet streets) and C09_S1 (75.93 %; group I (construction industries) and IV).
498 *Herbaspirillum* was not detected among the 3rd sampling campaign except for C05_S3 and

499 C06_S3 but with a very low relative abundance (0.01 and 0.02 %), and a high one for C14_S3
500 (31.48 %) (**Table S15**). Interestingly, the pollution observed at C14 was associated with an
501 enrichment in *Herbaspirillum*. C16_S2 (96.35 %) also showed an environmental burst in
502 *Herbaspirillum tpm* reads (**Table S15**). *Xanthomonas tpm* reads also had a heterogeneous
503 distribution pattern with relative abundances going from 0 to 50% (**Table S15**). The 3rd
504 sampling campaign led to high relative abundances of *Xanthomonas* reads at C01_S3 (48.86
505 %), C03_S3 (33.81 %) and C07_S3 (20.72 %). *Xanthomonas* reads were not detected during
506 the 2nd sampling campaign except at C18_S2 but in low abundance (0.08 %). This approach
507 also led to the recovery of *Stenotrophomonas* (0.65 %), *Shewanella* (0.30 %) and *Curvibacter*
508 (0.2 %) *tpm* DNA reads (**Table S15**).

509 At the species level, more than 50 % of the *tpm* reads could be allocated to 78 well-defined
510 species (**Table S16; Fig. 7**). DNA reads from several species were recovered in more than 50
511 % of the samples such as *Herbaspirillum aquaticum*, *Pseudomonas koreensis*, *Pseudomonas*
512 *rhodesiae*, *Pseudomonas stutzeri*, *Pseudomonas anguilliseptica* and *Pseudomonas aeruginosa*
513 (**Fig. 7**). These species were considered K-strategists. The most abundant species was
514 *Herbaspirillum aquaticum* (**Table S16**). *H. aquaticum tpm* reads represented 31.32 % of the
515 total number of reads obtained from the C14_S3 sample. *Pseudomonas koreensis* was the
516 second most abundant species with an average relative abundance of 4.7 %, followed by
517 *Xanthomonas cannabis* (3.85 %), *Pseudomonas anguilliseptica-like* (2 %), and *Pseudomonas*
518 *aeruginosa* (2 %) (**Table S16, Fig. 7**). Other *tpm*-harboring species showing more variable
519 distribution patterns like *Aeromonas jadensis*, *P. putida*, *P. mendocina*, *P. pseudoalcaligenes*
520 and *P. fluorescens* appeared r-like strategists taking benefits for their development of some
521 specialized C-sources available over the catchment. These latter bacteria could, in fact, be
522 associated to hydrocarbon degradation as inferred from Faprotax analyses. It is to be noted
523 that some of the above r/K-like taxa detected by the *tpm* meta-barcoding approach were found

524 to be well-known human, animal and plant pathogens e. g. *A. hydrophila*, *P. mendocina*, *A.*
525 *caviae*, *P. aeruginosa*, *P. syringae*, *Stenotrophomonas maltophilia*, *Xanthomonas axonopodis*,
526 and *X. cannabis*. The latter species was the most common *tpm*-harboring phytopathogen
527 recorded in the samples, and was previously described to infect more than 350 plant species
528 including *Cannabis sativa* (Netsu et al., 2014).

529 The *tpm* reads allocated to *P. aeruginosa* human pathogens were recurrent in the runoff
530 *tpm* dataset. They were recorded in high numbers at C21_S2 (19.25 %; group IV), C07_S1
531 (10.36 %; group III), C22_S1 (10.06 %; group III) and C19_S3 (9.90 %; group III) (**Table**
532 **S16**). Conversion of these latter relative abundances into *Pseudomonas* 16S rRNA gene
533 copies according to **Table S3** indicated an equivalence of about 10^4 *P. aeruginosa* 16S rRNA
534 gene numbers per 100 mL in these samples. These *P. aeruginosa tpm* reads were further
535 allocated to sub-lineages, and mapped over the urban watershed (**Fig. S9**). These reads (n =
536 5594) were divided into 72 OTUs, and 26 were allocated to the sub-clade PAO1 (n = 2273
537 reads), and 20 to the sub-clade PA14 (n = 214 reads). The *P. aeruginosa tpm* sequence types
538 were allocated to strain characterized by Multi Locus Sequence Typing (<https://pubmlst.org/>)
539 (**Table S17a**) using the correspondence table reported by Colin et al. (2020). The *tpm*
540 OTU00592 (n = 181 reads) was found to match ST252 isolates from non-CF (cystic fibrosis)
541 infections; the one of OTU00987 (n = 85 reads) matched ST2123/ST226 strains respectively
542 isolated from CF patients and clinical environments; and OTU00072 (n = 1802 reads)
543 matched ST175/ST2042 found in urinary tract and blood infections. This significant
544 occurrence of *P. aeruginosa tpm* reads over the catchment led us to isolate the matching
545 bacterial strains during the third sampling campaign. More than 200 hundred isolates were
546 collected, and their *tpm* sequences were sequenced after PCR amplification (**Table S17b**).
547 Most of the *P. aeruginosa* urban isolates harbored the *tpmG* type 12_15_17_18_35 which was
548 found to be the most abundant among the *tpm* metabarcoding dataset. However, strains from

549 some other dominant meta-barcoding *tpm* sequence types were found more difficult to isolate.
550 Only 7 strains harboring the *tpmG* type 08_24 (matching Otu00056 which showed 2256
551 sequences) were obtained, and 2 showing the *tpmG* type 1 sequence (matching OTU021
552 which showed 1878 sequences). It is to be noted that OTU numbers were attributed by using a
553 larger *tpm* meta-barcoding dataset than the one reported in this study, and this explains the
554 lack of match between OTU code numbers and number of reads per OTU among the dataset
555 presented here. Interestingly, some strains harbored *tpmG* types not detected by
556 metabarcoding such as *tpmG3*, *tpmG4*, *tpmG16-26*, *tpmG30* and *tpmG34* (**Table S17**). The
557 *tpm* reads allocated to *P. syringae* could also be further allocated at the level of pathovars
558 such as *P. s.* pathovar *aceris* (3875 reads distributed into 84 OTUs), *P. s. panici* (4 OTUs, 203
559 reads), *P. s. japonica* (3 OTUs, 167 reads), *P. s. aptata* (1 OTUs, 46 reads), *P. s. maculicola*
560 (1 OTUs, 26 reads) and *P. s. pisi* (1 OTUs, 7 reads). DESeq2 computations were performed to
561 evaluate changes in the *tpm* OTU patterns according to the urban typologies and activities.
562 Otu00056 affiliated to *P. aeruginosa* (subclade PA14-like) showed significantly more reads
563 among group I (large avenue) than group II, and among group III (high gasoline engines) than
564 IV. Otu00072 (subclade PAO1-like) reads were more abundant among samples from group
565 IV (those from dead ends) (**Table S18**).

566 The sources of *tpm* bacterial taxa among runoffs were explored by DNA sequence
567 comparisons with previously reported sequence types recovered from urban deposits and
568 sediments accumulating at C23 (which is the detention basin of the experimental site
569 collecting the runoff waters) (Aigle et al., 2021). 17 OTUs from the runoff water datasets
570 matched at 100 % identity with *tpm* sequences from sediments. Runoff *tpm* of Otu00013
571 (unclassified *Proteobacteria*, n=5891 reads), Otu00027 (Gp_DE, n=3791 reads) matched
572 respectively with ASV_17, ASV_32 found in old deposits containing high concentrations of
573 poorly degradable pollutants including metallic trace elements (Cd, Cr and Ni) and

574 naphthalene (**Table S19**). 14 additional *tpm* runoff OTUs matched with sequences reported
575 among recently mobilized urban deposits and containing high chrysene concentrations. To
576 illustrate, Otu00002 (*Pseudomonadaceae*, n=11433 reads), Otu00016 (unclassified
577 *Gammaproteobacteria*, n=5291), Otu00026 (unclassified *Gammaproteobacteria*, n=4410),
578 and Otu00028 (Proteobacteria, n=4388) sequences from runoff water matched Otu013,
579 ASV_38, ASV_51 and ASV_35 of Aigle et al. (2021) (**Table S19**). Otu00036 affiliated to *P.*
580 *syringae* pathovar *aceris* showed higher number of reads among old sediments containing
581 high concentrations of naphthalene, cadmium, chrome and nickel (Aigle et al., 2021).

582 - *correlation network analyses*

583 Co-occurrence networks computed by the MENA approach led to the identification of K-
584 like bacterial modules (termed Mod-*tpm*) supported by Spearman Rho factors > 0.7 (**Table**
585 **S21; Fig. 6b**). Mod1-*tpm* showed significant (p-value < 0.001) positive correlations with the
586 relative quantities of faeces (0.42) and hygienic wastes (0.42) differentiating social behaviors
587 associated with group IV (**Fig. 1**). Mod2-*tpm* species were negatively correlated with faeces (-
588 0.50), and Mod3-*tpm* species showed positive relationships with merchandise storage
589 activities (0.51) (**Fig. 6b**). The freight lift activities representing the c1 variables (**Fig. 3**) were
590 negatively related to Mod4-*tpm* (-0.90). Multiple linear regression analyses (performed as
591 described in **Fig. S8a**) were run to find r-like distribution patterns in the *tpm* dataset (**Fig.**
592 **S10**). Numbers of *tpm* reads allocated to several species were found correlated to physico-
593 chemical parameters: (i) those of *Xanthomonas hortorum* and *Pseudomonas amygdali* were
594 negatively correlated to conductivity values ($R^2 = -0.023$ and -0.05), and (ii) those of
595 *Pseudomonas sp. Gp_BS* were positively correlated to turbidity ($R^2 = 0.05$).

596 **4. Discussion**

597 Urban surface microbial communities are expected to be made of (i) opportunistic
598 colonizers (also termed r-strategists) coming from exogenous sources, and transiently taking

599 benefit of the nutrients available in these systems, and (ii) of core taxa (termed K-strategists)
600 adapted to the living conditions prevailing among the colonized catchment including high
601 variations in temperature, water content, and chemical pollutants. The ecology of these core
602 and opportunistic taxa among urban biomes remain to be defined in order to avoid growth of
603 undesirable species and hazards such as human exposure to pathogens. Furthermore, the
604 spread and impact of synurbic bacterial taxa over the earth need to be evaluated. The
605 Anthropocene era might be gradually leading to a major invasion of the earth's ecological
606 systems by these taxa. To evaluate these impacts, the core components of urban microbiomes
607 need to be defined, and be tracked over other systems.

608 Field surveys were carried out to identify key socio-urbanistic variables impacting the
609 investigated catchment and related microbiomes. This led to the identification of sub-
610 catchments (sampling points) with common economical and industrial activities, leading to
611 similar social behaviors, that could be used as references to infer relationships with particular
612 bacterial taxa. More than 500 industries, and more than 10 000 observations were considered
613 to assess the main socio-urbanistic forces impacting the investigated urban catchment. This
614 catchment was found to be divided into two groups (termed I and II) of industrial /
615 commercial activities, and two groups (termed III and IV) of social behaviors. Interestingly,
616 streets with high numbers of gasoline engines (group III sites) were significantly associated
617 with restaurants while areas with high numbers of pedestrians were associated with higher
618 numbers of wastes. Furthermore, quiet streets and dead ends showed more traces of fecal
619 matters, and night activities (group IV). Previous investigations have shown that traffic was a
620 major contributor of surface sediments and pollutants (Revitt et al., 2014). The above groups
621 and panels of activities were used to investigate relationships with FIB, MST (microbial
622 source tracking) DNA markers, and DNA meta-barcoding datasets. All these datasets

623 confirmed the informative character of runoff waters to estimate bacterial diversity over sub-
624 catchments, and identify K-bacterial strategists living in an urban catchment.

625 *Relationships between socio-urbanistic features and runoff contents in classical bacterial*
626 *indicators*

627 Significant contents in FIB were observed all over the investigated catchment among the
628 runoff waters. These taxa (*E. coli* and intestinal *Enterococci*) were clearly core K-like
629 bacterial strategists because of recurrent fecal contaminations coming from multiple sources
630 including man, making available the appropriate C-sources for their survival and growth.
631 Their high values were in line with those of Paule-Mercado et al. (2016) which showed an
632 increase in FIB among urban settings, and related these increases to surface
633 impermeabilization. Group IV sub-catchments (quiet streets) showed the highest FIB numbers
634 in their runoffs, and were associated with the highest occurrences of HF183 MST DNA
635 signatures indicative of human fecal matters. Group a1 variables grouping all sorts of gasoline
636 engines, and variables associated with merchandise storage areas were negatively correlated
637 to FIB contents of the runoffs. These results are in agreement with a previous study which
638 showed road pollutants such as PAHs to be negatively correlated to FIB contents (Bernardin-
639 Souibgui et al., 2018). Such pollutants were suggested to reduce their numbers (Ukpaka et a
640 al., 2014). In fact, only variables indicative of the occurrences of wastes and fecal matters
641 were found positively associated with high FIB contents in the runoffs. These observed high
642 contents of FIB in city runoff samples were not surprising, and similar trends had previously
643 been reported for other urban contexts (Ellis, 2004; Lee et al. 2020; Ahmed et al., 2010; Sauer
644 et al., 2011; Sidhu et al., 2012; Chong et al., 2013). However, the relationships between FIB,
645 urbanistic issues, and social behaviors were inferred for the first time.

646 Integrons of class I, previously shown to have an abundance positively correlated with the
647 ones of urban pollutants (Wright et al., 2008), were also observed in the collected runoff

648 waters. These integrons can harbor resistance genes toward antibiotic, metallic elements and
649 disinfectants. Class I integron DNA targets were detected in about 40% of the runoff water
650 samples, and showed a higher occurrence among group III sites associated with high numbers
651 of motor-engines generating petrol-related pollutants. As expected, the distribution of
652 integron 1 genetic elements did not match the one of fecal pollutions, and confirmed that
653 runoff waters DNA imprints can differentiate ecological trends between sub-catchments.

654

655 **K-like bacterial strategists among urban surface microbiomes**

656 - **Inferences from 16S rRNA meta-barcoding analyses**

657 As indicated above, *E. coli* and intestinal enterococci were clearly demonstrated, in this
658 study, to be part of the K-bacterial taxa colonizing urban surfaces. DNA meta-barcoding
659 analytical schemes further confirmed the success of such fecal bacterial taxa over the city. In
660 fact, fecal bacterial DNA reads reported by McLellan and Roguet (2019) and Noyer et al.
661 (2020) among the 16S rRNA meta-barcoding dataset could be tracked, and their distribution
662 patterns were in line with the ones of the FIB and HF183 MST marker for human fecal
663 contaminations. The 16S rRNA gene reads from *Bacteriodes*, *Cloacibacterium*, and
664 *Macellibacteroides* showed a high prevalence over the catchment, and were found to be part
665 of the core urban taxa likely harboring K-strategists. However, DNA sequence reads allocated
666 to fecal *Arcobacter* and the *Ruminococcus* showed more variations in their distribution
667 patterns, and these genera were considered to be part of the r-opportunistic taxa.

668 Overall, twenty-eight core genera likely harboring K-strategists were found conserved
669 among the sampling points of the catchment. Most of these genera had previously been
670 reported among other urban settings such as *Blastococcus*, *Massilia*, *Flavobacterium*, and
671 *Sphingomonas* (McLellan et al., 2015; Marti et al., 2017; Leung et al., 2014; Robertson et al.,
672 2013). Nevertheless, a few discrepancies in the bacterial taxa distribution patterns between
673 those recorded in this work and other reports were observed. In fact, *Spirosoma*, *Alkanindiges*,
674 *Rubellimicrobium*, and *Novosphingonium* 16S rRNA gene reads were recorded all over the
675 investigated catchment but not always in the other urban settings. Conversely, some taxa had
676 been described as recurrent in other reports among the urban microbiome but here showed
677 more versatile r-like patterns as observed for *Acidibacter*, *Psychrobacter* and
678 *Propionibacterium*. This is in line with the hypothesis of a selection of specialized taxa by
679 certain urban morphotypes as suggested by Saxena et al. (2015). Here, the investigated

680 morphotype showed high industrial and economical activities with little green areas and no
681 residential zones. This morphotype was previously shown to be associated with a high
682 occurrence of chemical pollutants and related microbial degraders through the analysis of
683 sediments and deposits transferred into the detention basin located at the extremity of the
684 catchment (Aigle et al., 2021; Marti et al., 2017). Correlation network analyses confirmed this
685 conclusion. The largest modules of correlated distribution patterns between genera (mainly
686 made of *Actinobacteria*) was associated to hydrocarbon-degraders. The most significant K-
687 like actinobacterial taxa among these modules were *Modestobacter*, *Williamsia*,
688 *Marmoricola*, and *Blastococcus*, but also involved three *Bacteroidetes* (*Cytophaga*,
689 *Flavobacterium* and *Pedobacter*) and a γ proteobacteria (*Pseudospirillum*). This module was
690 positively correlated with variables of group II (large avenue) and III (high number of
691 gasoline engines), but was inversely correlated to group IV variables related to hygienic
692 wastes. It is to be noted that bacterial taxa previously recorded in high numbers among road
693 sediments/deposits (*Peredibacter*, *Aquabacterium*, *Paludibacter* and *Cloacibacterium*) by
694 Marti et al. (2017) were found among the sampled runoffs. The broad distribution pattern of
695 these taxa confirmed their tropism for city surface habitats.

696 - **Inferences from *tpm* meta-barcoding analyses**

697 The *tpm* meta-barcoding marker allowed a deeper characterization of some of the K core
698 taxa down to the species level. Nine species were highly conserved over the catchment:
699 *Pseudomonas koreensis*, *Herbaspirillum aquaticum*, *P. rhodesiae*, *P. stutzeri*, *P. syringae*, *P.*
700 *anguilliseptica* and *P. aeruginosa*. Interestingly, three of these species, *P. stutzeri*, *P.*
701 *aeruginosa*, and *P. koreensis*, were also part of the most common taxa observed in urban
702 transit systems (Danko et al., 2021), confirming their synurbic status. Some of these K core
703 species were previously shown to use hydrocarbons as C-sources such as *P. aeruginosa* and
704 *P. stutzeri*. Moreover, *P. koreensis* was reported to degrade, among others, hexadecane,

705 engine oil, pyrene and phenanthrene (Bučková et al., 2013). *H. aquaticum* and *P. stutzeri* are
706 also known producers of polyhydroxyalkanoates and polyhydroxybutyrate storage molecules
707 allowing survival under extreme growth conditions (Yan et al., 2008; Langenbach et al., 2006;
708 Timm and Steinbuechel, 1992) such as those occurring over road surfaces. *H. aquaticum tpm*
709 reads were highest among the most polluted sites (those showing a drop in pH values). These
710 bacteria are known to be plant growth-promoting rhizobacteria (Marques et al., 2015) but had
711 not been associated with urban pollutants, so far. Correlation network analyses were
712 performed with the *tpm* metabarcoding dataset to further evaluate the occurrence of K-like
713 strategists, and revealed a large module of correlated species associated with hygienic wastes.

714 **r-like bacterial strategists among urban surface microbiomes**

715 Multilinear regression models and DESeq2 analyses highlighted r-like genera. Point source
716 pollutions revealed by changes in pH values were related to environmental bursts of
717 *Novosphingobium* and *Sulfurospirillum* in the runoff waters of a few sub-catchments. These
718 bursts were associated with an unusual drop in *Actinobacteria* (0.7 %). The BASOL database
719 of the French ministry of the ecological and solidarity transition ([https://](https://basol.developpement-durable.gouv.fr/)
720 basol.developpement-durable.gouv.fr/) indicated a recurrent pollution by trichloroethylene
721 and halogenated solvents in this area. *Sulfurospirillum* was previously found to be enriched by
722 organohalides, arsenate and selenite, and chlorinated ethenes (Luijten, 2003; Nijenhuis et al.,
723 2005; Buttet et al., 2018). Similarly, *Novosphingobium* was previously found to be enriched
724 by phenol, aniline, nitrobenzene and phenanthrene (Liu, 2005; Sohn, 2004; Jiao et al., 2017).

725 RDA of the *tpm* meta-barcoding dataset also revealed r-like taxa such as *Pseudomonas*
726 *extremaustralis*. In fact, one OTU of this species showed positive relationships with car
727 numbers and the quantity of food wastes over the catchment. This species was previously
728 shown to degrade phenols and alkanes (Tribelli et al., 2012). Other r-like *tpm*-harboring
729 species likely taking benefits from the occurrence of hydrocarbon pollutants over the

730 catchment were further detected by Faprotax analyses such as *Aeromonas jadensis*, *P. putida*,
731 *P. mendocina*, *P. pseudoalcaligenes* and *P. fluorescens*. Furthermore, three OTUs showed a
732 distribution pattern indicative of an opportunistic development among old deposits containing
733 high concentrations of poorly degradable pollutants including metallic trace elements (Cd, Cr
734 and Ni) and naphthalene, and fourteen among recent deposits/sediments previously shown
735 enriched in chrysene content (Aigle et al., 2021).

736 **Microbiological hazards associated with urban surface microbiomes**

737 Previous reports suggested an increase in virulence gene loads among K-strategists from
738 highly selective systems (e. g. Song et al., 2017). Bacterial pathogens were thus searched
739 among the *tpm* meta-barcoding dataset, and their distribution biases were analyzed. The
740 following bacterial pathogens were recorded over the catchment: *Aeromonas hydrophila*, *A.*
741 *caviae*, *Stenotrophomonas maltophilia*, *P. aeruginosa*, *P. anguilliseptica*, *P. mendocina*, *P.*
742 *syringae*, *P. amygdali*, *Xanthomonas axonopodis*, *X. hortorum* and *X. cannabis*. The latter
743 species, *X. cannabis*, was the most common *tpm*-harboring phytopathogen recorded in the
744 samples, and was previously described to infect more than 350 plant species including
745 *Cannabis sativa* (Netsu et al., 2014). However, most of these *tpm*-harboring bacterial
746 pathogens appeared to have r-like distribution patterns except *P. aeruginosa* and *P. syringae*.
747 Viable *P. aeruginosa* cells had previously been recorded among urban sediments of the
748 detention basin of this catchment at about 10^5 CFU g dw⁻¹ (Bernardin-Souibgui et al., 2018).
749 Furthermore, *P. aeruginosa* was found to have a conserved distribution pattern in these
750 sediments, whatever their pollution content, through the *tpm* meta-barcoding approach (Aigle
751 et al., 2021). This latter species was thus clearly part of the K strategists being able to survive
752 and grow over the city surfaces. Interestingly, variations in *P. aeruginosa* cell counts or of
753 their matching *tpm* DNA read numbers were not found correlated with the FIB dataset
754 suggesting distinct habitats over the catchment.

755 Urban socio-urbanistic patterns associated with the distribution of the *tpm* sequence types
756 of *P. aeruginosa* and *P. syringae* were investigated at the sub-species level. This led to the
757 first observation of *P. s. pathovar aceris*, *P. s. panici*, *P. s. japonica*, *P. s. aptata*, *P. s.*
758 *maculicola*, and *P. s. pisi* among urban runoffs. Otu00036 affiliated to *P. syringae* pathovar
759 *aceris* previously showed higher number of reads among old sediments containing high
760 concentrations in naphthalene, cadmium, chrome and nickel (Aigle et al., 2021). However,
761 this latter OTU did not show any particular distribution biases over the catchment. Regarding
762 *P. aeruginosa*, using the correspondence table reported by Colin et al. (2020), the recorded
763 *tpm* sequences could be attributed to PAO1- and PA14-like sub-clades and MLST groups
764 such as ST252 involved in non-CF (cystic fibrosis) infections, and ST2123/ST226 and
765 ST175/ST2042 found in lung, urinary tract and blood infections. The PA14 OTU00056
766 showed a tropism for sampling sites with high numbers of gasoline engines, suggesting a use
767 of petrol-derivatives as C-sources. The above classifications were validated through the
768 analysis of *P. aeruginosa* isolates obtained from runoffs. These analyses confirmed the
769 occurrence of bacterial pathogens among urban surface K-strategists. However, contribution
770 of their virulence properties in their establishment among urban surface microbiomes remains
771 to be demonstrated.

772 **5. Conclusions**

773 Understanding the complexity of urban surface microbiomes requires (i) the identification of
774 the sources of microbial taxa seeding these systems, and (ii) of the key selective forces
775 triggering changes in their assemblages, and favoring the emergence of efficient functional
776 units. Here, socio-urbanistic surveys were undertaken to better define these forces and
777 sources. The surveyed variables led to significant groupings of urban typologies matching
778 segregations observed among the microbiological datasets (FIB, MST, DNA meta-barcoding)
779 generated from runoff samples. This was clearly indicative of well-established K-like

780 synurbic bacterial taxa over the catchment. The most significant selective forces explaining
781 the success of these K taxa were related to urban pollutants. A large module of co-occurring
782 actinobacterial taxa involved in hydrocarbon degradation was resolved from the 16S rRNA
783 gene dataset. Among these K-like hydrocarbon degraders, bacterial pathogens were observed
784 such as *P. aeruginosa* and *P. syringae*. These urban surface bacterial assemblages were also
785 found to be made of opportunistic r-like taxa. In fact, an industrial point source pollution
786 likely involving trichloroethylene and halogenated solvents was associated with a significant
787 re-shuffle of the microbiota, and a burst in *Novosphingobium* and *Sulfurospirillum*. The high
788 impact of urban pollutants on these urban microbiomes was also further supported by the
789 observation of r-like *tpm*-harboring species known to play part in hydrocarbon degradation
790 such as *P. extremaustralis*.

791 The generated meta-barcoding datasets presented here, and the investigated long – term
792 experimental site, will be highly useful for studies to come on the evaluation of the ecological
793 benefits associated with a reduction of gasoline engines over a city. The evolving state of the
794 genetic structures of a surface urban microbiome is likely to be a very good indicator of the
795 ecological benefits associated with such changes.

796 **Credit authorship contribution statement**

797 **Rayan Bouchali:** conceptualization, data curation, formal analysis, writing - original draft.

798 **Claire Mandon:** conceptualization, data curation, formal analysis. **Romain Marti:**

799 conceptualization, data curation, formal analysis. **Jérôme Michalon:** conceptualization,

800 formal analysis. **Axel Aigle:** formal analysis. **Laurence Marjolet:** data curation, formal

801 analysis. **Sophie Vareilles:** conceptualization, data curation. **Gislain Lipeme-Kouyi:**

802 conceptualization, data curation and analyses, writing - review & editing. **Philippe Polomé:**

803 data curation and analyses, writing - review & editing. **Jean-Yves Toussaint:** data curation,

804 writing - review & editing. **Benoit Cournoyer:** conceptualization, data curation, formal

805 analysis, funding acquisition, project administration and supervision, writing - original draft,

806 writing - review & editing.

807 **Declaration of Competing Interest**

808 The authors declare that they have no known competing financial interests or personal

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1019 **Figure captions**

1020 **Fig. 1.** Graphical representations of (A) the Mi-plaine catchment of Chassieu (Suburbs of
1021 Lyon, France) and (B) of the adjusted Wallace inferences indicating organizational
1022 proximities between the observed industrial and commercial activities of this catchment. (A)
1023 Relative importance of shops, restaurants and industries in terms of number of employees was
1024 indicated by a grey scale (dark values = high numbers). Runoff water sampling sites (C code)
1025 are numbered and positioned on the map. The outlet of the watershed is located at C23, and
1026 corresponds to a stormwater infiltration system (SIS). Industrial, commercial, and social
1027 activities and their traces are listed in **Tables S1** and **S2**. These activities were analyzed over
1028 an area of 50 m of diameter around each sampling point. In (B), Adjusted Wallace inferences
1029 allowed to identify two large groups, I (brownish dotted lines) and II (dark green dotted lines)
1030 of industrial/commercial activities. In (A), groups III (red background – high intensity area)
1031 and IV (blue background – low intensity area) were defined according to significant
1032 relationships inferred from the behavioral variables. Representative sites of groups III and IV
1033 are shown in **Fig. 2**. **Fig. 3** indicates the main socio-urbanistic variables impacting these sites.
1034 Yellow circles in (A) indicate human fecal contaminations, yellow and green circles indicate a
1035 human fecal contamination associated with the presence of integron 1, and green circles of
1036 integron 1. *E. coli* and intestinal enterococci were recorded among all sub-catchments (**Table**
1037 **S3**).

1038 **Fig. 2.** Representative urban typologies for the socio-urbanistic groups III and IV observed
1039 over the Mi-plaine catchment. Group III was illustrated by sampling point C19, and group IV
1040 by C18. See **Table S1** for a description of the other sampling points. Aerial views show the
1041 runoff water flow directions which led to a delimitation of the sub-catchment. Economical
1042 activities, traffic intensity, human behaviors and wastes recorded during the socio-urbanistic
1043 surveys are also indicated by symbols defined on the figure. Photographs (the yellow arrow

1044 indicates the direction of the selected view) illustrate the main typologies observed over the
1045 selected sub-catchments, and were taken from the sections indicated by dotted yellow
1046 rectangles on the aerial views. Position of the sampling code numbers (C18 or C19) indicated
1047 the point of collect of runoff waters. Human fecal contamination is indicated by a small
1048 yellow circle while the yellow/green circle indicates a human fecal contamination associated
1049 with integron 1.

1050 **Fig. 3.** Spearman correlation tests exploring relationships between the repartition of industries
1051 and commercial activities, and of general descriptors of human activities, over the Mi-plaine
1052 urban catchment. Only Rho factors > 0.6 that linked significantly ($p < 0.05$) these variables
1053 were considered to draw these networks. Green circles represent the surveyed industries;
1054 orange ones represent general descriptors of human activities. Variables in agreement with the
1055 definition of Group II (shown in Fig. 1) are circled with a plain line. Socio-urbanistic
1056 variables with high correlations are shown with dotted circles, and the selected representative
1057 variables of these groups were circled with a plain line. The datasets are shown in Tables S2b,
1058 c.

1059 **Fig. 4.** NMDS representation of the Bray-Curtis dissimilarity matrix (**Table S13**) computed
1060 from the 16S rRNA gene OTU profiles (**Table S6**) of runoff water samples (except C14)
1061 according to the groups of sampling points defined by the socio-urbanistic surveys. (a) group I
1062 and II and (b) group III and IV. See **Figs. 1 and 2** and text for a description of these groups.
1063 Adonis statistical tests showed significant differentiation between 16S rRNA gene OTU
1064 profiles of (a) groups I and II, and (b) groups III and IV, ($p < 0.01$).

1065 **Fig. 5.** Chord diagram showing the distribution of the 19 dominant 16S rRNA-inferred genera
1066 (relative abundance $> 0.5\%$) among the runoff water samples from the investigated urban
1067 catchment. Full dataset of taxonomic allocations is shown in **Table S11**. These genera

1068 grouped about half of the reads and almost 80% of those that could be allocated to well-
1069 defined taxa. The average relative abundance (among the three sampling campaigns) is
1070 indicated for each genus. Genera in bold were affiliated to the core-microbiome (present in all
1071 the samples).

1072 **Fig. 6.** Correlation networks computed from the fast greedy modularity optimization method
1073 of the MENA pipeline for (a) the 16S rRNA gene inferred genera and (b) the *tpm*-harboring
1074 bacterial species. Only bacterial taxa detected in at least 25 (for the 16S rRNA gene dataset)
1075 or 10 (for the *tpm* one) runoff water samples were used to compute the correlation networks.
1076 Spearman correlations above 0.7 are presented. Global properties of the two networks are
1077 indicated in Tables S14 and S21. Module-EigenGene analyses were computed to highlight
1078 relationships between 16S rRNA-based or *tpm* modules, and the physico-chemical or socio-
1079 urbanistic variables. The significant correlation ratios between the modules and the
1080 explanatory variables are indicated in boxes. Only correlations over 0.5 (for the 16S rRNA
1081 gene dataset) or 0.4 (for the *tpm* one) are shown. Asterisks indicate Spearman correlations p-
1082 values < 0.001. Genera and specie showed in bold indicate taxa involved in hydrocarbon
1083 degradation.

1084 **Fig. 7.** Chord diagram showing the distribution of the 44 dominant *tpm*-harboring bacterial
1085 species (relative abundance > 0.1 %) among the runoff water samples from the Mi-Plaine
1086 catchment of Chassieu (**Table S16**). These species grouped 45 % of the *tpm* reads. Average
1087 relative abundances (among the three sampling campaign) of each *tpm*-harboring bacterial
1088 species are indicated. Species in bold were affiliated to the core-microbiome of the runoff
1089 water and had a prevalence >50%.

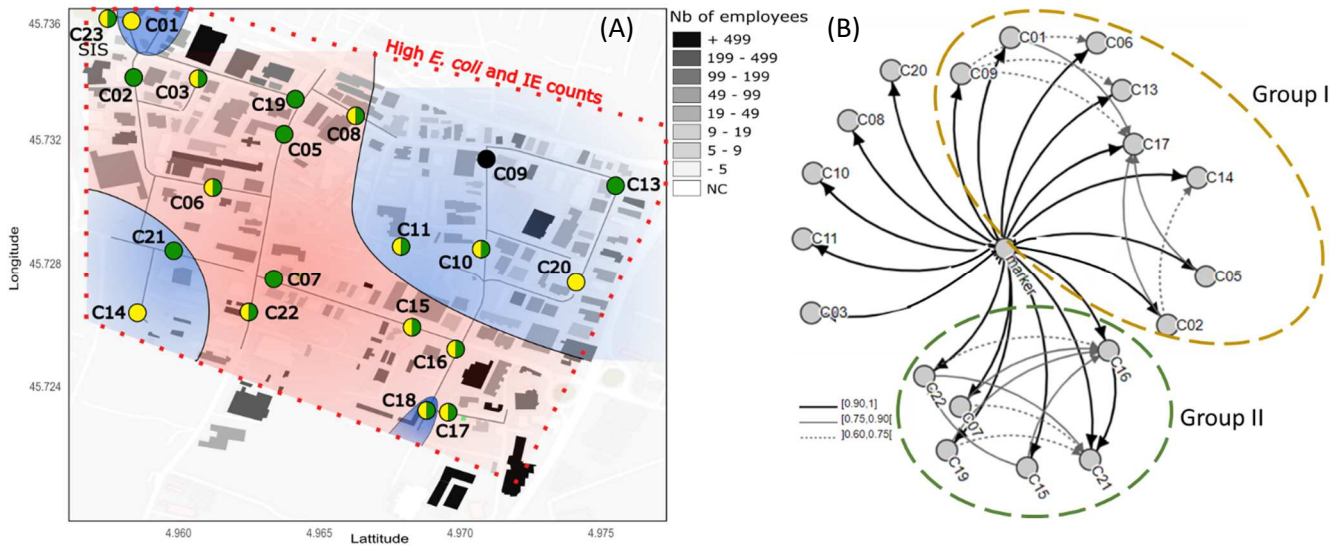
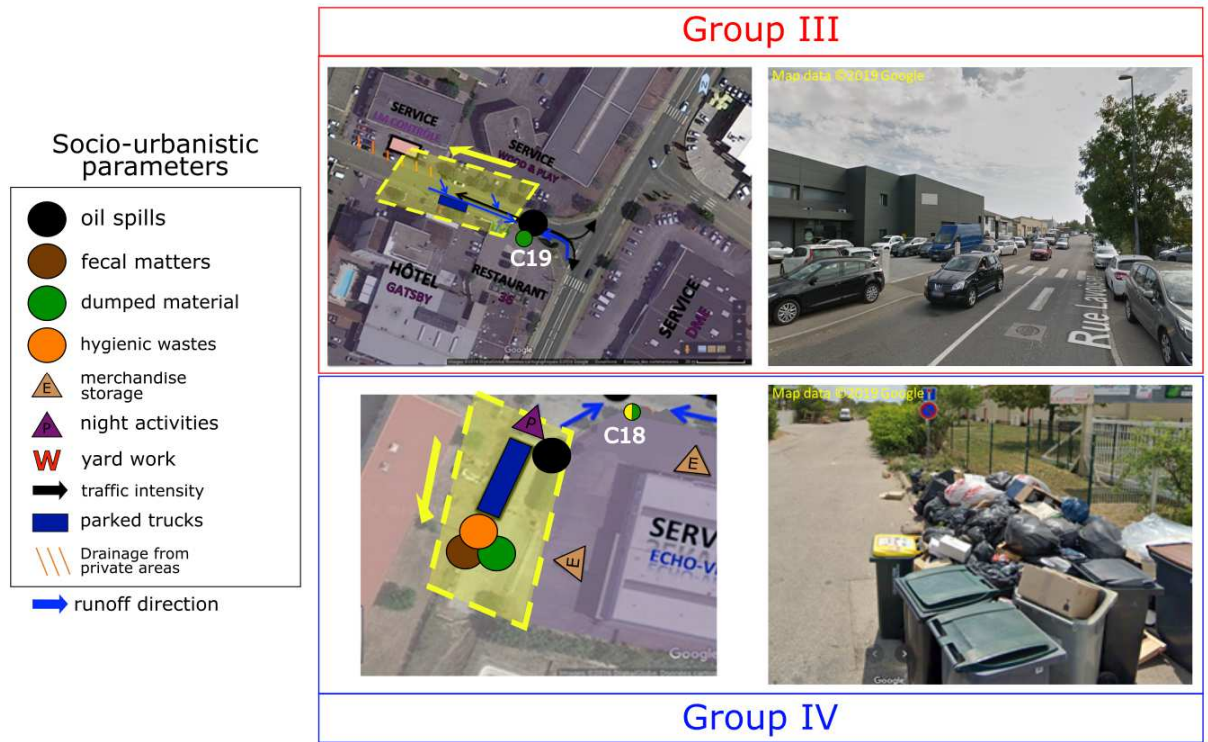
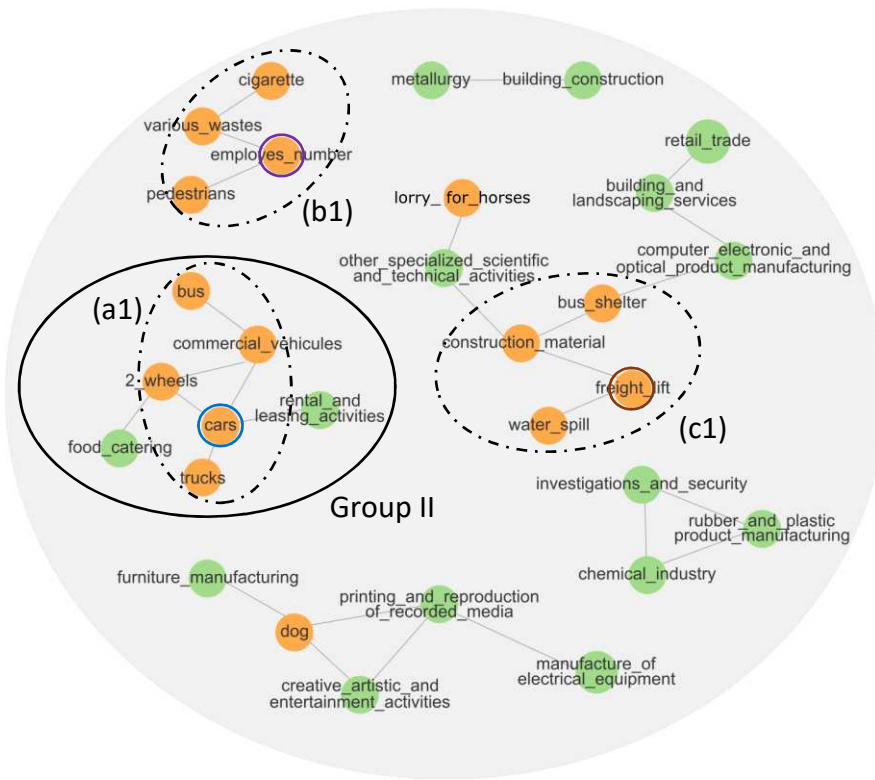


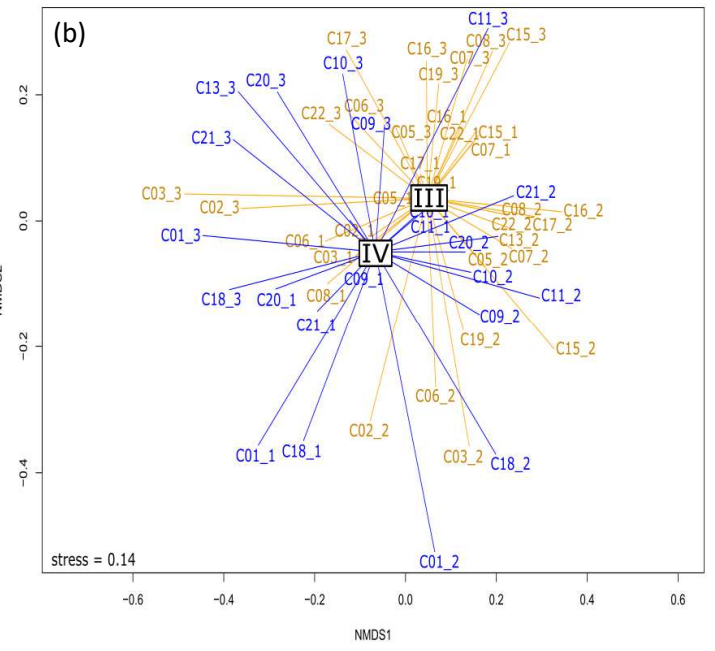
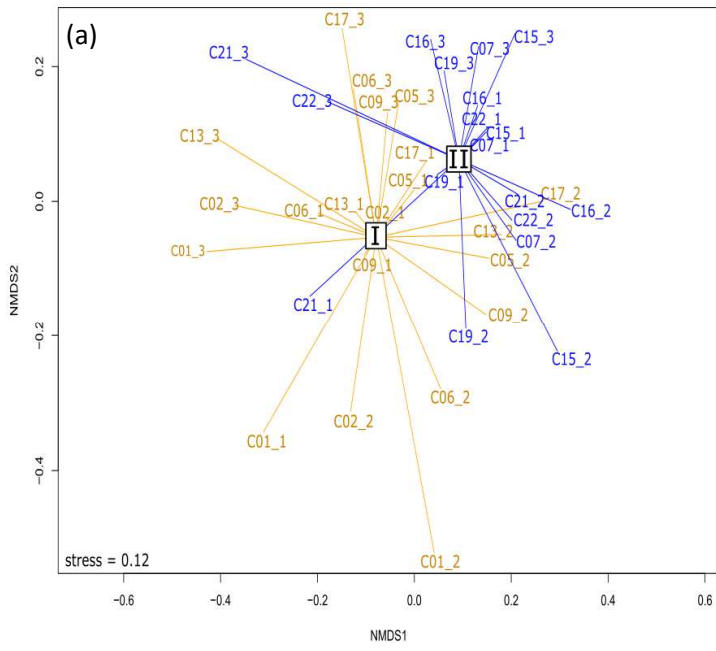
Figure 1. Bouchali et al.



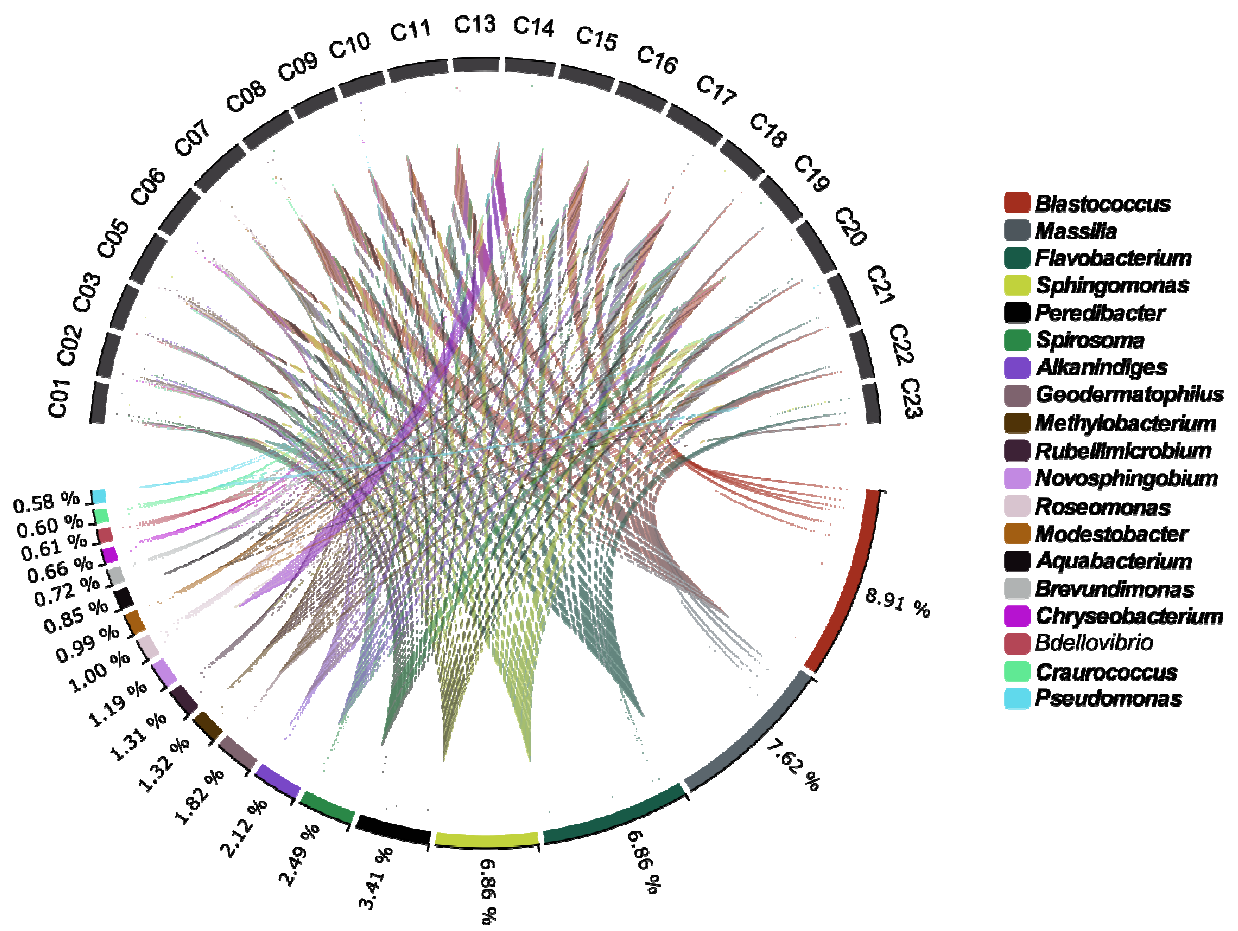
Bouchali et al. Fig. 2



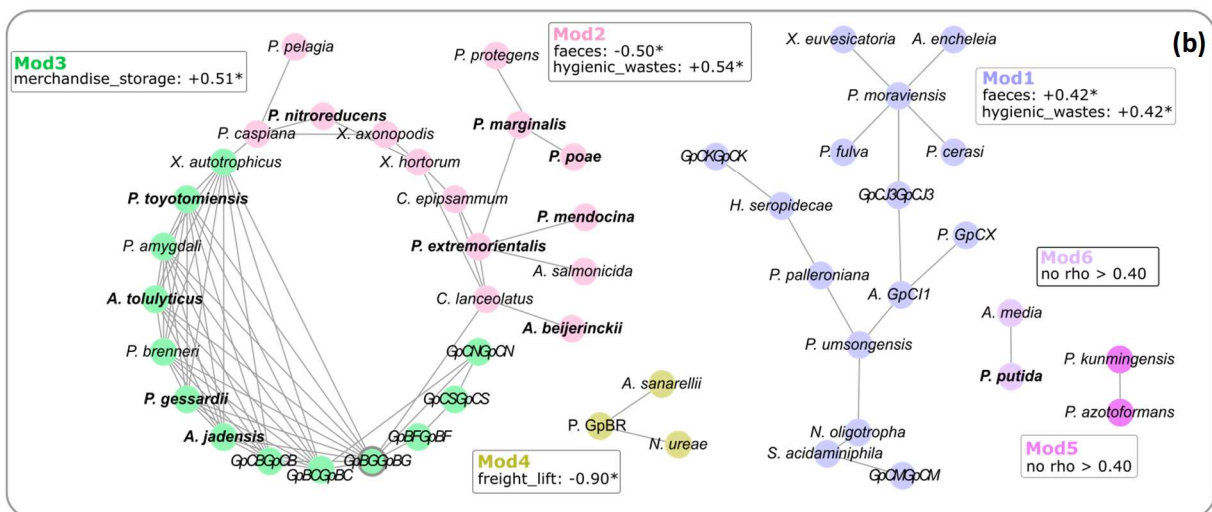
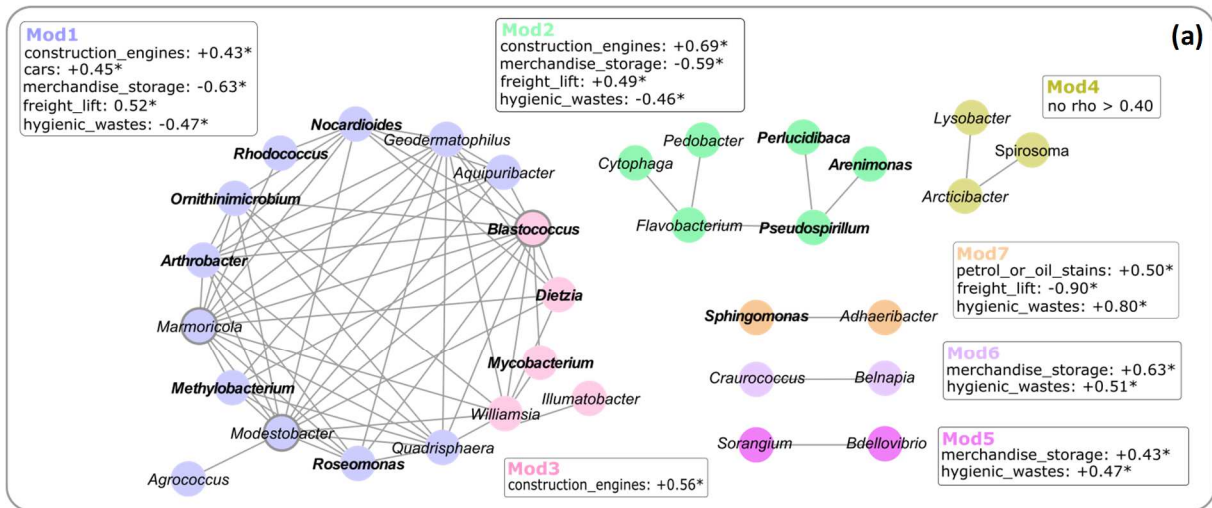
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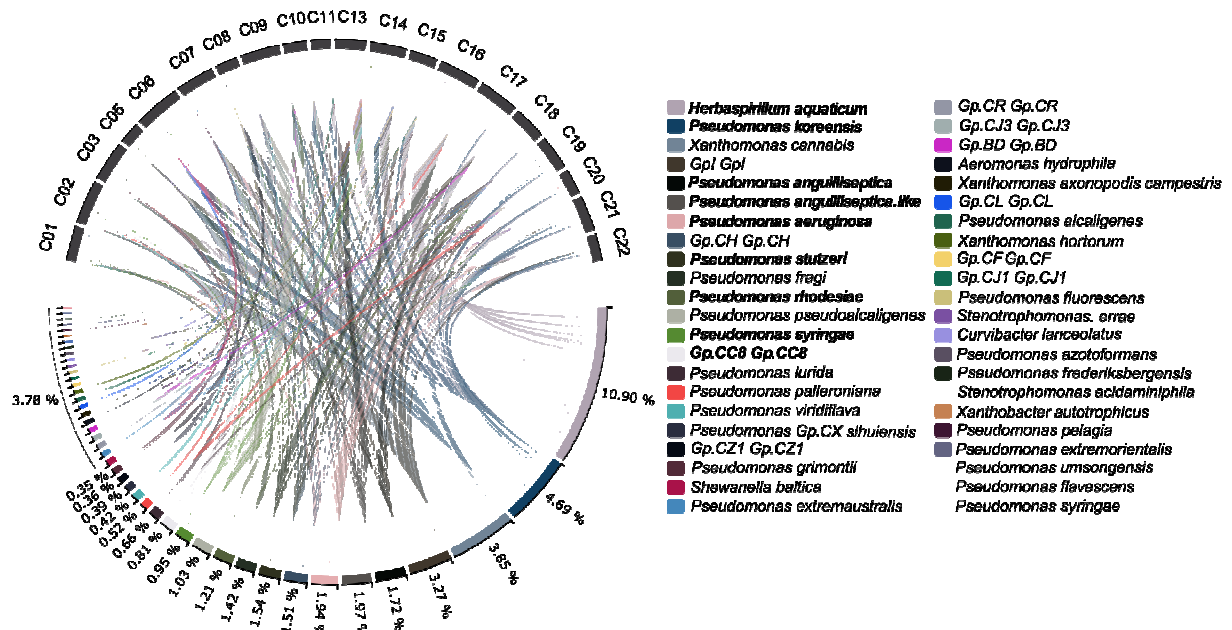
Bouchali et al. Fig.4



Bouchali et al. Fig. 5



Bouchali et al. Fig. 6



Bouchali et al. Fig. 7

GRAPHICAL ABSTRACT

