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1 **Pollution gradients shape microbial communities associated with *Ae. albopictus* larval**  
2 **habitats in urban community gardens**

3  
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16  
17 **Abstract**

18 The Asian tiger mosquito *Aedes albopictus* is well adapted to urban environments and takes  
19 advantage of the artificial containers that proliferate in anthropized landscapes. Little is  
20 known about the physicochemical, pollutant and microbiota compositions of *Ae. albopictus*-  
21 colonized aquatic habitats and whether these properties differ with noncolonized habitats.  
22 We specifically addressed this question in French community gardens by investigating  
23 whether pollution gradients (characterized either by water physicochemical properties  
24 combined with pollution variables or by the presence of organic molecules in water) influence  
25 water microbial composition and then the presence/absence of *Ae. albopictus* mosquitoes.  
26 Interestingly, we showed that the physicochemical and microbial compositions of  
27 noncolonized and colonized waters did not significantly differ, with the exception of N<sub>2</sub>O and  
28 CH<sub>4</sub> concentrations, which were higher in noncolonized water samples. Moreover, the

29 microbial composition of larval habitats covaried differentially along the pollution gradients  
30 according to colonization status. This study opens new avenues on the impact of pollution on  
31 mosquito habitats in urban areas and raises questions on the influence of biotic and abiotic  
32 interactions on adult life history traits and their ability to transmit pathogens to humans.

33

34 **Key words:** *Aedes albopictus*, pollution, urbanization, water, microbial communities, organic  
35 molecules, physicochemical parameters

36

37 **Running title:** Water microbial and chemical factors of larval habitats

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## 40 Introduction

41 Roughly 3% of the Earth's land surface is occupied by urban areas (CIESIN et al,  
42 2011). The rapid rise in the size, density and heterogeneity of urban areas has had deep  
43 impacts on urban populations, biodiversity and climate (Vlahov 2002; Li et al. 2022; Khanh et  
44 al. 2023). Rapid urban expansion has led to an increase in poverty, social inequality,  
45 temperature (through the urban heat island –(UHI) effect), and homogenization of  
46 biodiversity and has also generated much waste and pollution (Kalnay and Cai 2003;  
47 McKinney 2006; Dociu and Dunarintu 2012; Liddle 2017; Liang et al. 2019). A promising  
48 solution for reducing the negative impacts of urbanization is the creation of green areas and  
49 addition of vegetation in cities (Susca et al. 2011; Gago et al. 2013; Pascal et al. 2019).  
50 Urban green areas are efficient in remedying UHIs by cooling effects in cities (Norton et al.  
51 2015). In addition, vegetation and green spaces have been shown to reduce atmospheric  
52 pollution, and have had a positive impact on human health by attenuating mortality and  
53 improving mental health through increasing physical activities (Lepczyk et al. 2017; Kondo et  
54 al. 2018; Nunho Dos Reis et al. 2022; Royer et al. 2023). Green urban spaces include  
55 different types ranging from parks and gardens to cemeteries and derelict lands (Catalano et  
56 al. 2021). Among them, community gardens are increasingly implemented in European cities  
57 (Ochoa et al. 2019). The concept of the family garden (formerly known as workers' gardens)  
58 emerged during the Industrial Revolution, in a period of intense urbanization to mitigate the  
59 problem of the precariousness of workers (Keshavarz et al. 2016). Currently, diverse types of  
60 community gardens exist, e.g., family gardens, shared gardens and integrated gardens.  
61 These urban gardens are managed by different organizations, including metropolises,  
62 municipalities, private organizations and nonprofit associations (Holland 2004).

63 Urban community gardens play a crucial role in promoting biodiversity (Jha et al.  
64 2023). They often feature a variety of plant species that attract pollinators such as bees,  
65 butterflies, and other beneficial insects, ensuring a continuous supply of nectar and pollen for  
66 both bee and non-bee species (Schmack et al, 2023). This supports the health of urban  
67 ecosystems and facilitates the pollination of nearby plants and crops. For example, a study in

68 Dunedin, southern New Zealand, found Collembola, Amphipoda, and Diptera to be the most  
69 abundant taxa in 55 domestic gardens (Baratt et al, 2015). Mosquitoes, like other insects, are  
70 attracted to and feed on both floral and extrafloral nectar as an energy source (Jhumur et al,  
71 2008; Nyasembe and Torto, 2014). While male mosquitoes need nectar to survive, the  
72 sugars from nectar help female mosquitoes increase their life expectancy, survival rate, and  
73 reproduction (Foster, 1995). Interestingly, recent observations suggest that mosquitoes could  
74 also act as pollinators (Lahondere et al, 2020). In addition to the presence of diverse plant  
75 species, urban community gardens often contain a high density and diversity of still water  
76 containers (e.g., watering cans, flower buddies, or rainwater collectors), which are suitable  
77 oviposition habitats for mosquitoes, including the Asian tiger mosquito (Hayden et al. 2010,  
78 Duval et al. 2022). Oviposition site selection is a critical behavior that influences egg hatching,  
79 juvenile development rate, and larvae or pupae survival. To locate floral scents and suitable  
80 oviposition sites, mosquitoes utilize a highly sensitive olfactory system that integrates visual,  
81 gustatory, and olfactory cues (Bentley and Day, 1989; Barredo et al, 2020). Females can  
82 identify aquatic habitats conducive to the development and survival of their offspring based  
83 on characteristics such as color, size, and sunlight, assessing water quality through olfactory  
84 and tactile cues (Day, 2016). Recent studies have also highlighted the role of microbe-  
85 derived volatiles as environmental cues influencing mosquito foraging and oviposition  
86 behavior decisions (Afify and Galizia, 2015; Girard et al, 2021; Peach et al, 2021; Sobhy and  
87 Berry, 2024).

88 The proliferation of the Asian tiger mosquito *Aedes albopictus* constitutes a major  
89 public health challenge due to its ability to transmit more than 19 arboviruses, such as  
90 dengue, zika and chikungunya, in humans (Paupy et al. 2009; Duval et al. 2023). Previous  
91 studies demonstrated that the number of larval habitats and the development and survival  
92 rates of *Ae. albopictus* were positively impacted by the urban context (Li et al. 2014; Wilke et  
93 al. 2019; Westby et al. 2021). In urban areas, this species breeds in human-made  
94 containers, where water could be exposed to various sources of pollutants. The biotic and  
95 abiotic characteristics of larval habitats determine the choice by gravid females for

96 oviposition and obviously affect the development of offspring, from larvae to adults (Afify and  
97 Galizia 2015; Malassigné et al. 2020; Hery et al. 2021a; Dalpadado et al. 2022). For  
98 instance, physicochemical parameters such as pH, temperature, organic matter, phosphate,  
99 ammonia and potassium are known to guide females to choose egg-laying sites (Darriet  
100 2019). In addition, microbial communities produce volatile organic compounds that can be  
101 attractive or repulsive to mosquitoes (Díaz-Nieto et al. 2016; Weisskopf et al. 2021). More  
102 globally, the microbial structure in breeding sites partly shapes the larval microbiota and can  
103 have carryover effects on the physiology of adults and their ability to transmit pathogens  
104 (Dickson et al. 2017; Guégan et al. 2018; Zheng et al. 2023). However, reciprocal  
105 interactions between physicochemical parameters and microbial structure in artificial  
106 breeding sites and their impact on the mosquito life cycle are still poorly understood.

107 In mainland France (Europe), since the first identification of autochthonous cases of  
108 dengue in 2010, viral diseases transmitted by mosquitoes have tended to increase each  
109 year, as well as the number of regions at risk due to the continuous spread of the Asian tiger  
110 mosquito (Cochet et al. 2022). Following previous observations, the aim of this study was to  
111 evaluate whether and how human activities, particularly pollution gradients, impact the biotic  
112 and abiotic properties of *Ae. albopictus* larval breeding sites in community gardens in the  
113 Lyon metropolis, one of the largest and most populous cities in mainland France. This study  
114 mainly focused on containers mainly filled with rainwater but also occasionally supplemented  
115 with tap water, both of which may sometimes contain pollutants. For this purpose, we  
116 analysed the physicochemical and microbiological composition of water by comparing *Ae.*  
117 *albopictus*-colonized and *Ae. albopictus*-noncolonized waters as well as the microbiota  
118 composition of larvae. Microbial communities were analysed by characterizing bacterial and  
119 microeukaryotic communities through high-throughput sequencing. Physicochemical water  
120 properties (i.e., pH, temperature, conductivity, oxidation–reduction potential, turbidity and  
121 carbon content) and organic molecules were also characterized by using multiparameter  
122 probes, gas and ion chromatography and liquid chromatography coupled with high-resolution  
123 mass spectrometry (LC-HRMS). We then evaluated whether pollution gradients

124 (characterized either by water physicochemical properties combined with pollution variables  
125 reflecting proximity to polluted areas or by organic molecule presence in water) influence  
126 water microbial composition depending on the presence/absence of *Ae. albopictus*  
127 mosquitoes. While microbial and physicochemical compositions were very similar between  
128 colonized and noncolonized water samples, our results revealed differential effects of  
129 pollution gradients on microbial community structure according to water colonization status.

130

## 131 **Experimental procedures**

132

### 133 **Selection and delineation of the study areas**

134

135 By taking advantage of our recent database, in which we have inventoried a total of 288  
136 community gardens in the metropolitan area of Lyon (Duval et al. 2022), we characterized  
137 the gardens based on different criteria: the proximity to different pollution sources, the  
138 surface of nearby agricultural areas and the level of atmospheric pollution. The proximity with  
139 highways and agricultural lands were defined thanks to the BD CARTO® of French national  
140 mapping agency ([www.geoportail.gouv.fr](http://www.geoportail.gouv.fr)), while that of industrial areas was characterized  
141 using an open platform for French public data ([www.data.gouv.fr](http://www.data.gouv.fr)). Highways are significant  
142 sources of air pollution, including particulate matter, nitrogen oxides, and other pollutants.  
143 Therefore, normalization for proximity to a highway was considered, as it accounts for the  
144 significant pollution impacts highways may have on urban gardens.

145 The surface of agricultural areas was calculated from satellite images of Google Earth using  
146 QGIS ([www.qgis.org](http://www.qgis.org)). In addition, air pollution through emission data of NO<sub>2</sub> and particular  
147 matter (PM<sub>2.5</sub> and PM<sub>10</sub>) was provided by Atmo Auvergne-Rhône-Alpes ([www.atmo-  
auvergnerhonealpes.fr](http://www.atmo-<br/>148 auvergnerhonealpes.fr)) the local authority for air quality as 1 x 1 grid, in 2021.

149 While ensuring geographical representation across Lyon metropolis would have been ideal  
150 for capturing the full range of conditions affecting urban community gardens,

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151 sites were selected if they met the following conditions: proximity to one pollution source  
 152 (atmospheric, industrial or agricultural) (**Table 1**), surface area > 1km<sup>2</sup>, availability of access  
 153 authorization and presence of *Ae. albopictus* colonized and noncolonized waters.

154 Pollution variables reflecting each pollution source i.e. atmospheric (Var\_atmo), agricultural  
 155 (Var\_agri) or industrial pollution (Var\_indus) were then calculated for each garden using the  
 156 following formula:

$$Var_{atmo} = \frac{qNO_2 + qPM_{2.5} + qPM_{10}}{dH}$$

157 Where 'qNO<sub>2</sub>', 'qPM<sub>2.5</sub>' and 'qPM<sub>10</sub>' correspond to air pollution scores ranging from 1 (the first  
 158 quartile of gardens with the lowest pollution rates) to 4 (the last quartile of gardens with the  
 159 highest pollution rates) and 'dH' corresponds to the distance between the garden and the  
 160 nearest highway.

$$Var_{agri} = \frac{surface^2}{dA}$$

161 Where 'surface' corresponds to the area of the nearest agricultural area and 'dA'  
 162 corresponds to the distance between the garden and the nearest agricultural area.

$$Var_{indus} = \frac{1}{dl}$$

163 Where 'dl' corresponds to the distance between the garden and the nearest industrial area.

164 Atmospheric, industrial and agricultural pollution variables were normalized for each  
 165 community garden using the following formula:

$$\frac{X - min}{max - min}$$

166 Where 'X' corresponds to calculated pollution variables and 'min' and 'max' correspond to the  
 167 minimum and maximum variables for the same pollution context.

168

## 169 **Sample collection**

170

171 Field sampling was performed in 23 community gardens of Lyon metropolis between June  
 172 and September 2021. To minimize the influence of precipitation on pollution dispersion and



173 mosquito dynamics, sampling was conducted only after ensuring no rainfall had occurred in  
174 the previous 48 hours. Samples were collected in the morning within the same time frame,  
175 with air temperatures consistently similar. For each garden, *Ae. albopictus* larval habitats  
176 were sampled by collecting water from a colonized and a noncolonized reservoir (referred  
177 hereafter as C and NC, respectively) as well as *Ae. albopictus* 3<sup>rd</sup> and 4<sup>th</sup> instar larvae (n=50  
178 per breeding site) (referred hereafter as L). Colonized waters were identified by the presence  
179 of both *Ae. albopictus* larvae in water and adults flying around the reservoir. Larvae were  
180 identified using morphological identification keys of Darsie and Ward (1981) (Kline et al. 2006)  
181 and then verified by COI barcoding by randomly selected 4 larvae per colonized water, as  
182 previously described (Raharimalala et al. 2012). Polymerase Chain Reaction (PCR) products  
183 were sent to Sanger sequencing at Microsynth France SAS (Vaulx-en-Valin). Regarding  
184 noncolonized waters, the entire volume of water in the container was filtered to ensure the  
185 absence of larvae. For each selected container (C and NC), water was mixed thoroughly  
186 before sampling and the water temperature (°C), pH, dissolved oxygen (mg/L), oxidation-  
187 reduction potential (mV) and electrical conductivity (µS/cm) were measured directly in the  
188 field with a portable multiparameter water probe (Horiba, U-50, France). Water samples (1 L)  
189 were collected and split into one sterile 50 mL Falcon tube (Greiner, Germany) for microbial  
190 analysis (40 mL) and three HDPE bottles (VWR, Radnor, USA) for chemical analysis (400  
191 mL, 250 mL and 250 mL), respectively. Third- and fourth-instar larvae were collected with a  
192 sterile plastic pipette into a sterile 50 mL Falcon tube (Greiner) and then transferred  
193 individually to the laboratory in sterile 1.5 mL micro-centrifuge tubes (Sarstedt, Germany).  
194 Water samples were stored at -20°C and defrosted before each analysis except for ion  
195 chromatography analyses where samples were filtered at 0.22 µm before freezing. The  
196 process of field water sampling is detailed in **Figure 1**.  
197

198

199 **DNA extraction**

200

201 Water samples were homogenized by vortexing for 1 min and a 2 mL aliquot was centrifuged  
202 for 10 min at maximum speed (13 400 rpm) at 4°C. The supernatant was discarded and a  
203 new aliquot of 2 mL was added. This step was repeated 3 times for each sample. Pooled  
204 pellets were resuspended in 250 µL of CTAB buffer (2% hexadecyltrimethyl ammonium  
205 bromide, 1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris pH8, 0.2% 2-β mercaptoethanol). After  
206 incubation for 1h at 60°C in a shaker (300 rpm), 4 µL of RNase (100 mg/mL) was added and  
207 incubated at 37°C for 5 min. Mixtures of phenol:chloroform:isoamyl alcohol (25:24:1) and  
208 chloroform:isoamyl alcohol (24:1) were added and after vortexing for 5 min, samples was  
209 centrifuged for 30 min at 13 200 rpm at room temperature, DNA was precipitated using  
210 isopropyl alcohol and Glycoblue coprecipitant (15 mg/mL). DNA pellets were rinsed with 75%  
211 cold ethanol, air-dried and resuspended in 24 µL of sterile RNase free water. Prior to DNA  
212 extraction, larvae were surface-sterilized as previously described (Zouache et al. 2022).  
213 Each larva was crushed for 1min using a FastPrep-24™ 5G (MP Biomedical) into a sterile  
214 2 mL screw-cap tube containing 3 beads of 3 mm diameter (Merck) and 250 µL of CTAB  
215 buffer. Larvae DNA extractions were then performed as previously described for water  
216 samples. DNA extractions from larvae and water were performed in 23 batches (one DNA  
217 extraction per garden) and a negative control (i.e. extraction performed without biological  
218 matrix) was included in each batch. DNA extracts purity and concentration were estimated  
219 using the NanoPhotometer NP80 (Implen) and the Qubit dsDNA High Sensibility kit  
220 combined with the Qubit 4 fluorometer (Invitrogen), respectively.

221

222 **Library preparation and high throughput sequencing**

223

224 DNA samples from water and larvae were used as templates to systematically amplify three  
225 gene markers. The V5-V6 region of the 16S rRNA genes of Bacteria was amplified using the

226 universal primers 784F/1061R (Andersson et al. 2008), whereas the V1-V2 region of the 18S  
227 rRNA genes of Eukaryotes was amplified using the universal primers Euk82F/Euk516R  
228 (Thongsripong et al. 2018) (**Supplementary Table S1**). The fungal nuclear ribosomal  
229 internal transcribed spacer (ITS2 region) was amplified using the universal primers gITS7 /  
230 ITS4 (Ihrmark et al. 2012) (**Supplementary Table S1**). PCR amplifications were also  
231 performed on 10 larvae per garden for bacterial and fungal markers. Primers were tagged  
232 with the Illumina adapters 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACAG-3'  
233 and 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3', enabling a two-step  
234 PCR construction of amplicon libraries. PCR amplifications were conducted in duplicate in a  
235 Bio-Rad T1000 thermal cycler (Bio-Rad, Hercules, USA) with 5× HOT BioAmp master mix  
236 (Biofidal, France) containing 2 µL sample DNA and 1 µM of each primer as previously  
237 described (Zouache et al. 2022; Girard et al. 2023) (**Supplementary Table S2**). For  
238 recalcitrant samples, PCR additives such as 10× of GC rich Enhancer and 0.5 mg/mL of  
239 bovine serum albumin (New England Biolabs, Evry, France) were added to the PCR mix.  
240 Amplicons were checked by electrophoresis on 1.5% agarose 20 min at 100 V and UV  
241 visualization. The PCR products were then sent to Microsynth sequencing company  
242 (Balgach, Switzerland) for purification and second-step PCR, 2x300bp Miseq sequencing  
243 (Illumina). DNA matrix that did not amplify during the first PCR reaction was used as  
244 templates for quantitative PCR, as previously described (Girard et al. 2023).

245

#### 246 **Bioinformatics analysis**

247

248 A total of 7,325,894; 9,591,794 and 2,311,285 reads were obtained for each dataset (i.e.  
249 bacterial 16S rRNA genes, fungal ITS2 and eukaryotic 18S rRNA genes, respectively) and  
250 paired-end reads were demultiplexed in the different samples. R1 and R2 reads were  
251 merged using Fastp, based on 5 mismatches in the overlap region. Sequence quality control  
252 and analysis of sequence data were carried out using the FROGS pipeline (Escudié et al.  
253 2018). Briefly, denoising was carried out by discarding reads without the expected length of

254 that displayed ambiguous bases (N). Clustering was performed using SWARM (Mahé et al.  
255 2014) based on an aggregation distance of 3 for identification of operational taxonomic units  
256 (OTUs). Chimeric OTUs were discarded using VSEARCH (Rognes et al. 2016) and  
257 sequences of low abundance were filtered at 0.005% of all sequences (Bokulich et al. 2013).  
258 The alignment and OTU affiliation were performed using Silva v.138.1 database for Bacteria  
259 and Eukarya (Quast et al. 2012) and the UNITE 8.2 database for fungi (Nilsson et al. 2019).  
260 Reads that did not align were filtered out and contaminant OTUs were filtered out using  
261 negative control (negative control of extraction and PCR). OTUs were removed if they were  
262 detected in the negative control sample and their relative abundance was not at least 10  
263 times greater than that observed in the negative control (Minard et al. 2015). Normalization  
264 for sample comparison was performed by randomly resampling down to 4 103, 6 567 and  
265 7 204 sequences in the bacterial, fungal and eukaryotic datasets, respectively.

266

### 267 **Gas and ion chromatography**

268

269 For gas chromatography analysis, water samples were thawed at room temperature then  
270 transferred into internal flask gases and 50 mL of Helium was added. The flasks were  
271 incubated at 60°C for 3h at 150rpm. After incubation, gas present in water samples were  
272 measured using a gas chromatograph ( $\mu$ GC R990, SRA Instrument, Marcy L'Etoile, France)  
273 with MS5A SS columns TCD detector (10m\*0.25mm\*30 $\mu$ m BF) and PPQ ( $\mu$ m  
274 10m\*0.25mm\*8 $\mu$ m) and 48 channels custom automatic sample changer (SRA Instrument,  
275 Marcy L'Etoile, France). A calibration mix solution was prepared using helium combined with  
276 each dissolved gas, with concentrations ranging from 0 to 14 000 ppm for CO<sub>2</sub>, 0 to 50 000  
277 ppm for N<sub>2</sub>O, 0 to 100 000 ppm for CH<sub>4</sub>, 0 to 79 % for N<sub>2</sub> and 0 to 21% for O<sub>2</sub>. The ions  
278 present in water samples were analyzed by an ionic chromatograph (AQUION) with  
279 automatic sample changer (AS-AP 120). Anion concentrations were measured on pre-  
280 column and column (AS9-HC, 4\*250 mm) at 30°C and eluted with 9 mM Na<sub>2</sub>CO<sub>3</sub> with a flow  
281 rate of 1 mL/min and a 4 mm carbonate suppressor (AERS 500). Cation concentrations were

282 evaluated on pre-column and column (CS-19, 4\*250 mm) at 40°C and eluted with 30 mM  
283 methanesulfonic acid with a flow rate of 1 mL/min and a 4 mm suppressor (CDRS 600). Both  
284 ions were detected with a conductimeter (Thermofischer Electron SAS Courtaboeuf 91941).  
285 Calibration standards were prepared for each ion with concentrations ranging from 0 (ultra-  
286 pure water only) to 500 mg/L. Anions ( $F^-$ ,  $Cl^-$ ,  $NO_2^-$ ,  $Br^-$ ,  $NO_3^-$ ,  $PO_4^{3-}$ ,  $SO_4^{3-}$ ) were prepared by  
287 diluting a more concentrated stock solution (1 g/L) from NaF, KCl,  $NaNO_2$ , KBr,  $KNO_3$ ,  
288  $Na_3PO_4 \cdot 12H_2O$  and  $K_2SO_4$ . Cations ( $Na^+$ ,  $NH_4^+$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ) were prepared by diluting a  
289 more concentrated stock solution (1 g/L) from NaCl,  $(NH_4)_2SO_4$ , KCl,  $MgCl_2 \cdot 6H_2O$  and  
290  $CaCl_2 \cdot 2H_2O$ .

291

### 292 UPLC-HRMS analysis

293

294 In these analyses, the 23 gardens were classified into three groups according to their  
295 proximity to different pollution sources and the presence of atmospheric pollutants (**Table 1**).  
296 An aliquot of each water sample (5 mL) was prepared by liquid-liquid extraction with 5 mL  
297 acetonitrile acidified with 0.1 % formic acid. Each sample was fortified with a labelled internal  
298 standard (diuron-d6), then ultrasonicated, centrifuged and frozen overnight at -18°C. A 2.5  
299 mL aliquot was retrieved. A volume of 500  $\mu$ L of each sample extract was pooled to prepare  
300 a quality control sample (QC). Then the 2 mL left extracts were dried under a slight nitrogen  
301 flow at 40°C. Each dry extract was reconstituted in 200  $\mu$ L of water/methanol 90/10, v/v.  
302 Moreover, to ensure the reliability of the results, each sample was extracted and analyzed 3  
303 times. Extracts with ultra-pure water were prepared with the same protocol and considered  
304 as blank matrix. Analyses by LC-HRMS were performed on an Ultimate 3000 UHPLC system  
305 (Thermo Scientific®, MA, USA) coupled to a quadrupole time-of-flight mass spectrometer  
306 (QToF) (Maxis Plus, Bruker Daltonics®, Bremen, Germany) equipped with an electrospray  
307 ionization interface (ESI). Analyses were carried out in reverse phase (elution gradient)  
308 employing an Acclaim RSLC 120 C18 column (2.2  $\mu$ m, 100  $\times$  2.1 mm, ThermoScientific®),  
309 protected with a KrudKatcher Ultra In-Line Filter guard column from Phenomenex (Torrance,

310 CA, USA) and heated at 30°C. The injected volume was 5 µL. Mobile phases consisted of:  
311 an aqueous phase (90%/10% ultrapure water/methanol mixture with 5 mM ammonium  
312 formate and 0.01% formic acid) and an organic phase (methanol with 5 mM ammonium  
313 formate and 0.01% formic acid). All extracts were analyzed in positive electrospray ionization  
314 with the following settings: capillary voltage of 3600 V, end plate offset of 500 V, nebulizer  
315 pressure of 3 bar (N<sub>2</sub>), drying gas of 9 L/min (N<sub>2</sub>), and drying temperature of 200 °C. An  
316 external calibration of exact masses was systematically performed at the beginning of each  
317 run, using a solution of sodium formate and acetate (0.5 mL of 1 M NaOH, 25 µL of formic  
318 acid and 75 µL of acetic acid in H<sub>2</sub>O/isopropanol 50/50 v/v), generating cluster ions [M+H]  
319 + in the range 90.9766–948.8727 Da with high precision calibration (HPC) mode at a search  
320 range ± 0.05 m/z. Accepted standard deviations were inferior to 0.5 ppm. QCs were run  
321 several times at the beginning of the analytical sequence to equilibrate the column and at  
322 regular intervals throughout the sequence to check for instrument drift and to control  
323 analytical repeatability and sensitivity. Instrument control, data acquisition, and processing  
324 were performed using OTOF Control and Hystar™ (v4.1, Bruker Daltonics®) software.  
325 Samples were randomly analyzed in full-MS scan over a m/z range of 50-1000 Da. QCs  
326 were also analyzed in DDA (Data Dependent Analysis), a mode with alternative acquisition in  
327 MS and MSMS, with predefined parameters. The data preprocessing was performed with  
328 W4M and Metaboscape (Giacomoni et al. 2015). A feature matrix was generated for each  
329 sequence. It contained 1382 features for which the mass to charge ratio m/z in ion adduct  
330 form, the retention time RT of each detected peak, the intensity in each sample. Each feature  
331 was then normalized to the intensity of diuron-d6. It was discarded from the dataset when the  
332 coefficient of variation in the QCs (CVQC) was over 30%. Missing intensities in a replicate  
333 were replaced by the mean intensities in the two others replicate or by 0 if not available.  
334 Then for each group of gardens (**Table 1**), a detection rate was calculated. If it was less than  
335 100%, the feature wasn't considered afterwards. Signals that were not significantly higher in  
336 samples compared to blanks (intensity ratio > 10), and were not significantly different  
337 between C and NC (t-test p>0.05) were not considered. Annotation and identification were

338 performed using Metaboscope and MetFrag (Wolf et al. 2010) an *in silico* fragmentation tool,  
339 by comparing experimental MSMS spectra. Using these filters, a list of 147 LC-HRMS  
340 signals was kept on the 1382 features. Finally markers were putatively identified according to  
341 the level of identification confidence from 1 to 5 (Schymanski et al. 2015) (**Table 2**).

342

### 343 **Total organic carbon analysis and measurement**

344

345 Total Organic Carbon (TOC) was performed on a Shimadzu TOC 5050 (Kyoto, Japan). From  
346 16 to 54  $\mu\text{L}$  of samples were injected in a vertical combustion tube hold at  $680^{\circ}\text{C}$  and half  
347 filled with platinum-based catalysis in a flow of 150 mL/min. Total Carbon (TC) was  
348 converted into  $\text{CO}_2$  and measured on Non-Dispersive Infrared (NDIR)  $\text{CO}_2$  detector.

349 Inorganic Carbon (IC) was measured by the same amount of injection in a vessel containing  
350 phosphoric acid at a concentration of 25%. Carbon contained in carbonates was converted  
351 into  $\text{CO}_2$  and quantified on the same infrared detector. TOC concentration was obtained by  
352 the difference between TC and IC concentrations.

353

### 354 **Statistical and data analysis**

355

356 Data analysis and statistical tests were carried out with R Statistical Software (v4.1.1, R Core  
357 Team 2018), using the *vegan* package for diversity analyses (Oksanen et al. 2018) and the  
358 *ggplot2* package for graphical representations (Wickham 2016). Heatmap graphs were  
359 plotted with the *complexHeatmap* package (Gu et al. 2016). Prokaryotic, fungal and  
360 eukaryotic communities within-sample diversity also referred as alpha-diversity was  
361 estimated with the Shannon index ( $H'$ ) while community dissimilarity also referred as beta  
362 diversity was estimated with the Bray-Curtis index. Shannon index was tested using either  
363 nonparametric tests (Kruskal-Wallis and paired Wilcoxon signed rank tests with Benjamini-  
364 Hochberg correction) for bacteria or parametric tests (ANOVA and Tukey HSD post hoc tests)  
365 for fungi and other microeukaryotes. Beta diversity was analyzed using permutational

366 multivariate analysis (adonis2, R package *vegan*) (Legendre and Anderson 1999). Non-  
367 metric multidimensional scaling (NMDS) plots using Bray-Curtis dissimilarity matrices were  
368 used to visualize beta diversity of microbial communities. Comparison of physicochemical  
369 parameters and organic molecule occurrence between NC and C was performed with partial  
370 least squares discriminant analysis (PLS-DA) with *mixOmics*, R package (Rohart et al. 2017).  
371 The influence of the breeding site colonization status on the 5 most discriminant  
372 physicochemical parameters was tested by linear mixed model (lmm) after correcting for  
373 variations between inter-individual garden effect. Considering organic molecules, data were  
374 not analyzed with lmm because the residuals of the model were heteroskedasts and did  
375 follow a normal distribution. We therefore tested them using Kruskal-Wallis rank sum test, a  
376 non-parametric test. Principal Components Analysis (PCA) were performed for NC and C  
377 samples independently in function of either physicochemical combined with pollution  
378 variables or organic molecule water occurrence. Monte Carlo test on the sum of the singular  
379 values of a procrustean rotation was performed to test the concordance of each dataset. To  
380 analyze whether the abiotic factors of NC and C (either physicochemical and pollutant  
381 variables or organic molecule occurrence) were correlated with the microbial communities,  
382 we performed the same analysis. Then, we asked what was the proportion of the microbial  
383 communities within NC and C that could be explained by major variations in the abiotic  
384 factors. To that end, we have used the two first components (PC1\_poll/PC2\_poll or  
385 PC1\_mp/PC2\_mp, for physicochemical and pollution variables or organic molecules,  
386 respectively) of the abiotic factors' PCA as an explanatory variable for a PERMANOVA  
387 analysis of the microbial communities Bray Curtis dissimilarities. Then, we performed  
388 distance-based Redundancy Analyses (dbRDA) as constrained multivariate analyses to  
389 represent the microbial OTUs that were mostly influenced by PC1\_poll/PC2\_poll or  
390 PC1\_mp/PC2\_mp. Those OTUs were correlated with PC1\_poll/PC2\_poll or  
391 PC1\_mp/PC2\_mp using Spearman's rank correlations.

392 Then, the objective was to develop a method allowing to determine relationships  
393 between variables of different natures, including categorical, numerical, and not following



394 normal distributions, and then to visualize them with graph representations, in which nodes  
395 represent privileged relationships between those variables. To that end, we used a python  
396 code and visualized with the pyvis library. For each numerical variable (OTUs, Water T°, LC-  
397 HRMS data, etc.), we converted it into a binary value, such as exceptionally high values were  
398 identified as 1, and average or low values were identified as 0. Given the non-normal  
399 distribution of values, we used a robust variant of the z-score to identify exceptionally high  
400 values. More precisely, we computed for each variable the so-called modified z-score,  
401 computed using the median absolute deviation to the median instead of the standard  
402 deviation.

403 Each value  $x_i$  is thus transformed using the following formula:

$$\frac{x_i - \text{med}(x)}{MAD}$$

404 Where 'med(x)' corresponds to the median of variable 'x' and 'MAD' the median absolute  
405 deviation. We then defined a threshold above which a value is considered exceptional. In the  
406 second step, we used association rule scores to identify strong associations. More precisely  
407 we used Zhang's score to assess how strongly related a variable is compared with another  
408 one ([https://rasbt.github.io/mlxtend/user\\_guide/frequent\\_patterns/association\\_rules/](https://rasbt.github.io/mlxtend/user_guide/frequent_patterns/association_rules/)).  
409 Zhang's score is defined between -1 and +1, with 0 meaning no particular association. Here  
410 again, we defined a threshold to keep only values above a given threshold, i.e., having a  
411 strong enough association. Zhang's score does not include statistical significance, so we add  
412 a statistical test (Binomial test, p-value < 0.05) *a posteriori* to assess the robustness of  
413 potential associations. An edge is added to the graph if the strength of the association is  
414 above the threshold and statistically significant. We used this method to avoid pitfalls with  
415 existing approaches.

416

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417

## 418 **Results**

419

### 420 **Noncolonized and colonized waters exhibit slight differences in physicochemical and** 421 **organic molecule composition**

422

423         Following our selection criteria (see the Materials and Methods section), a total of 23  
424 gardens were selected for water sample collection. The characterization of each garden  
425 according to the level of atmospheric pollution, the calculated pollution variables and the  
426 distance from the garden to each pollution source is given in **Table 1**. The most common  
427 containers sampled were plastic rainwater collectors (87%), followed by plastic buckets (9%)  
428 and plastic watering cans (4%) (**Table 3**). Water physicochemical properties, i.e., ionic and  
429 gas composition, TOC, temperature, pH, conductivity, redox potential, and dissolved oxygen,  
430 are all summarized in **Table 3** and **Table 4**. We conducted a PLS-DA supervised analysis to  
431 identify the most discriminant physicochemical parameters between C and NC (**Figure 2A**).  
432 Among the physicochemical properties, N<sub>2</sub>O and CH<sub>4</sub> were significantly higher in NC than in  
433 C according to linear mixed model ( $\chi^2 = 4.702$ ,  $p = 0.03$  and  $\chi^2 = 4.674$ ,  $p = 0.03$ , respectively)  
434 (**Figure 2B**). In fact, the average N<sub>2</sub>O and CH<sub>4</sub> were two and nearly seven times higher in NC  
435 than in C, respectively. In a second analysis, NC and C samples were separated (**Figure 2C-**  
436 **D**) to compare their physicochemical composition through a Procrustean analysis. The  
437 abiotic factors (physicochemical factors and pollution variables) had no significant effects on  
438 colonization status (NC or C) (Monte Carlo permutation test, observation = 0.345,  $p = 0.135$ ).  
439 Regarding organic molecule analysis, the application of a nontargeted approach involving  
440 liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QToF)  
441 allowed the detection of compounds that were found to be significantly different between C  
442 and NC from the same garden (t-test  $p < 0.05$ ) (**Supplementary Table S3**). Among them, 22  
443 features were putatively identified (**Table 2**). However, PLS-DA performed with all 147

444 features did not reveal significant differences between C and NC regarding organic molecule  
445 presence ( $p > 0.05$ ) (**Figure 3A-B**). When NC and C samples were separated (**Figure 3C-D**)  
446 through the Procrustes analysis, no significant effect was found depending on the  
447 colonization status (NC or C) (Monte Carlo permutation test, observation = 0.06,  $p = 0.985$ ).

448

449 **Fungal composition differs between noncolonized and colonized waters while**  
450 **microbial community structure is similar between larvae and water samples,**  
451 **regardless of colonization status**

452

453 A total of 1373 bacterial OTUs belonging to 27 phyla and 329 genera were identified  
454 from the 23 gardens (23 NC, 23 C and their associated larvae). NC and C exhibited similar  
455 bacterial compositions at the phylum level (**Supplementary Figure S1A**). The predominant  
456 phylum was Proteobacteria (37% and 34%), followed by Actinobacteriota (20% and 24%)  
457 and Firmicutes (17% and 12%) for NC and C, respectively (**Supplementary Figure S1A**). In  
458 larvae, the most abundant bacterial phylum was Proteobacteria (35%), followed by  
459 Actinobacteriota (24%) and Bacteroidota (17%) (**Supplementary Figure S1A**). At the genus  
460 level, 40%, 34% and 31% of the bacterial genera displayed low abundance (<1%) in NC, C  
461 and L, respectively (**Supplementary Figure S1B**). Among the 42 most abundant genera, 7  
462 were common (e.g., *Rhizobium*, *Legionella*, and *Abditibacterium*), and others were specific to  
463 NC (6), C (7) and larvae (14) (**Supplementary Figure S1B**). Interestingly,  
464 *Actinomycetospora* and *Actinoplanes* were abundant in C and L but in low abundance in NC  
465 (<1%). The alpha diversities of the bacterial communities in NC and C were comparable  
466 (Wilcoxon pairwise;  $p = 0.53$ ), while that in L was significantly lower than that in C (Wilcoxon  
467 pairwise;  $p = 0.0012$ ) and NC (Wilcoxon pairwise;  $p = 0.0005$ ) (**Supplementary Table S4**).  
468 Regarding the fungal community (i.e., ITS sequencing), a total of 18 gardens were analysed  
469 out of the 23 sampled gardens (with 18 NC, 18 C and their associated larvae). If the ITS  
470 sequencing preferentially targeted and revealed the presence of fungi (56.4%, 56.6% and  
471 57.8%), other eukaryotic kingdoms such as Viridiplantae were also detected (15.4%, 16.4%

472 and 15.8%) for NC, C and L, respectively, followed by 3 minority kingdoms (Rhizaria, Protista  
473 and Ichthyosporia) (data not shown). Regarding fungal communities, a total of 716 fungal  
474 OTUs belonging to 8 phyla and 264 genera were identified. *Ascomycota* was the  
475 predominant phylum (47%, 55% and 72% in NC, C and L, respectively), followed by  
476 Basidiomycota (27%, 24% and 16%) (**Supplementary Figure S2A**). The phylum  
477 Rozellomycota was the third most abundant phylum in NC and C (7% and 6%) but  
478 represented only 0.5% of the total fungi in L (**Supplementary Figure S2A**). At the genus  
479 level, 40%, 34% and 31% of the fungal genera displayed a low abundance (<1%) in NC, C  
480 and L, respectively (**Supplementary Figure S2B**). Only *Dioszegia* was common to NC and  
481 C, while *Phoma* was shared among NC, C and L (**Supplementary Figure S2B**). Interestingly,  
482 *Dacrymyces* and *Pyrenochaetopsis* were abundant in C and L but very low in abundance in  
483 NC (0.18% and 0.04%, respectively) (**Supplementary Figure S2C**). Comparison analysis  
484 performed with the Shannon index indicated no significant differences in the alpha diversity  
485 of fungal communities between NC and C (Tukey HSD post hoc tests,  $t$  ratio = 0.559,  $df$  = 51,  
486  $p$  = 0.84) or between C and L (Tukey HSD post hoc tests,  $t$  ratio = -1.914,  $df$  = 51,  $p$  = 0.14)  
487 (**Supplementary Table S4**). Conversely, the alpha diversity of fungal communities was  
488 significantly higher in L than in NC (Tukey HSD post hoc test,  $t$  ratio = 2.473,  $df$  = 51,  $p$  = 0.04)  
489 (**Supplementary Table S4**). Regarding eukaryotic communities, a total of 739 eukaryotic  
490 OTUs belonging to 38 phyla and 181 genera were identified among 13 gardens (13 NC and  
491 13 C). In contrast to bacteria and fungi, the main eukaryotic phyla differed between NC and  
492 C. The predominant phylum was Ciliophora (22.5%), followed by Chlorophyta (18.4%) and  
493 Ochrophyta (10.2%), in NC, while the predominant phylum was Bicosoecida (19.5%),  
494 followed by Ochrophyta (14.6%) and Chlorophyta (9.8%), in C (**Supplementary Figure S3A**).  
495 At the genus level, 10.3% and 14.9% of the eukaryotic genera displayed low abundance  
496 (<1%) in NC and C, respectively (**Supplementary Figure S3B**). Among the 28 most  
497 abundant genera, three were common (*Sterkiella*, *Scotinosphaera* and *Goniomonas*), and  
498 others were specific to NC (13) and C (11) (**Supplementary Figure S3B**). The alpha  
499 diversities of the eukaryotic communities in NC and C were comparable

500 (Tukey HSD post hoc test,  $p = 0.9$ ) (**Supplementary Table S4**). Nonmetric multidimensional  
501 scaling based on Bray–Curtis dissimilarity revealed no differences in microbial composition  
502 between NC and C (*adonis2*-PERMANOVA,  $R^2 = 0.02$ ,  $p = 0.77$  for bacteria;  $R^2 = 0.04$ ,  $p =$   
503  $0.7$  for eukaryotes) (**Figure 4A, 4B and 4C**). However, significant differences in fungal  
504 composition were found between NC and C (*adonis2*-PERMANOVA,  $R^2 = 0.04$ ,  $p = 0.03$ ).  
505 Moreover, significant differences in bacterial composition were observed between NC and L  
506 (*adonis2*-PERMANOVA,  $R^2 = 0.03$ ,  $p = 0.001$ ) as well as between C and L (*adonis2*-  
507 PERMANOVA,  $R^2 = 0.03$ ,  $p = 0.001$ ). Heatmaps showed contrasting patterns with some  
508 OTUs unique (specific) to certain categories of samples or shared (OTUs that are common  
509 among several categories of samples) (**Figure 4D-F**). Among fungi, OTU 3 (family  
510 *Didymellaceae*) was present in all samples. Conversely, the bacterial OTUs 50, 51, and 52  
511 (family Beijerinckiaceae), 62 (family Anaeromyxobacteraceae), 84 (family Bacillaceae), 144  
512 (family Parachlamydiaceae) and 202 (family Clostridiaceae) as well as the fungal OTU 54  
513 (not identified) were found in substantial proportions in NC and C but absent from L. Two  
514 other bacterial OTUs (OTU 13 and OTU 14) belonging to family Pseudonocardiaceae were  
515 enriched in L compared to C

516

### 517 **Organic molecules and physicochemical properties differentially affect microbial** 518 **communities in noncolonized and colonized waters**

519

520 According to previous analyses, coordinates of the two first components of PCA  
521 (PC1\_poll and PC2\_poll from **Figure 2C-2D** and PC1\_mp and PC2\_mp from **Figure 3C-D**)  
522 were extracted and subsequently used to reflect the distribution of samples according to  
523 either physicochemical and pollution variables or the occurrence of organic molecules in  
524 terms their microbial composition. We then evaluated whether those variables shape the  
525 microbial structure of NC and C. For this purpose, distance-based redundancy analyses (db-  
526 RDAs) were conducted to maximize the separation of water microbial communities based on  
527 differences in either their physicochemical composition and pollution context (PC1\_poll and

528 PC2\_poll) or organic molecule structure (PC1\_mp and PC2\_mp) (**Figure 5 and 6**). In  
529 colonized water samples, the structure of bacterial communities was significantly correlated  
530 with PC1\_poll (*adonis2*-PERMANOVA,  $R^2 = 0.07$ ,  $P = 0.001$ ), PC2\_poll (*adonis2*-  
531 PERMANOVA,  $R^2 = 0.06$ ,  $P = 0.015$ ) and PC2\_mp (*adonis2*-PERMANOVA,  $R^2 = 0.44$ ,  $P =$   
532  $0.007$ ), while that of fungal communities varied only according to PC1\_poll (*adonis2*-  
533 PERMANOVA,  $R^2 = 0.07$ ,  $P = 0.006$ ). The most important microbial OTUs involved in these  
534 separations were bacterial OTU 13 (identified as *Actinomycetospora*) and fungal OTU 11  
535 (unidentified Rozellomycota), which correlated with high values of PC1\_poll ( $p = 0.017$ ,  $Rh\hat{o} =$   
536  $0.5$  for OTU 13 and  $p = 0.003$ ,  $Rh\hat{o} = 0.66$  for OTU 11), as well as bacterial OTU 39  
537 (identified as Rhizobiaceae) (**Figure 5A-B**) and fungal OTU 44 (identified as *Ascochyta*  
538 *phacae*), which correlated with low and high values of PC2\_mp, respectively ( $p = 0.008$ ,  $Rh\hat{o}$   
539  $= 0.54$  for OTU 39 and  $p = 0.04$ ,  $Rh\hat{o} = 0.48$  for OTU 44) (**Figure 5C-D**). Regarding  
540 noncolonized water samples, the structure of microbial communities was significantly  
541 influenced by PC1\_poll (*adonis2*-PERMANOVA,  $R^2 = 0.06$ ,  $P = 0.027$  for bacteria and  
542 *adonis2*-PERMANOVA,  $R^2 = 0.1$ ,  $P = 0.04$  for eukaryotes) as well as by PC1\_mp (*adonis2*-  
543 PERMANOVA,  $R^2 = 0.08$ ,  $P = 0.025$  for fungi). The most important clusters involved in these  
544 separations were OTU 24 (identified as *Haematococcus*), which correlated with PC1\_poll ( $p$   
545  $= 0.017$ ,  $Rh\hat{o} = 0.6$ ) (**Figure 6A**), and OTU 30 (unidentified Rozellomycota) and OTU 163  
546 (identified as *Neochloris aquatica*), which correlated with PC1\_mp ( $p = 0.03$ ,  $Rh\hat{o} = 0.5$  and  $p$   
547  $= 0.04$ ,  $Rh\hat{o} = 0.48$  for OTU 30 and OTU 163, respectively) (**Figure 6B**).

548

#### 549 **Organic molecules and microbial communities are highly connected in colonized** 550 **water samples**

551

552 A network analysis was performed on colonized water samples to explore  
553 associations between microbial OTUs (i.e., bacterial, fungal and eukaryotic OTUs),  
554 physicochemical parameters, pollution variables and organic molecules (**Figure 7**). Two  
555 independent networks of positive correlations that contained inter-kingdoms associations and

556 organic molecules were characterized (**Figure 7**). In the first network, microbial co-  
557 occurrence patterns included the eukaryotic OTU 9 (identified as Ostracoda), 5 bacterial  
558 OTUs (i.e., OTU 77 identified as the genus *Azohydromonas*, OTU 355 identified as the class  
559 Acidimicrobiia, unidentified OTU 630, and OTUs 186 and 659 identified as the class  
560 Clostridia), 5 fungal OTUs (i.e., OTU 8 identified as the species *Neochloris aquatica*, OTU  
561 188 identified as the species *Humicola grisea* and unidentified OTUs 5, 7, and 105) and one  
562 unidentified feature (M104T45) (**Figure 7A**). The second network showed module  
563 associations between 9 bacterial OTUs (i.e., 89, 93, 112, 130, 275, 590, 684 and 1018  
564 identified as belonging to the genera *Bacillus*, *Bacteriovorax*, *Brevundimonas*, *Candidatus*  
565 *Methylopumilus*, and *Flavobacterium*, the families *Yersiniaceae* and *Rhizobiaceae* and the  
566 genus *Tyzzarella*, respectively), 7 fungal OTUs (i.e., OTUs 37, 68, 114, 127, 132 and 298  
567 identified as belonging to the order Pleosporales and the genera *Coniochaeta*,  
568 *Vishniacozyma*, *Filobasidium*, *Coniochaeta* and *Coniochaeta*, respectively), 8 eukaryotic  
569 OTUs (i.e., OTUs 7, 8 and 736 identified as belonging to the phylum Bicosoecida and OTUs  
570 68, 94, 125, 265 and 423 identified as belonging to the class Klebsormidiophyceae, the  
571 genus *Coniochaeta*, the phylum Ascomycota, the genus *Gyrodinium* and the class  
572 Chytridiomycetes) and 3 unidentified features (M305T477, M209T480 and M285T707). In  
573 this second network, we focused on the eukaryotic OTU 68 that was associated with 2  
574 eukaryotic OTUs (i.e., OTU 94 identified as belonging to the class Sordariomycetes and OTU  
575 125 to the class Chytridiomycetes), the fungal OTU 132 identified as Sordariomycetes and 3  
576 bacterial OTUs associated with 3 different classes (OTU 89 identified as Bacilli, OTU 590  
577 identified as Gammaproteobacteria and OTU 1018 identified as Clostridia) (**Figure 7B**).  
578 Finally, the eukaryotic OTU 7 belonging to the phylum Bicosoecida was associated with 3  
579 features (M305T477, M209T480 and M285T707).  
580

581

582 **Discussion**

583

584 Whether biotic and abiotic factors directly or indirectly impact the ability of mosquitoes  
585 to thrive in aquatic habitats remains poorly investigated. We addressed this question by  
586 specifically studying *Ae. albopictus*, one of the most invasive mosquito species in the world.  
587 As the type of container was shown to influence water physicochemical parameters (Hery et  
588 al. 2021b), our analysis mainly focused on rainwater collectors. Rainwater harvesting is a  
589 widely used method to collect and store rainwater for garden water supply and leads to  
590 common breeding sites for *Aedes* mosquitoes (Ritchie et al. 2002; Duval et al. 2022). We  
591 first investigated whether the presence/absence of *Ae. albopictus* larvae in water habitats  
592 could be related to specific biotic and abiotic features. With the exception of  $N_2O$  and  $CH_4$   
593 concentrations, our results showed no significant differences in water physicochemical  
594 properties and microbial composition between colonized and noncolonized water samples.  
595 We showed that the average water temperature and pH values of colonized larval habitats  
596 were 21.6 °C and 7.4, respectively. The temperature ranged between 15 °C and 26 °C, while  
597 the pH values were between 5.7 and 8.8. Consistent with the findings of other studies, the  
598 average electrical conductivity and dissolved oxygen were 0.19 S/m and 6.45 mg/L,  
599 respectively (Rao et al. 2011; Garcia-Sánchez et al. 2017; Dalpadado et al. 2022). These  
600 values are optimal for the development of *Ae. albopictus* (Delatte et al. 2009; Medeiros-  
601 Sousa et al. 2018; Reinhold et al. 2018). When measured as a percentage of the calculated  
602 (or expected) amount at saturation, referred to as dissolved saturation (%DO), dissolved  
603 oxygen was on average 2.1%. This contrasted with previous measures of %DO of  
604 approximately 1-1.5% in *Aedes* breeding sites (Gopalakrishnan et al. 2013; Medeiros-Sousa  
605 et al. 2020). Interestingly, it was previously shown that %DO is positively correlated with  
606 mosquito larval density and can influence the forms and toxicity of dissolved metals as well  
607 as the concentrations of certain chemicals, such as sulphides and ammonia (Khalid et al.  
608 1978; Gopalakrishnan et al. 2013). Regarding redox potential, higher values were observed



609 in *Ae. aegypti* breeding sites (Garcia-Sánchez et al. 2017) and could be explained by  
610 variation in microbial communities or ionic composition (DeLaune and Reddy 2005;  
611 Søndergaard 2009). With the exception of oxygen, dissolved gases in larval breeding sites  
612 remain poorly studied. Our study is the first to examine the presence and concentration of  
613 several gases in *Ae. albopictus* water habitats. Interestingly, it was shown that the presence  
614 of methane in the larval habitats of *Culex pipiens* increased oviposition, while that of CO<sub>2</sub> did  
615 not seem to affect mosquito colonization or fitness (Bentley and Day 1989) or *Aedes* hatching  
616 stimulation (Judson 1960). In addition, methane and dissolved CO<sub>2</sub> exposure could also  
617 affect water microbial communities (Brankovits et al. 2017). Nitrate, potassium and chloride  
618 ions were detected at higher concentrations than those found in tap water, which could be  
619 linked to the use of nitrate fertilizers and the importance of fertilizer inputs in intensive urban  
620 agriculture (Amerasinghe et al. 1995; Panno et al. 2006; Wakejo et al. 2022). Although  
621 previous studies showed that mosquitoes are able to reproduce over a wide range of ion  
622 concentrations (Sehgal and Pillai 1970; Bashar et al. 2016; Garcia-Sánchez et al. 2017), the  
623 impact of water ionic composition on mosquito physiology remains unclear. Due to  
624 confounding factors in a complex field environment, further studies are required to  
625 investigate the impact of ionic and gas composition alone or in combination with other  
626 physicochemical parameters on different mosquito traits. For instance, the bioavailability of  
627 contaminants in water may be strongly influenced by the chemical environment (in particular  
628 ionic composition) and could in turn affect mosquito larvae by either directly modulating their  
629 physiology or indirectly modulating the microbial communities of breeding sites (Adams et al.  
630 2020; Hery et al. 2021b). Recent studies have shown that ion content in water is linked to  
631 conductance capabilities and can influence the growth rates of larvae and pupae,  
632 potentially impacting the microbial community composition in both the larval diet and water  
633 environment over time (Ukubuiwe et al. 2020, Mamai et al. 2021). Conducting controlled  
634 experiments in the laboratory could be beneficial for assessing the causality between these  
635 factors, determining whether gases and ionic compositions influence the presence of *Ae.*

636 *albopictus* larvae in water habitats or whether colonization leads to differential abundance of  
637 these compounds.

638 Consistent with previous reports, the dominant bacterial phyla in colonized water samples  
639 were Proteobacteria (currently named Pseudomonadota), Actinobacteria (currently named  
640 Actinobacteriota) and Bacteroides (currently named Bacteroidota) (Wang et al. 2018; Scolari  
641 et al. 2021), while Ascomycota, Basidiomycota and Cryptomycota (previously named  
642 Rozellomycota) were the dominant fungal phyla (Tawidian et al. 2021). The microalga  
643 *Picochlorum* and the protozoan flagellate *Spumella* were found to be specific to colonized  
644 waters. Interestingly, polysaccharides extracted from *Picochlorum* were shown to exhibit  
645 antiviral activity against human enterovirus 71 (Guo et al. 2021). It could be interesting to  
646 evaluate whether this effect persists in larvae and adult mosquitoes and could interfere with  
647 arbovirus transmission. Surprisingly, Egizi et al. (2014) observed the opposite tendency, with  
648 a negative correlation between the density of *Culex quinquefasciatus* larvae and that of  
649 *Spumella*. Overall, our results confirmed that a fraction of microorganisms present in larval  
650 breeding water are maintained in the larval stage with higher microbial diversity in water  
651 breeding sites compared to in larvae (Dada et al. 2014; Coon et al. 2016; Wang et al. 2018;  
652 Strand 2018; Scolari et al. 2021). We identified bacteria such as *Rhizobium* and fungi such  
653 as *Dacrymyces* and *Pyrenochaetopsis* that were shared between water and larvae. However,  
654 whether these taxa play a role in mosquito biology is still unknown. *Rhizobium* was listed as  
655 part of the core microbiota in different *Anopheles* mosquito tissues (Tchioffi et al. 2016;  
656 Guégan et al. 2018). Another study showed that *Ae. albopictus* females were not attracted to  
657 or repelled by *Rhizobium huautlense* isolated from canebrake bamboo, but we cannot  
658 exclude a role in larval development (Ponnusamy et al. 2015). Interestingly, noncolonized  
659 water exhibited higher fungal diversity and specific fungal composition as well as higher  
660 concentrations of N<sub>2</sub>O compared to colonized samples. This could be explained by the fact  
661 that N<sub>2</sub>O production is a widespread trait in fungi (Maeda et al. 2015).

662 Physicochemical parameters and microbial communities interact with each other in  
663 aquatic ecosystems (Bojarczuk et al. 2018; Rahman et al. 2021) and can also be influenced

664 by urban pollution. Physicochemical factors and pollution exposure could directly alter  
665 bacterial communities by impairing microbial physiological activities or indirectly by  
666 establishing conditions that adversely affect microorganisms. In community gardens,  
667 rainwater tanks are mainly filled with rainwater and in some cases may be supplemented  
668 with tap water, which can be occasionally contaminated by organic molecules in trace  
669 quantities (Kim and Zoh 2016). Although rainwater is considered a contamination-free source,  
670 human activities, particularly in the industrial and agricultural sectors, pollute this pure form of  
671 water (Guidotti et al. 2000; Samuel et al. 2012). Over the last decade, a wide range of  
672 contaminants have been detected in surface water, including pharmaceutical, industrial and  
673 pesticide compounds (Rosen 2007; Verlicchi et al. 2010; Yang et al. 2022). Some of them,  
674 such as microplastic particles and antibiotic residues, were shown to alter water microbial  
675 communities (Qing et al. 2020; Yang et al. 2022; Edwards et al. 2023). To the best of our  
676 knowledge, no study has characterized the presence of organic molecules in breeding sites  
677 without *a priori* information. In our study, we identified organic molecules, although  
678 uncertainties remain regarding some candidate compounds. Further studies could be  
679 conducted using a suspect screening approach to better identify organic molecules  
680 (Pinasseau et al. 2023). Among the identified organic molecules, we found pharmaceutical  
681 residues such as anti-inflammatories, hormones or antioxidants (3-tert-butyl-4-  
682 hydroxyanisole), as well as fertilizers (dimethylenetriurea) and plastics (phthalate derived).  
683 Due to their high prevalence in aquatic ecosystems, further experiments are needed to test  
684 the influence of these compounds on the physiology of *Ae. albopictus* (Yang et al. 2022).  
685 Interestingly, a previous study showed an effect of fertilizer (i.e., ammonium sulphate  
686 fertilizer) on *Aedes* and an impact on larval development, survival rates and larval density  
687 (Mutero et al. 2004; Muturi et al. 2016; Darriet 2019). Moreover, Prud'homme et al. (2017)  
688 also revealed an impact of ibuprofen and benzo[a]pyrene on life history traits and insecticide  
689 tolerance in *Ae. aegypti* (Prud'homme et al. 2017). More recently, Edwards et al. (2023)  
690 showed that microplastics decreased the bacterial and fungal diversity of the *Ae. albopictus*  
691 and *Ae. aegypti* microbiota (Edwards et al. 2023).

692 We classified the community gardens according to their pollution context, which  
693 allowed us to define pollution gradients that combined either the water physicochemical  
694 parameters and the pollution variables (e.g., proximity to pollution sources) or water organic  
695 molecule occurrence. Our results showed differential effects of pollution gradients according  
696 to the water colonization status. While no relationship between physicochemical and pollution  
697 variables and bacterial composition was evidenced in noncolonized water samples, the  
698 opposite was observed in colonized waters. Industrial pollution and ionic composition were  
699 positively correlated with OTUs belonging to the bacterial genus *Actinomycetospora* and  
700 fungal phylum *Rozellomycota*. Similarly, we found correlations between the presence of  
701 organic molecules in water-colonized samples and OTUs belonging to the bacterial family  
702 *Rhizobiaceae* and the phytopathogenic fungus *Ascochyta phaca*. *Actinomycetospora* is  
703 common in *Aedes* breeding sites (Zouache et al. 2022) and was found in this study to be  
704 highly prevalent in larvae. The Rhizobiaceae family contains phenotypically diverse  
705 organisms, including N<sub>2</sub>-fixing legume symbionts (known as rhizobia), plant pathogens,  
706 bacterial predators, and other soil bacteria (Kuzmanović et al. 2021). Interestingly, members  
707 of this family were already shown to be involved in the degradation of bisphenol A and 4-  
708 nonylphenol, both ubiquitous pollutants with oestrogenic activity in the aquatic environment  
709 (Cai et al. 2016; Gayathri and Da 2021). In noncolonized water samples, *Haematococcus*  
710 was positively correlated with physicochemical and pollution variables. Interestingly,  
711 *Haematococcus* are known to help to reduce water pollution, which may explain their  
712 presence in polluted environments (Krishnamurthi et al. 2023). We also found specific  
713 patterns highlighting correlations between the organic molecule gradient and OTUs identified  
714 as the green alga *Neochloris aquatica* or as belonging to the fungal phylum *Rozellomycota*.  
715 Interestingly, *Rozellomycota* was also correlated with pollution related to industrial sources  
716 and specific physicochemical profiles in colonized waters. The *Rozellomycota* form a lineage  
717 basal or sister to fungi, the ancestor of Microsporidia (Corsaro et al. 2014). Their biodiversity  
718 is very rich but remains poorly characterized. However, we know that they are common in  
719 aquatic habitats and on pollen (Wurzbacher et al. 2014). They may play a key role in the

720 decomposition of organic matter (Grossart et al. 2016). The few known species are all  
721 parasites, including water moulds and algae (*Rozella*), crustaceans (*Mitosporidium*), and  
722 endonuclear parasites of amoebae (*Nucleophaga*, *Paramicrosporidium*) (Corsaro et al. 2020;  
723 Chung et al. 2020; Gonçalves et al. 2022). Interestingly, *Neochloris aquatica* was shown to  
724 have a negative effect on the larval development of *Cx. quinquefasciatus* mosquitoes (Gil et  
725 al. 2021). A study also showed their ability to reduce or remove chemical oxygen demand  
726 (used as a measurement of pollutants in water), nitrite, and phosphate from water  
727 (Ummalyma et al. 2023). Therefore, we could assume that this species could modulate water  
728 physicochemical parameters that could in turn have consequences for colonization by *Ae.*  
729 *albopictus*. Further studies under experimental conditions would help disentangle potential  
730 interactions between the microbial taxa found to be correlated and the level of anthropization  
731 as well as their impact on mosquito biology. Despite the influence of water properties on  
732 mosquito colonization, it is important to consider bidirectional relationships, as mosquito  
733 colonization itself can directly impact water chemistry and microbiology through various  
734 mechanisms. During their development, larvae metabolize organic compounds such as  
735 detritus and excrete nitrogenous waste products like ammonia (Clements 1992), which can  
736 affect nutrient levels and pH in the water. Ammonium appears in water as an excretory  
737 product of nitrogenous wastes (Cochran 1985; Clements 1992) or as a metabolite from  
738 microbially mediated decomposition of nitrogen-containing organic matter (Ward  
739 2007). Microbial communities in water are involved in nutrient cycling and decomposition  
740 processes (Juma et al, 2021). The presence of larvae can influence the abundance and  
741 activity of these microbial communities, leading to changes in water chemistry (Kaufman et al.  
742 1999). Additionally, larvae may consume certain microbes, influencing their abundance and  
743 composition in the water. Egg-laying by females has also been shown to shape bacterial  
744 communities in breeding sites (Mosquera et al. 2023). Furthermore, larvae actively move and  
745 filter water as they feed and navigate through aquatic habitats (Clements 1992). This  
746 physical disturbance can stir up sediment particles, release nutrients trapped in sediments,  
747 and enhance oxygen exchange at the water surface. These actions can modify nutrient

748 availability, sedimentation rates, and oxygen levels, thereby impacting water chemistry. In  
749 summary, mosquito colonization can significantly affect water properties by altering nutrient  
750 levels, pH, microbial communities, and physical characteristics of aquatic habitats.  
751 Understanding these interactions is crucial for assessing the ecological impacts of mosquito  
752 populations on aquatic ecosystems.

753

## 754 **Conclusion**

755

756 Our study confirmed that larval habitat is an important determinant of microbial  
757 community structure. It also revealed that  $N_2O$  and  $CH_4$  were significantly higher in  
758 noncolonized water samples than in colonized water samples. Then, we found that pollution  
759 gradients differentially contributed to the microbial ecology of *Ae. albopictus*-colonized and  
760 *Ae. albopictus*-noncolonized water samples. The relationships between microbial  
761 communities and water quality presented here are a first and simplified step towards  
762 longitudinal sampling in the field and delimitation of the type of larval breeding sites in the  
763 urban mosaic to determine their environmental exposome. Further studies are needed to  
764 determine the influence of biotic and abiotic factors on the larval productivity of breeding sites  
765 in highly anthropized environments and their carry-over impact on adult life history traits and  
766 vector competence. Taking advantage of the wide range of parameters identified in this study,  
767 artificial breeding sites could be replicated in laboratory settings to simulate specific field  
768 conditions. These controlled experiments will provide an opportunity to investigate whether  
769 pollution directly impacts microbial composition and subsequently influences mosquito  
770 colonization through measures of oviposition behavior and other life history traits.

771

## 772 **Authors' contributions**

773 All authors contributed intellectually to and agreed to this submission. PD and CVM designed  
774 the experiments. PA, AS, EM, LV, PL, CVM, GM and PD conducted the experiments and  
775 sampling collection. LW, AF, CB and EV performed UPLC–HRMS analysis. PJ and EB

776 rperformed total organic carbon measure and stable isotopic ratio. RC constructed  
777 association networks and analyzed the data. AC and JG conducted ionic and gases  
778 chromatography. GM, DA, MG and PD made statistical analyses. PD and CVM wrote the  
779 initial draft of the manuscript, while GM and RC provided substantial feedback. The authors  
780 read and approved the final manuscript.

781

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785

## 786 **Conflict of interest statement**

787 All authors declare that they have no conflicts of interest.

788

## 789 **Data availability statement**

790 The COI gene sequences were deposited in GenBank under the accession numbers  
791 OR391757 to OR391848. All FastQ files were deposited in the EMBL European Nucleotide  
792 Archive (<https://www.ebi.ac.uk/ena>) under the project accession number PRJEB65881,  
793 PRJEB65912, PRJEB65914 for, 16S, 18S and ITS sequencing, respectively. Scripts used for  
794 analysis and figure generation are available in the following Github Repository  
795 (<https://github.com/penelopeduval/Pollution-gradients-shape-microbial-communities-associated-with-Ae.-albopictus-larval-habitats>). All other relevant data supporting the findings  
796 of this study are available within the article and its supplementary information.

797

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805

806

## 807 **Abbreviations**

808 dbRDA: distance-based Redundancy Analyses

809 C: Colonized water samples

810 L: Larvae

811 Lmm: linear mixed model

812 NC: Noncolonized water samples

813 OTU : Operational Taxonomic Unit

814 PLS-DA: partial least squares discriminant analysis

815

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818

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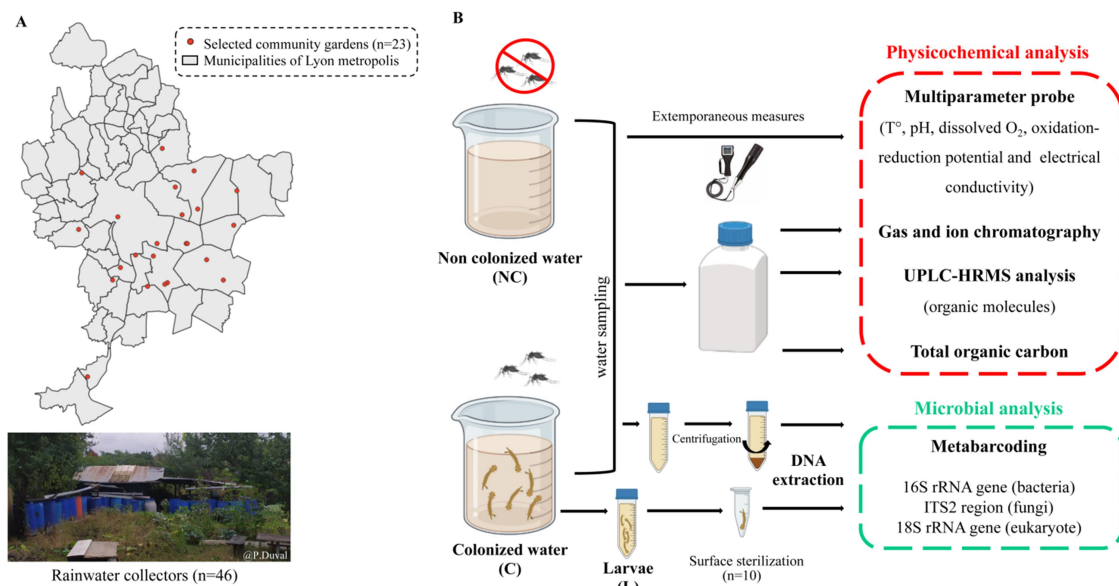
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1288

Fig. 1



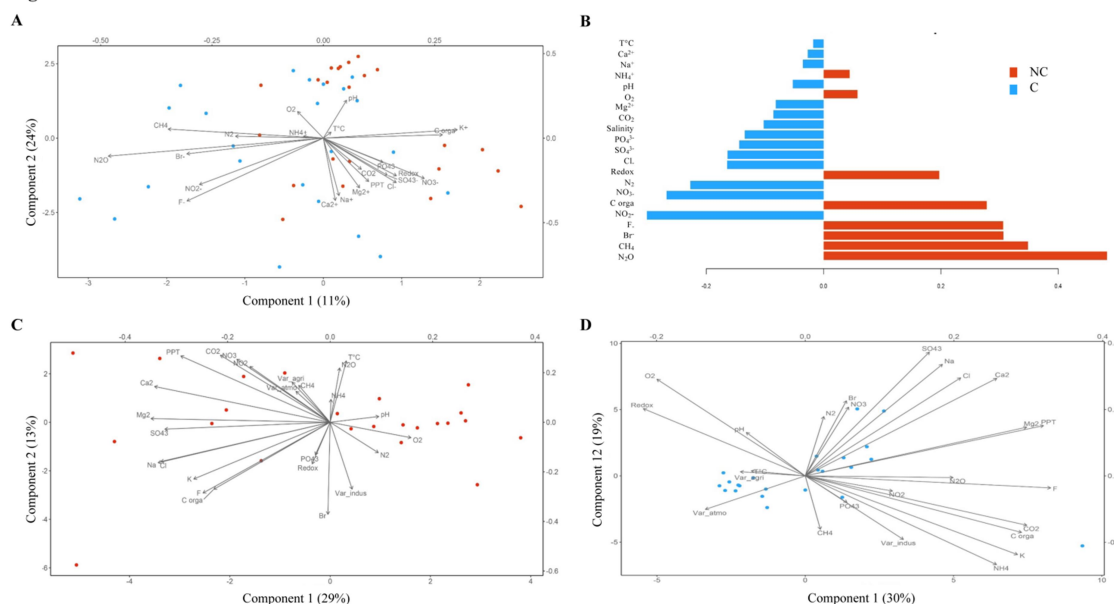
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**Figure 1. Sample collection overview.** (A) Distribution of community gardens in the Lyon metropolis, outlining the sites where water sampling collection was performed. The map of the Lyon metropolis was created using QGIS ([www.qgis.org](http://www.qgis.org)) (B) Sample processing describing physicochemical and microbial analyses of selected samples.

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Fig. 2



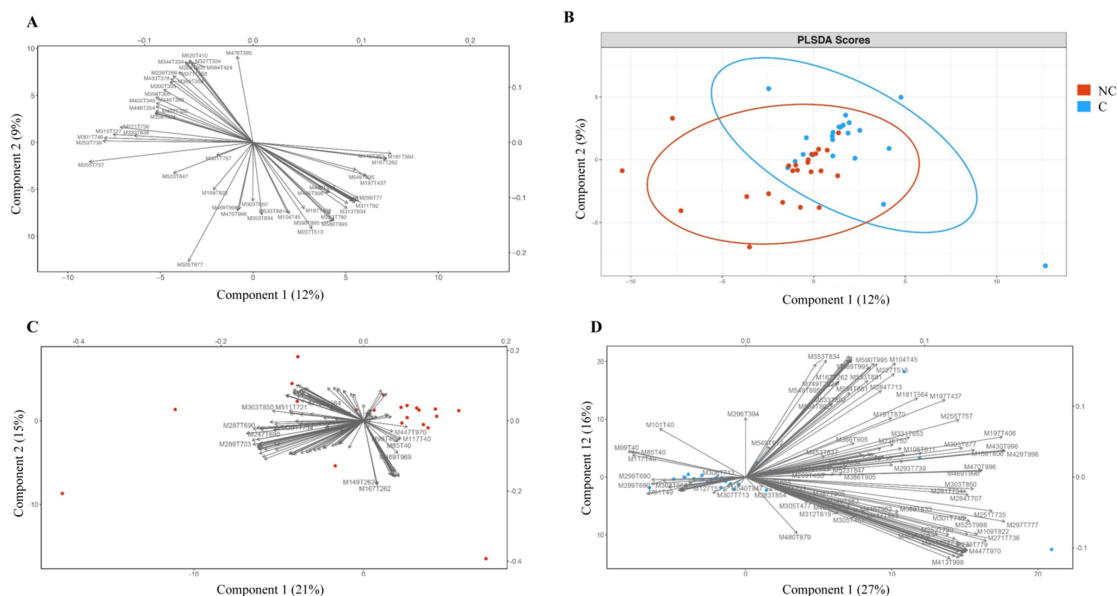
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**Figure 2. Variability of physicochemical parameters associated with noncolonized (NC) and colonized (C) water samples.** (A) Partial least squares-discriminant analysis (PLS-DA) showing physicochemical parameters for NC (red circle) and C (blue circle). (B) Loading plot from the PLS-DA showing the physicochemical parameters with higher values in either the NC or C category for the first component of the PLS-DA (C) and (D) principal coordinate analysis (PCA) plots of the measured physicochemical parameters (black vectors) for NC (C) and C (D). 'Var\_indus', 'Var\_agri' and 'Var\_atmo' indicate calculated industrial, agricultural and atmospheric pollution variables, respectively. 'C Organ' corresponds to organic carbon content.

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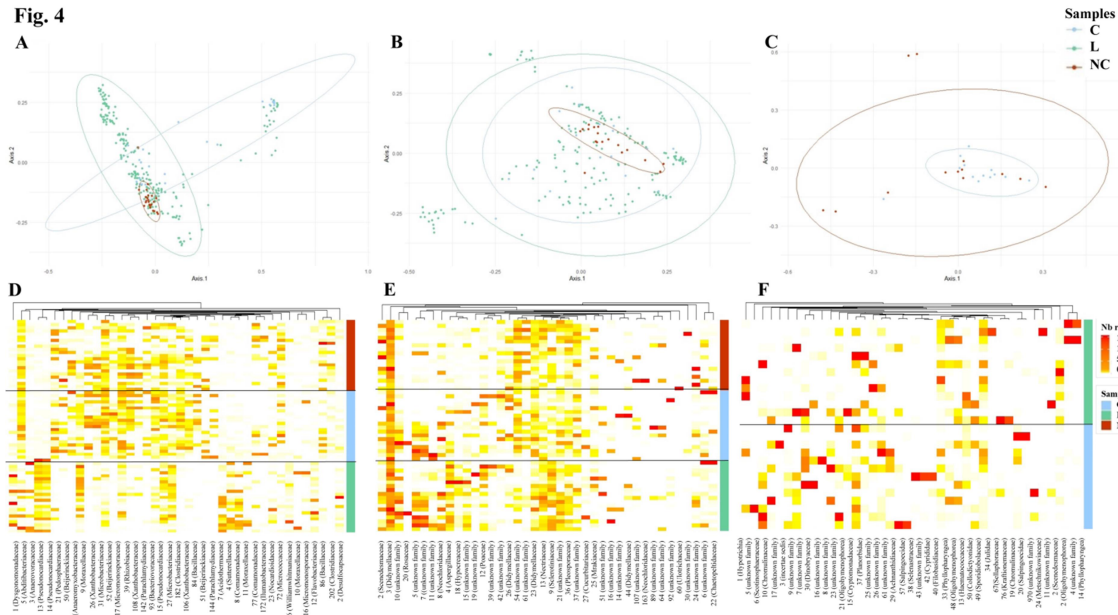
Fig. 3



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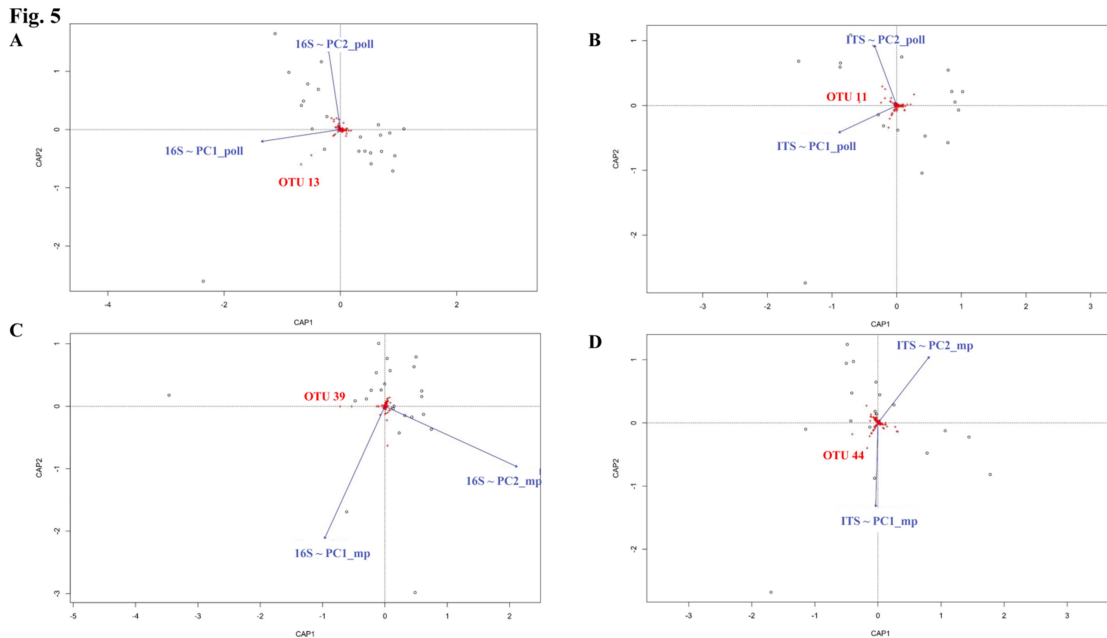
**Figure 3. Variability of organic molecule occurrence associated with noncolonized (NC) and colonized (C) water samples. (A) Loading plot from the PLS-DA showing organic molecule variables most strongly correlated with latent variables. (B) Score plot showing organic molecule composition for NC (red circle) and C (blue circle). (C) and (D) Principal component analysis (PCA) plots of organic molecules (black vectors) for NC (C) and C (D).**

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**Figure 4. Microbial community composition and structure associated with noncolonized waters (NC), colonized waters (C) and larvae (L).** Nonmetric multidimensional scaling (NMDS) ordinations displaying operational taxonomic unit (OTU) composition across NC, C and L are shown for each category of microorganisms: (A) bacteria, (B) fungi and (C) microeukaryotes. Heatmaps with cluster categorization representing the number of reads of the 40 most abundant OTUs among the total (D) bacterial, (E) fungal and (F) microeukaryotic OTUs (columns) for each type of sample (noncolonized, colonized, larvae (average of 10 larvae)) (rows). The intensity of the colour corresponds to the relative abundance of OTUs for each sample.

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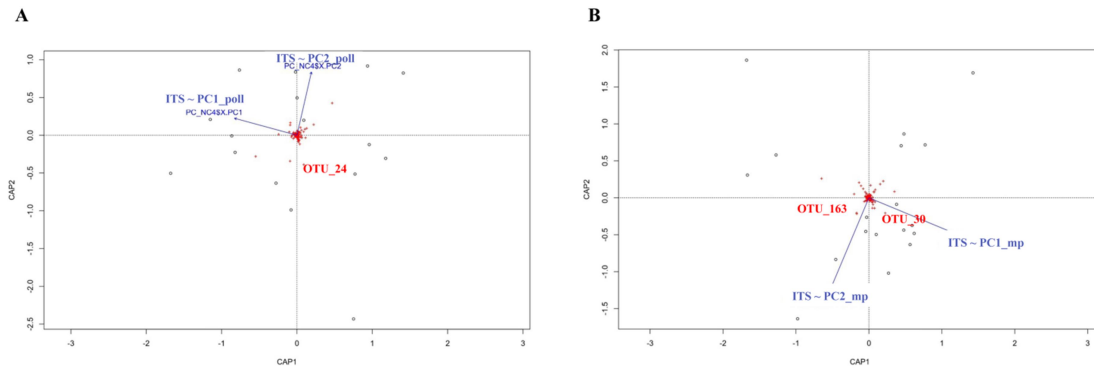
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**Figure 5. Effect of pollution gradients on the structure and variability of microbial communities associated with colonized (C) water samples.** Microbial community distances among samples (dots) were constrained based on physicochemical and pollution parameters (blue vectors) and are represented by distance-based redundancy analysis (dbRDA) plots. PC1\_poll/PC2\_poll represents the combination of physicochemical and pollution variables, and PC1\_mp/PC2\_mp represents the organic molecule occurrence. OTU names significantly correlated ( $p < 0.05$ ) with PC1\_poll/PC2\_poll or PC1\_mp/PC2\_mp are highlighted in red. Only significant effects are shown. (A) and (B) PC1\_poll/PC2\_poll dbRDA was performed for microbial community distances obtained from 16S rRNA and ITS sequencing, respectively. (C) and (D) PC1\_mp/PC2\_mp dbRDA was performed for microbial community distances obtained from 16S rRNA and ITS sequencing, respectively.

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**Fig. 6**



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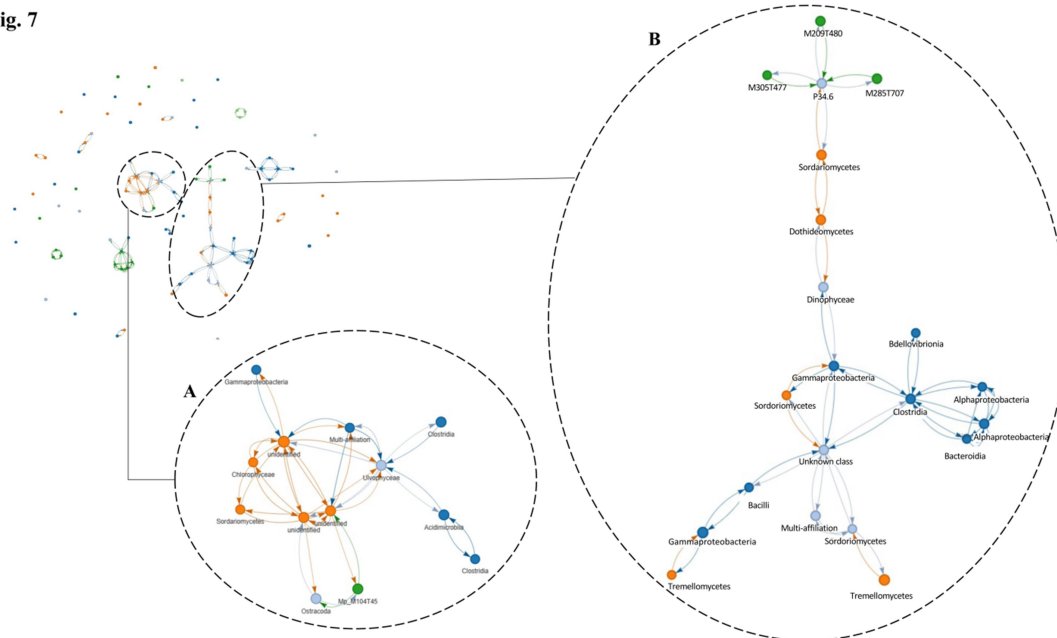
**Figure 6. Effect of pollution gradients on the structure and variability of microbial communities associated with noncolonized (NC) water samples.** Microbial community distances obtained from ITS sequencing (dots) were constrained based on physicochemical and pollution parameters (blue vectors) and represented by distance-based redundancy analysis (dbRDA) plots. (A) PC1\_poll/PC2\_poll represents the combination of physicochemical and pollution variables. (B) PC1\_mp/PC2\_mp represents the organic molecule occurrence. OTU names significantly correlated ( $p < 0.05$ ) with PC1\_poll/PC2\_poll or PC1\_mp/PC2\_mp are highlighted in red.

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Fig. 7



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**Figure 7. A network analysis showing positive correlations between microbial OTUs, i.e., bacteria (dark blue dots), fungi (orange dots) and eukaryotes (light blue dots), organic molecules (green dots) and physicochemical parameters (light green dots). Each link represents a correlation with  $\rho > 0.25$ . For this analysis, we kept the samples with positive PCR amplification for each genetic marker, for a total of 13 colonized waters among the 23 sampled.**

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Table 1. Characterization of selected community gardens. For each garden, the level of atmospheric pollution, distance from the garden to each pollution source (agricultural, industrial and atmospheric pollution) and calculated pollution variables (Var\_indus, Var\_agri and Var\_atmo) are given.

Garden	Pollution group	Atmospheric pollution scores	Distance from the garden to each pollution source (m)			Calculated pollution variables		
			Agricultural	Industrial	Atmospheric	Var_indus	Var_agri	Var_atmo
MID	AGRI	8	2624	1167	183	0.0009	4993	0.0437
MOU	INDUS	6	1311	1829	544	0.0005	386	0.011
PER	AGRI	5	158	3442	121	0.0003	228	0.0209
RECU	ATMO	12	1228	4598	4	0.0002	1365	0.1958
REB	ATMO	12	2624	1986	56	0.0005	191	0.2159
BIL	ATMO	12	4066	1818	26	0.0004	92	0.1534
GAR	AGRI	6	327	2209	476	0.0005	1144	0.011
ARK	INDUS	8	2640	528	500	0.0019	192	0.0152
ALST	ATMO	8	1549	3967	464	0.0003	80	0.0172
FRA	INDUS	3	1584	787	3068	0.0013	48	0.0009
TAS	INDUS	6	2044	802	1587	0.0012	923	0.0038
JUST	ATMO	12	3541	2999	24	0.0003	5	0.0587
DEC	AGRI	4	200	3422	59	0.0003	246	0.0382
FOR	ATMO	12	2759	2022	23	0.0005	182	0.2352
SYT	INDUS	11	3317	1974	204	0.0005	113	0.0538
QUA	INDUS	3	1545	590	2495	0.0017	585	0.0012
COR	AGRI	5	164	8643	1430	0.0001	193	0.0034
ESP	AGRI	9	669	1963	814	0.0005	559	0.0111
EDF	INDUS	12	7059	631	14	0.0016	2508	0.0147
BIG	AGRI	3	465	1457	620	0.0007	1099	0.0044
AVIA	AGRI	6	319	1491	298	0.0007	1659	0.0148
BONN	AGRI	6	682	1373	220	0.0007	771	0.0209
VOIL	ATMO	12	3371	2288	16	0.0004	560	0.3004

Table 2. Putative identification of organic molecules in noncolonized and colonized water samples

Id micropollutant	Exact mass m/z (Da)	tR (min)	Formula	Level of identification confidence	Micropollutant affiliation	Detection in water samples	
						C	NC
M181T564	181.1225	9.4	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	2B	3-tert-butyl-4-hydroxyanisole	VOIL, JUST, ARK, FRA, BIL	SYT, FRA, EDF, ALST, TAS
M197T437	197.1175	7.28	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	2A	Loliolide	VOIL, ARK, JUST, FRA, BIL	SYT, QUA, FRA, ALST, VOIL
M227T64	227.0867	1.07	C <sub>5</sub> H <sub>12</sub> N <sub>6</sub> O <sub>3</sub>	2B	Dimethylenetriurea	ARK, GAR	PER
M233T692	233.154	11.53	C <sub>15</sub> H <sub>20</sub> O <sub>2</sub>	2A	Costunolide, Indicanone, Alantolactone, Eremofrullanolide	ARK	
M277T743	277.2165	12.38	NA	5	Glechomanolide, Frullanolide family of phthalates	VOIL	FOR, QUA, TAS, GAR
M279T761	279.2321	12.68	C <sub>18</sub> H <sub>32</sub> O <sub>3</sub>	2B	13(S)-HODE, alpha-dimorphecolic acid laetiseric acid 9(10)-EpOME, (+)-vernolic acid	VOIL	QUA, FRA, FOR, COR, BIL, ALST, GAR
M284T713/M284T707	284.2225	11.89/11.78	C <sub>16</sub> H <sub>29</sub> NO <sub>3</sub>	2B	N-dodecanoyl-L-homoserine lactone		BIG
M127T51	127.0729	0.85	C <sub>3</sub> H <sub>6</sub> N <sub>6</sub>	2A	Melamine	FOR, JUST	FOR
M313T834	313.2741	13.89	C <sub>19</sub> H <sub>36</sub> O <sub>3</sub>	2B	(2E,18R)-18-hydroxynonadec-2-enoic acid	ARK	
M317T759/M317T743	317.2093	12.38/12.66	C <sub>20</sub> H <sub>28</sub> O <sub>3</sub>	2B	9-cis-4-hydroxyretinoic acid inumakoic acid, 15-deoxy-Delta(12,14)-prostaglandin (A2)	VOIL	QUA, FRA, GAR, TAS, FOR, ARK

M331T729	331.1885	12.16	C20H26O4	2B	or J2) (7Z)- lobohedleolide, hedychilactone D	VOIL
M289T703	289.1781	11.97	C14H20N6O	4	Unidentified	VOIL
M298T690	298.2382	11.51	C17H31NO3	4	Unidentified	BIG
M299T90	299.1191	1.49	C14H16F2N2O 3	4	Unidentified	ARK
M236T50	236.1496	0.83	C10H21NO5	4	Unidentified	VOIL QUA, FRA
M371T92	371.1516	1.54	C17H24N4O2S	4	Unidentified	ARK, GAR FRA,
M429T996	429.373	16.6	C29H50O3	2B	13-hydroxy- alpha-tocopherol	ARK, GAR, QUA, ROC VOIL
M533T881	533.2548	14.68/14.8 7	C33H34N4O3	2B	pyropheophorbid e a	ARK, FRA, FRA, ALST VOIL, , QUA, SYT, JUST EDF
M301T746	301.2156	12.38/12.4 3	C16H22O4	2A	Dibutyl phthalate	QUA, FRA
M419T933	419.316	15.34/15.5 5	C26H42O4	2A	Diisononyl phthalate	BIL COR
M447T983	447.3471	16.17/16.3 8	C28H46O4	2A	Di-n-decyl phthalate, Diisodecyl phthalate	QUA, BIL, COR FOR, VOIL
M167T262	167.0342	4.36	C8H6O4	2A	phthalic acid	ARK, FRA

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Table 3. Characteristics of water samples and extemporaneous measures of physicochemical parameters. For each sample, a description of the colonization status, the type of water habitat and measures of physicochemical properties are given. T(°C), salinity (PPT), pH, O2 dissolved (mg/L) are given for each sample individually and are also expressed per container as mean  $\pm$  SE

Sample ID	Garden	Sample type	Date	Type of sample	Material	Color	T(°C)	Mean $\pm$ SE	Salinity	Mean $\pm$ SE	pH	Mean $\pm$ SE	O2 dissolved (mg/L)	Mean $\pm$ SE	Redox	Mean $\pm$ SE
ALST_NC	ALST		2021-07-22	Rainwater collector	Plastic	Blue	25.17		0		8.49		3.48		49	
ARK_NC	ARK		2021-08-02	Rainwater collector	Plastic	Blue	19.09		0		7.93		8.6		190	
AVIA_NC	AVIA		2021-07-05	Bucket	Plastic	Red	20.42		0		6.95		7.1		305	
BIG_NC	BIG		2021-07-01	Rainwater collector	Plastic	Blue	20.8		0		7.28		5.45		300	
BIL_NC	BIL		2021-07-27	Rainwater collector	Plastic	Blue	25.64		0.2		7.45		4.78		18	
BONN_NC	BONN	Noncolonized	2021-07-05	Rainwater collector	Plastic	Green	22.18	21.5 (+/- 4)	0	0.1 (+/- 0.14)	7.4 (+/- 0.6)		7.46	6.4 (+/- 2)	283	198 (+/- 112)
COR_NC	COR		2021-09-22	Rainwater collector	Plastic	White	14.95		0.3		7.35		8.51		250	
DEC_NC	DEC		2021-06-16	Rainwater collector	Plastic	Blue	28.09		0.3		7.29		6.74		265	
EDF_NC	EDF		2021-07-20	Rainwater collector	Plastic	Blue	26.38		0		7.99		2.64		105	
ESP_NC	ESP		2021-07-08	Rainwater collector	Plastic	Blue	20.13		0		8.28		6.91		188	
FOR_NC	FOR		2021-07-	Watering can	Plastic	White	21.3		0		6.15		6.6		285	

					19								
FRA_N C	FRA	202 Rainwa 1- ter Plast Blue	09- collecto r	ic	17. 7	0	6.9 8	7.86	245				
GAR_N C	GAR	202 Rainwa 1- ter Plast Blue	08- collecto r	ic	17. 7	0	7.9 8	9	206				
JUST_N NC	JUST	202 Rainwa 1- ter Plast Blue	06- collecto r	ic	20. 23	0.1	7.5 9	3.28	70				
MID_N C	MID	202 Rainwa 1- ter Plast Blac	08- collecto r	ic k	19. 2	0.4	7.0 4	12.25	271				
MOU_N C	MOU	202 Rainwa 1- ter Plast Gra	07- collecto r	ic y	23. 02	0	6.4 1	7.94	214				
PER_N C	PER	202 Rainwa 1- ter Plast Blue	09- collecto r	ic	21. 8	0.1	7.6 4	3	257				
QUA_N C	QUA	202 Rainwa 1- ter Plast Whit	09- collecto r	ic e	24. 93	0.1	7.4 4	6.56	192				
REB_N C	REB	202 Rainwa 1- ter Plast Blue	09- collecto r	ic	20. 72	0.1	6.3 8	6.14	288				
RECU_N NC	REC U	202 1- Bucket Plast Whit	09- collecto r	ic e	17. 01	0.2	6.5 8	6.42	254				
SYT_N C	SYT	202 Rainwa 1- ter Meta Gra	07- collecto r	l y	24. 94	0.1	7.4 6	3.91	-158				
TAS_N C	TAS	202 1- Bucket Plast Blac	09- collecto r	ic k	14. 82	0.1	8.1 7	5.96	228				
VOIL_N C	VOIL	202 Rainwa 1- ter Plast Blue	06- collecto r	ic	29. 12	0.2	8.0 5	5.83	245				
ALST_N C	ALST	202 Rainwa 1- ter Plast Blue	07- collecto r	ic	25. 51	0.3	0.1 8.1 9 9	5.04	230	215			
		Colonized											
ARK_N C	ARK	202 Rainwa 1- ter Plast Blue	08- collecto r	ic	22. 25	0.4	0.1 6.7 9 2	3.12	-64	88			

		02	r						
AVIA_C	AVIA	202	Rainwa	1-	ter	Plast	Gre	20.	
		07-	collecto	ic			en	92	0
		05	r						7.9
									5
									7.17
									252
BIG_C	BIG	202	Rainwa	1-	ter	Plast	Blue	21.	
		07-	collecto	ic				98	0
		01	r						8.0
									2
									8.85
									317
BIL_C	BIL	202	Rainwa	1-	ter	Plast	Blue	23.	
		07-	collecto	ic				4	0
		27	r						8.0
									5
									3.21
									86
BONN_	BON	202	Rainwa	1-	ter	Plast	Blue	21.	
C	N	07-	collecto	ic				22	0
		05	r						7.3
									6.79
									199
COR_C	COR	202	Rainwa	1-	ter	Plast	Blue	15.	
		09-	collecto	ic				97	0.3
		22	r						6.7
									8.64
									231
DEC_C	DEC	202	Rainwa	2-	ter	Plast	Blue	26.	
		06-	collecto	ic				29	0.012
		16	r						7.6
									3
									10.12
									194
EDF_C	EDF	202	Rainwa	1-	ter	Plast	Blue	23.	
		07-	collecto	ic				81	0
		20	r						7.8
									5.02
									248
ESP_C	ESP	202	Rainwa	1-	ter	Plast	Blue	20.	
		07-	collecto	ic				29	0
		08	r						7.9
									9
									7.39
									165
FOR_C	FOR	202	Rainwa	1-	ter	Plast	Gra	23.	
		07-	collecto	ic			y	71	0
		19	r						7.8
									5
									5.75
									296
FRA_C	FRA	202	Wateri	1-	ng	Plast	Gre	18.	
		09-	ng can	ic			en	13	0.1
		29							5.7
									10.07
									264
GAR_C	GAR	202	Rainwa	1-	ter	Plast	Blue	18.	
		08-	collecto	ic				45	0.3
		31	r						7.9
									7
									6.82
									160
JUST_	JUST	202	Rainwa	1-	ter	Plast	Blue	20.	
C		06-	collecto	ic				83	0.1
		25	r						7.6
									7
									6.91
									260
MID_C	MID	202	Rainwa	1-	ter	Plast	Blue	20.	
		08-	collecto	ic				43	0
		03	r						7.2
									8
									4.56
									284
MOU_C	MOU	202	Rainwa	1-	ter	Plast	Blue	23.	
		07-	collecto	ic				68	0.3
									7.7
									9
									7.02
									237

		26	r						
PER_C	PER	202	Rainwa						
		1-	ter	Plast	Blue	21.	0.1	7.2	1.95
		09-	collecto	ic		5		6	71
		07	r						
QUA_C	QUA	202	Rainwa						
		1-	ter	Plast	Blue	21.	0	7.1	5.09
		09-	collecto	ic		86		7	254
		06	r						
REB_C	REB	202	Rainwa						
		1-	ter	Plast	Blue	21.	0	5.7	8.32
		09-	collecto	ic		21		2	328
		01	r						
RECU_C	REC U	202	Rainwa						
		1-	ter	Plast	Blue	19.	0.2	6.1	5.03
		09-	collecto	ic		91		2	277
		13	r						
SYT_C	SYT	202	Rainwa						
		1-	ter	Meta	Gra	23.	0.2	7.6	4.11
		07-	collecto	l	y	24		3	207
		21	r						
TAS_C	TAS	202							
		1-	Bucket	Plast	Whit	16.	0.2	7.7	7.55
		09-		ic	e	21		8	256
		23							
VOIL_C	VOIL	202	Rainwa						
		1-	ter	Plast	Whit	25.	0	8.7	9.23
		06-	collecto	ic	e	97		6	199
		17	r						

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1316 **Table 4. Measures of ion and gas composition as well as total organic carbon in noncolonized and colonized water**  
1317 **samples. The concentrations of each ion (F-, Cl-, Br-, NO<sub>2</sub>-, NO<sub>3</sub>-, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) and each gas**  
1318 **(O<sub>2</sub>, N<sub>2</sub>, CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub>O) as well as total organic carbon (TOC) are given for each sample individually and are also**  
1319 **expressed per container as mean ± SE.**

Sample ID	Sample type	Water ionic composition												Gas composition of water																
		F <sup>-</sup>	Me an ±S	Cl <sup>-</sup>	Me an ±S	N O <sub>2</sub> -	Me -	Br	Me 3-	NO	PO	SO	Na	NH	Mg	Ca	O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub> (ppm vol)	CO <sub>2</sub> (ppm vol)	N <sub>2</sub> O (ppm vol)	Total Me organ ±S								
		F <sup>-</sup>	F <sup>-</sup>	Cl <sup>-</sup>	Cl <sup>-</sup>	NO <sub>2</sub> -	NO <sub>2</sub> -	NO <sub>2</sub> -	NO <sub>3</sub> -	NO <sub>3</sub> -	NO <sub>3</sub> -	NO <sub>3</sub> -	NO <sub>3</sub> -	NH <sub>4</sub> <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	Mg <sup>2+</sup>	Mg <sup>2+</sup>	Mg <sup>2+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Ca <sup>2+</sup>	Ca <sup>2+</sup>	Ca <sup>2+</sup>	O <sub>2</sub> (%)	O <sub>2</sub> (%)	O <sub>2</sub> (%)	O <sub>2</sub> (%)	CH <sub>4</sub> (ppm vol)	CO <sub>2</sub> (ppm vol)	N <sub>2</sub> O (ppm vol)
ALST_NC_01		0.3	0.0	0.0	0.1				1.2	0.8	0.5	0.6	0.2	4.8	2.1	5.0	18.7	2373.	3.67	4.37										
ARK_NC_03		0.2	0.0	0.0	0.0	2.0	0.0	0.0	0.4		0.0	0.5	0.1	2.9	2.0	4.6	0.17	770.9	0.14	1										
AVIA_NC_03		1.5	0.0	0.0	0.6	0.8	3.8	1.7	0.4	3.4	0.9	11.	2.1	5.1	2071.		2071.		2.74	7.9										
BIG_NC_02		0.5	0.0	0.0	0.2	0.1	0.9	1.5	0.8	0.7	0.2	3.8	2.1	5.0	2065.		2065.		0.5	5.77										
BIL_NC_07		8.5	0.0	0.0	0.5	0.5	17.	6.7		4.8	4.9	44.	1.8	4.2	198.	4200.		4200.	0.73	11.6										
BONN_NC_04		0.3	0.0	0.0	0.0	0.0	0.8		0.0	0.3	0.2	5.2	1.8	4.4	5512.		5512.	0.04	6.84											
COR_NC_11		31.	0.0	0.0	2.2	0.3	33.	20.	0.4	5.5		62.	1.7	5.5	2674.		2674.	0.24	24.9											
DEC_NC_04		25.	0.0	0.0	10.		18.	15.	0.0		6.2	65.	2.1	4.9	1166		1166	0.12	7.8											
EDF_NC_02		0.2	0.0	0.0	0.2	0.0	0.5	0.4	0.2	0.5	0.2	4.3	2.0	4.3	2098.		2098.	1.01	6.56											
ESP_NC_02		0.1	0.0	0.0	0.1		0.4	0.5	0.0	0.1		9.5	6.4	21.	365.1		365.1	0.37	3.18											
FOR_NC_02		0.8	0.0	0.0	0.4	0.6	1.3	1.7	0.0	1.4	0.3	4.5	2.0	5.2	1904.		1904.	0.14	12.7											
FRA_NC_24	NC	0.6	0.0	0.0	1.0	0.3	8.1	1.2	3.0	0.5	2.0	10.5	26.7	5.9	14.9	8.4	3.1	5.2	15.9	14.6	35.5	1.12	1.1	2.10	39.4	9.9				
GAR_NC_03		0.7	0.0	0.0	0.0	0.0	1.8	1.6	2.3	1.2	0.2	6.1	6	42	2.8	6.4	1947.92		1947.92	0.64	8									
JUST_NC_09		1.2	0.0	0.0	0.7		2.8	1.5	0.9	4.3	0.6	16.	2.0	4.5	11.2	2212.		2212.	4.79	9.09										
MID_NC_05		20.	0.0	0.0	6.5	0.0	26.	13.	0.1	5.7	11.	71.	4.0		1207		1207	0.18	5.5											
MOU_NC_01		0.2	0.0	0.0	0.0	0.0	0.7	0.3		0.2	0.1	5.6	2.2		33.86		33.86	0.11	3.98											
PER_NC_04		2.0	0.0	0.0	0.5	0.5	2.9	1.3	0.0	3.0	0.6	18.	2.1		1978.		1978.	0.18	22.8											
QUA_NC_18		12.	0.0	0.0	0.0	0.0	33.	8.7		2.9	6.5	24.	1.9	4.6	417.3		417.3	0.17	14.5											
REB_NC_07		4.2	0.0	0.0	2.7	0.5	11.	3.2	0.0	1.9	2.0	25.	2.1	4.6	1358.		1358.	0.23	5.12											
RECU_NC_11		11.	0.0	0.0	16.	9.7	0.1	2.0	7.4	38.	5.1	3593.		28.0			3593.	0.24	28.0											
SYT_NC_1		7.3	0.0	0.0	8.8	5.5	4.1	4.2	29.	0.6	4.6	69.2	1222		16.5		1222	2.76	16.5											
TAS_NC_06		3.1	0.0	0.0	9.3	3.4	0.4	1.0	2.1	23.	1.9	5.5	2383.		20.0		2383.	0.36	20.0											
VOIL_NC_07		11.	0.0	0.0	30.	7.8	4.4	6.0	5.0	48.	2.1	5.0	3248.		17.2		3248.	1.52	17.2											
ALST_C_08		17.	0.0	1.0	4.1	34.	13.	0.0	7.8	64.	3.6	10.	8966.		7.65		8966.	0.46	7.65											
ARK_C_16		16.	0.0	0.0	0.0	0.9	3.2	6.1	3.0	125.	11.	39.	0.3	5.2	2855		2855	9.04	66											
AVIA_C_02	C	0.3	10.0	0.0	0.0	0.1	0.6	1.8	0.1	0.4	1.4	13	6.1	0.2	0.5	0.5	9.1	3.4	2.9	2.1	2.1	5.2	2.4	2038.	39.86	0.6	10.			
BIG_C_02		0.4	0.0	0.0	0.0	0.3	0.5	0.1	0.8	0.8	0.8	1.4	7.4	0.8	0.4	0.4	26	0.1	3.7	3.3	24	3	0.6	6.5	1.3	8.5	1192.57	0.22	2.51	8.4
BIL_C_03		0.5	0.0	0.0	0.3	0.0	1.2	0.9	0.8		0.2	3.8	1.9		40.5	4233.		40.5	4	84					0.54	6.33				
BONN_C_02		1.1	0.0	0.0	0.8	0.5	2.4	0.7	0.0	2.6	0.9	9.9	2.3	5.3	1735.		1735.	0.06	31.6						0.13	5				



COR_	0.	33.	0.0		3.1		34.	19.		2.4	5.9	45.		4.4		1809.		32.9
C	06	53	7	0	6	0	2	89	0	7	9	37	2.1	1	0.01	48	0.05	7
DEC_	0.	0.6	0.0	0.	0.4		0.9	1.8	0.0	0.4	0.1	4.4	2.0	5.0		2967.		
C	02	2	2	01	8	0.1	8	2	9	6	6	4	4	7	0.95	18	0.02	3.05
EDF_	0.	18.	0.0			0.0	27.	13.			10.	45.	1.9			4611.		
C	04	65	2	0	6	1	6	24	0	2.4	28	43	6	4.1	0.24	83	0.05	3.77
ESP_	0.	4.1	0.0	0.	2.0	0.1	4.6	1.9		1.0	0.7	14.	2.4	5.2		789.2		
C	02	6	2	04	4	9	1	6	0	1	3	45	2	3	0.06	8	0.51	5.2
FOR_	0.	0.2	0.0			0.0	0.7	0.8		0.1	0.1	2.4		4.9		1577.		
C	01	3	1	0	0.4	9	9	6	0	9	2	6	2.2	3	0.23	37	0.26	4.25
FRA_	0.	3.3	0.0	0.	0.0	0.2	3.9	1.0	0.3	21.	3.9	26.	1.9	5.0		1516.		35.7
C	11	2	7	01	8	4	4	1	9	46	7	81	1	8	0.14	77	0.02	6
GAR_	0.	29.	0.0	0.		0.7	28.	5.5	0.1	8.2	4.3	72.	1.7	4.5		6583.		29.6
C	05	23	5	04	3.6	9	99	1	2	6	3	61	8	1	0.25	94	0.82	3
JUST	0.	4.2	0.0	0.	12.	3.4	12.	6.8		16.	2.3	32.	1.9	4.6		3391.		
_C	05	1	6	02	04	1	95	6	0	66	7	71	1	8	0.12	08	0.59	17.4
MID_	0.	0.2	0.0	0.	0.6	0.0	1.2	0.7	0.0	0.3		5.6	2.4	5.5		1694.		
C	02	5	1	05	4	6	4	4	8	2	0.2	3	6	5	0.24	02	0.74	3
MOU_	0.	62.			3.3	0.0	65.	29.		1.8	8.2	75.	2.2	4.9		4190.		
C	07	57	0	0	2	7	65	4	0	2	3	23	4	6	0.02	09	0.06	7.84
PER_	0.	0.5	0.0	0.	0.1	0.4	0.9	1.0	0.0	2.4	0.8	19.	2.3	5.7		4109.		22.0
C	07	9	7	02	2	5	2	8	9	9	3	95	4	1	1.48	99	0.7	6
QUA_	0.	0.3	0.0	0.	0.8	1.1	0.9	0.4	0.1	5.1	0.3	2.6	2.1	4.7		2264.		11.9
C	02	7	2	01	2	5	8	2	8	4	5	5	2	6	0.3	26	0.48	7
REB_	0.	0.4	0.0	0.		0.2	0.6	0.6		0.2	0.1	1.8	2.1	4.3		731.8		
C	02	1	1	01	0.7	4	3	8	0	4	1	9	1	7	0.04	8	0.01	2.14
RECU	0.	13.	0.0		0.4	0.1	9.2			3.0	8.4	34.	2.0	4.8		3726.		21.3
_C	06	21	7	0	5	3	6	9.8	0	1	4	69	7	8	0.5	21	0.48	9
SYT_	0.	19.	0.0	0.	1.7		29.	13.			4.0	37.	2.1	4.8		977.7		15.1
C	07	25	3	02	3	0	7	3	0	6.2	3	31	1	1	0.18	3	3.01	2
TAS_	0.	10.	0.0	0.			30.		0.9	3.3	6.3	34.	1.9			2757.		24.1
C	06	81	4	52	0.5	0	44	8.8	1	1	6	97	5	4.4	0.21	52	0.77	5
VOIL_	0.	1.4	0.0	0.	0.0	0.3	1.1	2.2	0.1	0.8	0.3	5.2	1.9	4.6		1270.		
C	04	2	2	01	7	3	6	9	1	6	4	7	1	6	0.5	64	0.01	14.1

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