Population structure determined by comparative amplicon gene sequencing in biofilm and planktonic bacterial communities from pesticide-contaminated water

Population structure in biofilm and planktonic bacterial communities from contaminated water

1 **Jhenifer Yonara Lima¹, Cassiano Moreira¹, Gessica Costa¹, Paloma Nathane**
2 **Nunes Freitas¹, Luiz Ricardo Olchanheski², Sonia Alvim Veiga Pileggi¹, Rafael**
3 **Mazer Etto¹, Christopher Staley³, Michael Jay Sadowski⁴ and Marcos Pileggi^{1*}**

4 ¹ Department of Structural and Molecular Biology and Genetics, Ponta Grossa State
5 University, Ponta Grossa, Paraná, Brazil

6 ² Department of Microbiology, University of São Paulo, Sao Paulo, Brazil

7 ³ The Biotechnology Institute, University of Minnesota, St. Paul, Minnesota, United
8 States of America

9 ⁴ Department of Soil, Water, and Climate and The Biotechnology Institute, University
10 of Minnesota, St. Paul, Minnesota, United States of America

11 * Corresponding Author

12 E-mail: pileggimarcos@gmail.com

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15 **Abstract**

16 Molecular and bioinformatics tools for research are very important for
 17 biodiversity characterization in contaminated environments due the presence of a high
 18 percentage of nonculturable microorganisms. The wide use of pesticides in agriculture
 19 exposes microbiomes to stressful and selective conditions that demand survival
 20 strategies such as biofilm formation. The purpose of this work was to evaluate the
 21 bacterial population structure in planktonic and biofilm communities of water that was
 22 used for washing the packaging of herbicides and stored for six months in tanks. This
 23 substance is highly contaminated waste. Samples of water and biofilms from tanks and
 24 biofilms developed for short times in flasks were used for DNA isolation and 16S rRNA
 25 gene sequencing. The physicochemical conditions imposed by water used for washing
 26 the containers were inadequate for killing the bacterial genera identified according to
 27 water and wastewater standards. The variation in population structure and diversity was
 28 lower in the planktonic samples than in the biofilm samples, indicating a possible
 29 combination of genetic drift and subsequent selection of individuals surviving under
 30 stressful water conditions, such as heating and contact with agrochemicals, over a six-
 31 month period. The biofilm formation in water tanks contaminated with pesticides
 32 enabled the survival of bacterial genera harboring the essential processes for adaptation
 33 to these environments; the presence of these processes was determined according to
 34 descriptions obtained from the genomic databases. This study suggests the potential of
 35 bacterial genera identified in biofilms obtained from tanks to adapt to contaminated
 36 environments through their metabolic complexity. Thus, herbicide biodegradation
 37 kinetics can be accessed through a culturable collection obtained from these
 38 communities.

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40 **Introduction**

41 Environmental microbiology has been gaining ground in academia over the last
42 few years, bringing with it an interest in understanding composition, structure and
43 function in nature [1]. Notably, contaminated environments expose microbiomes to
44 stressful and selective conditions, and once microorganisms exhibit structural and
45 metabolic adaptations, they can overcome these conditions and survive. These adaptive
46 strategies are linked at the cellular level and to specific populations, allowing different
47 responses to compounds such as pesticides [2,3] and enabling their use in
48 biotechnological processes such as agrochemical degradation [4–6]

49 However, microbial diversity is high and poorly elucidated by culture
50 techniques, even with different types of culture media, since a large percentage of these
51 microorganisms are nonculturable [7,8]. Therefore, it is necessary to create new
52 research tools that allow the characterization of microbiological biodiversity, i.e.,
53 molecular microbial ecology, through the use of high-throughput sequencing
54 technologies with the aid of bioinformatics techniques for data analysis [1,9–11].

55 The knowledge and use of molecular technologies are fundamental to
56 understanding the structure and functions of microbiomes and nonculturable
57 microorganisms. Advances in genomics and metagenomics, marker genes, sequencing
58 of the 16S rRNA gene and other molecular approaches are essential for predicting soil
59 microbiome functions and improving agriculture production [12]. These approaches are
60 fundamental to the creation of genomic libraries that allow us to identify and isolate
61 enzymes with different biocatalytic activities from culturable strains, permitting their

62 biotechnological exploitation [13,14]. These techniques allowed Krohn-Molt et al.
63 (2017) to evaluate the genes of an association of algae essential to the carbon cycle with
64 a unique and specific microbiome. Knowledge of the taxonomic structure of the
65 microbiome increases the reliable handling of its latent biotechnological potential
66 [16,17].

67 Thus, the composition and relative abundance of soil microbiomes in the
68 Amazon were correlated with different types of land use, such as deforestation, which
69 leads to carbon cycle disturbances and, consequently, climate change [18]. Therefore,
70 microbiomes, which play key roles in biogeochemical cycles, are affected by
71 anthropogenic action, particularly by the use of xenobiotic compounds, leading to
72 changes in sustainability and environmental quality [18,19]. High-density DNA
73 microarray and 16S rDNA amplicon next-generation sequencing were used to study the
74 impact of the insecticide chlorpyrifos, the herbicide isoproturon and the fungicide
75 tebuconazole on the soil bacterial community, and significant differences have been
76 found in field experiments but not in soil microcosms exposed to these pesticides [20].
77 These methods were considered a guide for more specific studies in this area. A
78 molecular approach was also used to analyze the effects of combinations of glyphosate
79 and different herbicides on the rhizobacterial community of transgenic plants; this study
80 indicated that glyphosate alone seems to be the less aggressive [21]. Molecular
81 approaches also helped Dennis et al., 2018, reach the conclusion that the herbicides
82 glyphosate, glufosinate, paraquat, and paraquat-diquat did not significantly interfere
83 with soil microbial diversity and function under laboratory conditions.

84 In aqueous environments, which are also subject to contamination by
85 agrochemicals, microbiomes present two possible lifestyles: planktonic and biofilm.
86 The first term refers to the cells of microorganisms suspended in water (e.g., in an ocean

water column) [22]. However, one of the main adaptive strategies found in microbiomes in contaminated or extreme environments is the formation of biofilms, in which cells adhere to a substrate or one another by secreting a gelatinous matrix of extracellular polymeric substances (EPS), which is configured as an extension of the cell itself [23,24]. The community can thus capture organic compounds for metabolization by extracellular enzymes through organized gene expression mediated by cell-cell communication or quorum sensing. This structure is also responsible for increased adaptability, stability, and cellular integrity [25–27]. 16S rRNA gene amplicon sequencing was used to verify the impact of herbicides and fertilizers on planktonic marine microbial communities at the Great Barrier Reef. Water characteristics, such as salinity, rainfall, temperature and water quality, have a great influence on the composition of microbial communities [28]. Aquatic environments are complex and subject to various physicochemical interferences, making it difficult to understand the impact of agrochemicals on microbiomes.

Thus, the objective of this study was to identify the population structure of planktonic and biofilm bacterial communities in an environment consisting of tanks used for storing washing water from the packaging of herbicides, which results in highly contaminated waste; thus, another objective was to glimpse adaptive strategies through the composition of genes present in their taxonomic units.

Materials and methods

Study area

The object of study was water used to wash the containers (Fig 1) of 30 agrochemicals (S1 Table). The water used for the washing of agrochemical packages

110 came from an artesian well. After washing, the water was kept in two 10,000 L tanks for
111 6 months at the Capão da Onça Farm School, State University of Ponta Grossa, State of
112 Paraná, Brazil (25°05'31.3"S; 50°03'28.0"W) (altitude: 1000 m). The first tank (T1) had
113 water from the washing of agrochemical containers that had lost some volume by an
114 evaporation process at 100 °C for 190 min and was then stored in a second tank (T2)
115 until being transported and discarded.

116 **Figure 1:** Evaporation of the water from the washing of pesticide containers used for
117 DNA collection in this study. S: Sanitary sink; TR: Tractor spray washing trough; SH:
118 Shed where T1 and T2 are; T1: Tank 1; T2: Tank 2; W: Water filters; TI: Timer for
119 controlling the flow of water; TC: Solar heating temperature control panel; So: Solar
120 heating system.

121 **Physicochemical analyses of water**

122 The following physicochemical characteristics of water samples collected from
123 the tanks and the artesian well were analyzed according to the *Standard methods for the*
124 *examination of water and wastewater* (1998): chlorine, conductivity, color, fluorine,
125 pH, total dissolved solids and turbidity, biochemical oxygen demand (BOD) and
126 chemical oxygen demand (COD).

127 **Collection of samples and extraction of DNA**

128 Four liters of water were collected from T1 and T2 to obtain planktonic samples.
129 The water was passed through autoclaved glass fiber membranes in 750 mL triplicates:
130 first, a 5-µm-thick prefilter; then, 1.2 µm; and last, 0.45 µm (Merck Millipore,
131 Carrigtohill, Ireland). After the last membrane, the samples were stored at -80 °C. The
132 samples were named M1 (preheating) and M2 (postheating).

133 The biofilm samples were collected in triplicate from the wall surface of the
134 tanks in contact with the water line using 3 sterile swabs for each triplicate. The samples
135 were named B1 (preheating) and B2 (postheating). Likewise, biofilms were collected
136 from the 4 L flasks in which water samples were collected after being kept immobile for
137 five days. The samples were named G1 (preheating) and G2 (postheating). The material
138 collected in triplicate was stored at -80 °C.

139 Samples of planktonic origin (retained in the membranes) were macerated in
140 liquid nitrogen with the aid of a pestle. The swabs used for the biofilm collections were
141 submerged in 1 mL of saline buffer (0.9% NaCl) and homogenized for 5 min to extract
142 biological material from the cells in the buffer. DNA extractions were performed using
143 the PowerSoil DNA extraction kit (MO BIO Laboratories Inc., Carlsbad, USA), and the
144 material obtained was stored at -80 °C.

145 **Sequencing**

146 Sequencing of the samples was performed using 515F/806R primers, which are
147 specific for the V4 region [29]. Amplification and sequencing were performed using the
148 dual index method reported by the University of Minnesota Genomics Center
149 (Minneapolis, MN, USA) [30]. A negative control containing sterile water was included
150 in each amplified and sequenced sample plate. Sequencing was performed on the
151 Illumina MiSeq platform using an iSeq 100 System (Illumina, Inc. San Diego, USA)
152 [29]. Sequence data are available from the Sequence Read Archive of the National
153 Center for Biotechnology Information under accession number SRA: PRJNA528924.

154 **Bioinformatics**

Sequence processing was performed using mothur software (version 1.29.2). The prokaryotic sequence data were trimmed to the first 160 nucleotides (nt), and end pairing was carried out using fastq-join software [31]. The sequences were trimmed for quality following a previous description for V5-V6 data [32]. The overall alignment was performed in relation to the SILVA database ver. 119 [33], the sequences were subjected to a 2% preclustering to remove sequence errors [34], and the chimeric sequences were identified and removed by UCHIME software [35]. Operational taxonomic units (OTUs) were assigned at $\geq 97\%$ identity by complete linkage clustering. Taxonomic attributions were made by comparing the Ribosomal Database Project ver. 14 to a bootstrap limit of 60%, as previously described [36].

Bacterial communities were ordered based on OTU abundance using multidimensional scaling. The relative abundance of the taxonomic groups and the functional abundances inferred in PICRUSt were regressed in relation to the ordering scores to determine significant correlations with the ordering scores of the bacterial communities [37]. The 16S rDNA data were analyzed as indicated by the PICRUSt genome prediction software [<http://picrust.github.io/picrust/>] from raw sequence reads in the following environment: NumPy (1.7.1), biom-format (1.3.1), PyCogent (1.5.3), PICRUSt (1.0.0-dev), and PICRUSt script (1.0.0-dev). Functional predictions were assigned up to KO tier 3 for all genes. Results from the MG-RAST QIIME report were compared with predictions from PICRUSt using the “compare_biom.py” subroutine with normalization and observations not in the “expected data” file ignored. Results were compared using both shotgun metagenomic and PICRUSt predictions as the expected data. Statistical analyses were performed using the vegan package in R [38].

Results and discussion

179 This work discusses three levels of results to account for interferences in the
180 adaptive strategies presented by the different bacterial communities linked to the
181 contaminated environment in this study: the physicochemical characteristics of the
182 environment, the taxonomic composition at the time of collection, and the genes
183 associated with the identified taxonomic units.

184 **Physicochemical characteristics of the tank water**

185 The physicochemical data are analyzed in this section because they provide the
186 conditions under which the adaptation of the present microbiome to the environment
187 occurs. The data for the water from T1 and T2 and the artesian well whose water was
188 used in the washing are shown in Table 1.

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200 **Table 1:** Physicochemical analyses of tanks 1 (T1) and 2 (T2) and the artesian well
 201 (AW). Parameter values are presented as the means of triplicate measurements. WHO:
 202 World Health Organization; SD: standard deviation; TDS: total dissolved solids; BOD:
 203 biochemical oxygen demand; COD: chemical oxygen demand; ND: not detected. The
 204 letters A, B, C, and D represent significant differences within each row.

Parameters	T1	SD	T2	SD	AW	SD	WHO
Chlorine (mg/L)	1.66 ^A	0.1	1.57 ^A	0	0 ^B	0	0.7 ^C
Conductivity (μS/cm)	418.3 ^A	2	413 ^A	0.7	13.57 ^B	0.564	10 – 100 ^B
Color (Pt/Co)	245 ^A	1.5	250 ^B	0	0 ^C	0	0 ^C
Fluorine (mg/L)	0.82 ^A	0	0.81 ^A	0	0.05 ^B	0	1.5 ^C
pH at 25°C	5.45 ^A	0.1	5.32 ^A	0	4.75 ^B	0.045	6.5 – 8.5 ^C
TDS (ppm/NaCl)	186.3 ^A	1	183.2 ^A	0.3	0 ^B	0	100 - 600 ^A
Turbidity (NTU)	204 ^A	5.8	206.3 ^A	3.6	0.25 ^B	0	1 ^C
BOD	80.3 ^A	1.5	69 ^B	4.6	ND	ND	0 ^C
COD	1352.3 ^A	29	1105 ^B	35.5	ND	ND	0 ^C

205 According to the parameters shown in Table 1, the water characteristics of tanks
 206 1 and 2 are not significantly different from each other.

207 The data obtained in this work indicate that the heating process did not interfere
 208 with the physicochemical parameters of the water in the tanks. Nevertheless, there are

209 significant differences between tank water and the water from the artesian well in every
210 analyzed item, mainly due to the presence of pesticides that significantly altered the
211 characteristics (Table 1) and independent of the microbiome of these communities.

212 The chlorine and fluorine contents of T1 and T2 are noteworthy since, according
213 to the World Health Organization [39], these specific values are close to the acceptable
214 concentrations for drinking water, even though the water from the artesian well and that
215 from the abovementioned tanks had not been added to these samples (Table 1).
216 Moreover, according to the WHO, the pH is also at inadequate levels for bacterial
217 growth, since the pH values are close to that of drinking water. In addition to these
218 parameters, the increase in conductivity and COD indicated important changes in the
219 physicochemical characteristics of the water of both tanks from those of water from the
220 artesian well, most likely related to the nonbiological oxidation processes of the
221 pesticides. The BOD data indicate biological activity, even in an environment
222 unfavorable to the development of microorganisms. COD values are usually higher than
223 BOD values due to the greater ease of compound oxidation by the chemical route than
224 by microorganisms [40].

225 The physicochemical data obtained in our study were compared to those of
226 water with different origins and from environments with different characteristics to
227 determine which water the T1 and T2 waters most resemble. Such environments were
228 that of drinking water [39], the interior transition zone of the ocean [41], the Red Sea
229 [42], southwest part of the Gulf of Mexico [43], a hot water spring in Siloam, South
230 Africa [44], an integrated system of anaerobic-aerobic reactors for the treatment of
231 wastewater (WW) in Ethiopia [45] and the Yeongsan River Basin of South Korea [46].
232 The two tanks do not significantly differ in chlorine level, but these levels are
233 statistically higher than those found for seawater and potable water (Table 2). However,

the levels found for the well water are statistically similar to those of the sea. Fluorine levels are statistically similar in both tanks; however, they are lower than those of drinking water and higher than those of the artesian well water (Table 2) [44]. The tanks have statistically similar pH values but are more acidic than the artesian well, residual tributaries, ocean, fresh water and drinking water and are statistically significantly different from these sources in pH (Table 2). In general, when total dissolved solids (ppm/NaCl, corresponding to salinity) are analyzed, our data present statistical similarities to the levels in WW, drinking water and a hot water spring in Siloé, South Africa. The BOD and COD rates are higher in T1 than in T2, indicating that there are probably more contaminants and microorganisms in the former. For the same reason, the rates in T1 and T2 are higher than those in the artesian well. The tanks have lower BOD and higher COD than WW and a wastewater tributary (WT), probably because the methods used in the treatment of these effluents have microorganisms that increase their BOD values but not their COD values in relation to their values in the tanks studied in this work (Table 2).

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257 **Table 2:** Comparison of physicochemical analyses of tanks 1 (T1) and 2 (T2) and the
 258 artesian well (AW) with different environments. Data were obtained in the literature for
 259 environments such as the oceanic transition zone (OC), drinking water (DW), the Red
 260 Sea (RS), Siloé Hot Spring (SHS), wastewater (WW), wastewater tributary (WT), the
 261 Yeongsan River Basin (YRB), the Gulf of Mexico (GLF) and freshwater aquaculture
 262 systems (FAS). No information (-). The letters A, B, C, D, E, and F represent significant
 263 differences within each column.

Environment	Chlorine	Fluorine	BOD	COD	TDS (ppm/NaCl)	pH
T1	1.66 ^B	0.82 ^C	80.33 ^C	1352.33 ^A	186.27 ^D	5.45 ^E
T2	1.57 ^B	0.81 ^C	69.00 ^D	1105.00 ^B	183.17 ^D	5.32 ^E
AW	0.00 ^D	0.05 ^D	0.00 ^F	0.00 ^D	0.00 ^D	4.75 ^F
OC	0.00 ^D	-	-	-	-	7.78 ^C
DW	0.70 ^C	1.50 ^B	-	-	600.00 ^A	7.50 ^C
RS	0.94 ^C	-	-	-	39826.47 ^D	-
SHS	44.35 ^A	6.11 ^A	-	-	197.32 ^D	9.50 ^B
WW	-	-	4886.26 ^A	308.91 ^C	2593.69 ^D	7.66 ^C
WT	-	-	12547.50 ^B	395.00 ^C	9470.50 ^C	10.40 ^A
YRB	-	-	3.28 ^E	-	-	7.61 ^C
GLF	-	-	-	-	23602.07 ^B	-

FAZ	-	-	-	-	-	7.09 ^D
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264 In this way, tanks T1 and T2 were characterized as environments with
 265 inadequate levels of chlorine and fluorine for the survival of the genera identified in this
 266 study. However, these genera were probably able to tolerate these conditions, either by
 267 inactivity in planktonic form or by organic and metabolic activity in biofilms [47].

268 It is important to remember that these physicochemical analyses reflect the
 269 planktonic community more directly than they do biofilms. Biofilms, although
 270 interfacing with the environment, most likely present modifications that improve
 271 metabolism and the adaptability of microorganisms to the studied environment [26], as
 272 will be discussed next.

273 Biodiversity and bacterial taxonomy

274 The identification of the taxonomic groups present in the different niches
 275 evaluated in this study may indicate changes in bacterial population structures adapting
 276 to the stress produced by the physicochemical conditions and contamination. Sample
 277 coverage data in terms of the Simpson indices from the 16S rRNA gene sequencing and
 278 diversity calculations are shown in Fig 2.

279 **Figure 2:** Measurements of alpha diversity in the planktonic triplicate (M1A, M1B,
 280 M1C and M2A, M2B, M2C) and biofilm triplicate (B1A, B1B and B2A, B2B) samples
 281 from the tanks and biofilm samples from the container's triplicates (G1A, G2B, G2C
 282 and G2A, G2B, G2C).

Analyses of the sequences obtained in every community studied in this work showed that the most representative classes are Gammaproteobacteria, Alphaproteobacteria and Betaproteobacteria, as shown in Fig 3.

Figure 3: Abundance of bacterial classes in the biofilm samples from the containers and planktonic samples from tanks 1 and 2. B1 and B2: taxonomic units identified from pre- and postheating biofilms, respectively; M1 and M2: taxonomic units identified from pre- and postheating planktonic samples, respectively; G1 and G2: taxonomic units identified from biofilms formed in containers with pre- and postheating water, respectively.

The most representative families identified by the sequences obtained in every community are shown in Fig 4.

Figure 4: Abundance of bacterial families in biofilm samples from tanks 1 and 2. B1 and B2: taxonomic units identified from pre- and postheating biofilms, respectively; M1 and M2: taxonomic units identified from pre- and postheating planktonic samples, respectively; G1 and G2: taxonomic units identified from biofilms formed in containers with pre- and postheating water, respectively.

The number of sequences assigned by community and by taxonomic category and their respective percentages within each community are shown in S1 Fig.

Simpson's diversity index demonstrates the distribution of alpha diversity (Fig 1). The sequences obtained and identified as the genera of the planktonic communities (M1 and M2) are represented in the biofilm communities (B1 and B2, G1 and G2) but have different diversity indices that depend on selection and adaptation to the environment (Figs 1 and S1 Fig).

306 The data shown in Figs 3 and 4 indicate that there is great variation in the
 307 population structure in different communities in relation to classes and families. The
 308 event of water heating is a nondirectional selective process since none of the taxonomic
 309 groups identified in this study have resistance responses for high temperatures, but it
 310 changes the number of taxonomic units, resulting in decreased diversity in the
 311 postheating samples for every community and less intense changes for the planktonic
 312 communities than for the biofilm communities. The effect of this genetic drift that
 313 began in the planktonic community can be observed in Fig 2 and leads to different
 314 diversity and abundance among the communities that settled in the biofilm structure in
 315 the tanks and in the flasks before and after the heating process. The planktonic
 316 community has a direct influence on biofilm formation [48]. Chronologically, the
 317 communities are formed in the following order: first T1, followed by B1 and then G1.
 318 This ordering is valid for postheating communities, with the proviso that the T2
 319 community is formed after the T1 community.

320 A genetic drift effect is more conspicuous in the biofilm population structures in
 321 flasks G1 and G2, formed from the planktonic strains, than in the structures in other
 322 communities. This is probably because they had less time to consolidate population
 323 structures that are more adapted to the environment, as observed in B1 and B2.

324 Population structure variation is lower in the planktonic samples than in the
 325 biofilm samples in tanks and flasks, which may indicate that there was a combination of
 326 the genetic drift of individuals who survived the heating and subsequent selection of
 327 differentiated characteristics to allow individuals to adapt to the tank environment. This
 328 environment for water contaminated with 30 different agrochemicals and with
 329 physicochemical conditions unfavorable to the survival of microorganisms identified in
 330 this work was considered stressful (Table 1 and S1 Table). The organization of bacteria

331 in biofilms in tanks and containers, with their cooperative or antagonistic interactions,
332 may lead to a specific combination of species with increased adaptability to the
333 environment [49]. Thus, the most dominant sequences in B2, corresponding to the
334 genera *Enterobacter* and *Acinetobacter*, are poorly represented in the other communities
335 studied, including the biofilms in B1, G1 and G2 (Fig 2).

336 Theoretically, the transition from planktonic to biofilm life involves changes in
337 the regulation of different groups of genes and can be seen as a transition phase before
338 the colonization of a new environment [50]. Therefore, this transition should not present
339 significant changes in the population structure (Fig 3). Throughout the establishment of
340 the community, the most abundant genera tend to retain most of the genes related to
341 basic cellular processes, leading to the same function being shared by individuals of
342 different species and not only being distributed by the selection of the fittest [51].

343 Nevertheless, the reduction in diversity after the water heating event possibly led
344 to genetic drift, altering the fine structure of the M2, G2 and B2 in relation to that of the
345 M1, G1 and B1 communities and the retention of genes involved in central adaptive
346 processes. If the genetic drift and the functional diversity of different bacterial
347 communities were less preponderant, the diversity indices of B1 and B2, B2 and G2,
348 and G1 and G2 should be closer [48] than what was observed in Fig 2. In addition, the
349 data shown in S1 Fig indicate that the same genus but with different 16S sequences may
350 contribute to different communities adapted to the environments studied in this work
351 [52]. Thus, the genetic drift hypothesis was established from the sequences identified
352 and not by the taxonomic units.

353 Aquatic environments are complex [28]; therefore, the analysis of changes in the
354 structure of communities of microorganisms that are made to adapt to environmental

contaminants is difficult. Restricted environments with a high number of agrochemicals, such as those presented in this paper, allow basic hypotheses to be tested with increased safety before being tested at a larger environmental scale.

Functional diversity

The comparative data in Fig 5 cluster genes by the sequences of the 16S rRNA gene identified in this study. This analysis was carried out as a survey of biotechnological potential [18,19] mainly related to biodegradation in the storage tanks of water contaminated with agrochemicals, which are considered a significant environmental liability.

Figure 5: Ordering the sites based on OTU abundance by multidimensional scaling. Only the bacterial genus (green), genetic process (red), cellular process (black), environmental process (blue) and metabolic pathways (yellow) with $p < 0.05$ after 999 permutations are displayed. Each vector shows the direction of increase for a given variable, and its length indicates the strength of the correlation between the variable and the ordination scores. Legend: M: Motility; GD: Growth and Death; TP: Transportation in Catabolism; MT: Membrane Transport; SMI: Signaling Molecule Interaction; ST: Signal Transduction; TL: Translation; TC: Transcription; RR: Replication and Repair; FSD: Folding, Sorting and Degradation; AA: Amino Acid; SM: Secondary Metabolites; C: Carbohydrate; E: Energy; EZ: Enzyme; G: Glycan; L: Lipid; CV: Cofactors and Vitamins; OA: Other Amino Acids; TP: Terpenoids and Polyketides; N: Nucleotide; XB: Xenobiotic Biodegradation.

Nonmetric multidimensional scaling (NMDS) shows the correlations between the number and types of 16S rRNA gene sequences grouped by the 6 different

378 communities. Genes related to bacterial motility have a high correlation with the two
379 planktonic communities (S2 Table).

380 On the other hand, genes associated with other cellular processes, environmental
381 processes, gene information processes and metabolic processes have a strong correlation
382 with the biofilm community B2 (Table 1), which is predominantly composed of the
383 genera *Acinetobacter* and *Enterobacter*.

384 To picture the potential for the degradation and bioremediation of pesticides and
385 relate the hypotheses about adaptation to the environment described in this study, the
386 degradation capacity of the pesticides (S1 Table) was related to the genera of the most
387 prevalent bacteria identified by the 16S rRNA gene sequencing (Fig 2).

388 Bacteria from the genus *Pseudomonas* (representing 45% of the structure in B1
389 and 8% of the structure in B2, Fig 4) present in communities identified in Hawaiian soil
390 can degrade pyraclostrobin, which is present in several fungicides [53]. A strain of
391 *Pseudomonas aeruginosa* isolated from rice-cultivated soil has the capacity to degrade
392 propiconazole [54]. Other strains of the genus *Pseudomonas* isolated from agricultural
393 soil degrade thiamethoxam [55].

394 Strains of the genus *Enterobacter* that were identified in Egyptian soil
395 (representing 63% of the structure in B2, Fig 4) degraded the herbicide Topik [56].
396 *Pseudomonas* and *Acinetobacter* (representing 15% of the structure in B2, Fig 4) from
397 communities where there was tar processing were identified as potential degraders of
398 mineral oil [57]. A strain of the genus *Burkholderia* was isolated from paddy fields with
399 pesticide application in Koppal, India, and was a degrader of propiconazole, a
400 component of the fungicide Tilt [54].

401 The genera in the literature that are most related to agrochemical biodegradation
402 are the same as those that dominate B2. Thus, the genera identified in this study have
403 agrochemical degradation potential and the capacity for structuring biofilm
404 communities, as shown in Fig 2, suggesting the viability of searching for promising
405 strains among the culturable collection obtained in this study. The high temperature to
406 which the planktonic community was subjected is bactericidal for any genus observed
407 in this study [39]. Therefore, the nondirectional selective effect induced by the high
408 temperature was independent of the genomes of the identified genera.

409 **Comparative 16S rRNA gene sequencing**

410 In this work, a model was proposed to explain a possible range of characteristics
411 whose expression could confer adaptive advantages over the stressful environment of
412 water tanks used in the washing of agrochemicals. The population fluctuations observed
413 were the result of genetic drift and the capacity for biofilm formation, which involves
414 membrane transport, interaction of signaling molecules and signal transduction, and
415 functional bacterial versatility [58]. The biofilm structure depends on the presence of
416 EPS, which involves the production of glycans and lipid transportation in catabolism.
417 Biofilms confer genomic plasticity and phenotypic flexibility to the species that
418 compose them, providing successful reproduction in stressful environments (Fig 5)[23].

419 Theoretically, these two characteristics, plasticity and flexibility, and community
420 diversity, which are higher in the planktonic community (Fig 2) than in the biofilm
421 community, may lead to species fixation over time or even extinction of the less
422 efficient. Some mutations may be randomly lost by genetic drift; however, significant
423 advantageous mutations are more likely to fix in the population, as is the case of
424 motility in a planktonic community, the only feature that has a high correlation with this

community (Fig 5). If the selection pressure in a community is strong, the frequencies of genotypes that improve competence increase in the population [59]. This model can be applied for mutations in populations with one species or populations of different species but with gene flow potential, as might occur in the B2 community.

The environments studied in this work passed through a sequence of physicochemical changes involving chemical contamination and heat over six months. During these events, genera that had the capacity to develop useful functions [58] to consolidate biofilms, such as *Enterobacter* spp. and *Acinetobacter* spp., dominated the B2 community [48,60], which was formed after the communities M1, M2 and B1 by selection and genetic drift.

Environmental biofilms have a level of metabolic complexity higher than that of their individual components [61]. Thus, the study of biofilm formation in environments contaminated with pesticides, which can be stressful to microorganisms, has the potential to produce information about response systems for adaptation. This information provides a possible way to exploit enzymatic potentials, mainly those related to biodegradation and bioremediation of herbicides [50], in the kind of waste used in this study. New knowledge can be obtained for the exploitation of the enzymatic potential of bacterial communities under these environmental conditions, mainly the potential related to the biodegradation kinetics of herbicides.

Conclusions

The population structure and abovementioned functional profile derived from amplicon sequencing of bacterial communities from tanks that contained water used for washing the packaging of herbicides and that had been stored for six months to form a

highly contaminated waste were studied in this work. The communities were characterized as tank biofilms, flask biofilms, and planktonic samples, all from pre- and postheating treatments and formed at different periods of time. Only two genera identified in this study, *Acinetobacter* and *Enterobacter*, were dominant in the tank biofilms after water heating, which probably acted as a genetic drift agent for the planktonic community. A database was accessed after the amplicon sequencing to compare genomic compositions, and this analysis showed that these genera have a metabolic potential for survival under these environmental conditions by possessing genes such as those related to biofilm formation, structure and membrane transport, quorum sensing and xenobiotic degradation. The genetic drift, induced by water heating, decreased the diversity of the bacterial biofilm community, which probably survived as large bacterial populations under the harmful conditions found in the tank due their genomic diversity and adaptation to environmental responses, since it is known that biofilms have a level of metabolic complexity higher than that of their individual components. Thus, the hypotheses about metabolic abilities, generated for a small and controlled environment as that studied in this work, can be tested with communities isolated from the biofilms formed in these tanks. Thus, new knowledge can be obtained for the exploitation of the enzymatic potential of bacterial communities, mainly the potential related to the biodegradation kinetics of herbicides.

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References

- 471 1. Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-cohen L. High-Throughput
472 Metagenomic Technologies for Complex Microbial Community Analysis: Open
473 and Closed Formats. CEUR Workshop Proc 2015;1542:33–6.
474 doi:10.1128/mBio.02288-14.Copyright.

- 475 2. Dobrzanski T, Gravina F, Steckling B, Olchanheski LR, Sprenger RF, Espírito
476 Santo BC, et al. *Bacillus megaterium* strains derived from water and soil exhibit
477 differential responses to the herbicide mesotrione. PLoS One 2018;13:1–24.
478 doi:10.1371/journal.pone.0196166.

- 479 3. Prione LP, Olchanheski LR, Tullio LD, Santo BCE, Reche PM, Martins PF, et al.
480 GST activity and membrane lipid saturation prevents mesotrione-induced cellular
481 damage in *Pantoea ananatis*. AMB Express 2016;6:1–12. doi:10.1186/s13568-
482 016-0240-x.

- 483 4. Martins PF, Martinez CO, Carvalho G De, Irajara P, Carneiro B, Azevedo RA, et
484 al. Selection of Microorganisms Degrading S-Metolachlor Herbicide
485 2007;50:153–9.

- 486 5. Olchanheski LR, Dourado MN, Beltrame FL, Zielinski AAF, Demiate IM,
487 Pileggi SA V, et al. Mechanisms of tolerance and high degradation capacity of
488 the herbicide mesotrione by *Escherichia coli* strain DH5- α . PLoS One 2014;9:1–
489 8. doi:10.1371/journal.pone.0099960.

- 490 6. Silva TM, Stets MI, Mazzetto AM, D.Andrade F, Pileggi SAV, Fávero PR, et al.
491 Degradation of 2,4-D herbicide by microorganisms isolated from Brazilian
492 contaminated soil. Brazilian J Microbiol 2007;38:522–5. doi:10.1590/S1517-
493 83822007000300026.

- 494 7. Deng Y, Deng C, Yang J, Li B, Wang E. Novel Butane-Oxidizing Bacteria and
 495 Diversity of bmoX Genes in Puguang Gas Field. *Front Microbiol* 2018;9:1–13.
 496 doi:10.3389/fmicb.2018.01576.

- 497 8. Oueriaghli N, Castro DJ, Llamas I, Béjar V, Martínez-Checa F. Study of bacterial
 498 community composition and correlation of environmental variables in Rambla
 499 Salada, a hypersaline environment in South-Eastern Spain. *Front Microbiol*
 500 2018;9:1–17. doi:10.3389/fmicb.2018.01377.

- 501 9. Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. The ecology
 502 and biogeochemistry of stream biofilms. *Nat Rev Microbiol* 2016;14:251–63.
 503 doi:10.1038/nrmicro.2016.15.

- 504 10. Gutarowska B, Celikkol-Aydin S, Bonifay V, Otlewska A, Aydin E, Oldham AL,
 505 et al. Metabolomic and high-throughput sequencing analysis-modern approach
 506 for the assessment of biodeterioration of materials from historic buildings. *Front*
 507 *Microbiol* 2015;6:1–13. doi:10.3389/fmicb.2015.00979.

- 508 11. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the
 509 microbiome: Advantages of whole genome shotgun versus 16S amplicon
 510 sequencing. vol. 469. Elsevier Ltd; 2016. doi:10.1016/j.bbrc.2015.12.083.

- 511 12. Fierer N. Embracing the unknown: disentangling the complexities of the soil
 512 microbiome. *Nat Rev Microbiol* 2017. doi:10.1038/nrmicro.2017.87.

- 513 13. Al-Amoudi S, Razali R, Essack M, Amini MS, Bougouffa S, Archer JAC, et al.
 514 Metagenomics as a preliminary screen for antimicrobial bioprospecting. *Gene*
 515 2016;594:248–58. doi:10.1016/j.gene.2016.09.021.

- 516 14. Madhavan A, Sindhu R, Parameswaran B, Sukumaran RK, Pandey A.
517 Metagenome Analysis: a Powerful Tool for Enzyme Bioprospecting. Appl
518 Biochem Biotechnol 2017;183:636–51. doi:10.1007/s12010-017-2568-3.

- 519 15. Krohn-Molt I, Alawi M, Förstner KU, Wiegandt A, Burkhardt L, Indenbirken D,
520 et al. Insights into Microalga and bacteria interactions of selected phycosphere
521 biofilms using metagenomic, transcriptomic, and proteomic approaches. Front
522 Microbiol 2017;8:1–14. doi:10.3389/fmicb.2017.01941.

- 523 16. Ji P, Rhoads WJ, Edwards MA, Pruden A. Impact of water heater temperature
524 setting and water use frequency on the building plumbing microbiome. ISME J
525 2017;11:1318–30. doi:10.1038/ismej.2017.14.

- 526 17. Ofaim S, Ofek-Lalzar M, Sela N, Jinag J, Kashi Y, Minz D, et al. Analysis of
527 microbial functions in the rhizosphere using a metabolic-network based
528 framework for metagenomics interpretation. Front Microbiol 2017;8:1–14.
529 doi:10.3389/fmicb.2017.01606.

- 530 18. Kroeger ME, Delmont TO, Eren AM, Meyer KM, Guo J, Khan K, et al. New
531 Biological Insights Into How Deforestation in Amazonia Affects Soil Microbial
532 Communities Using Metagenomics and Metagenome-Assembled Genomes.
533 Front Microbiol 2018;9:1–13. doi:10.3389/fmicb.2018.01635.

- 534 19. Imfeld G, Vuilleumier S. Measuring the effects of pesticides on bacterial
535 communities in soil: A critical review. Eur J Soil Biol 2012;49:22–30.
536 doi:10.1016/j.ejsobi.2011.11.010.

- 537 20. Storck V, Nikolaki S, Perruchon C, Chabanis C, Sacchi A, Pertile G, et al. Lab to
538 field assessment of the ecotoxicological impact of chlorpyrifos, isoproturon, or

- 539 tebuconazole on the diversity and composition of the soil bacterial community.
540 Front Microbiol 2018;9:1–13. doi:10.3389/fmicb.2018.01412.
- 541 21. Valverde JR, Marín S, Mellado RP. Effect of herbicide combinations on bt-maize
542 rhizobacterial diversity. J Microbiol Biotechnol 2014;24:1473–83.
543 doi:10.4014/jmb.1405.05054.
- 544 22. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al.
545 Structure and function of the global ocean microbiome 2015;348:1–10.
- 546 23. Decho AW, Gutierrez T. Microbial extracellular polymeric substances (EPSs) in
547 ocean systems. Front Microbiol 2017;8:1–28. doi:10.3389/fmicb.2017.00922.
- 548 24. Maunder E, Welch M. Matrix exopolysaccharides; the sticky side of biofilm
549 formation. FEMS Microbiol Lett 2017;364:1–10. doi:10.1093/femsle/fnx120.
- 550 25. Anutrakunchai C, Bolscher JGM, Krom BP, Kanthawong S, Chareonsudjai S,
551 Taweekaisupapong S. Impact of nutritional stress on drug susceptibility and
552 biofilm structures of Burkholderia pseudomallei and Burkholderia thailandensis
553 grown in static and microfluidic systems. PLoS One 2018;13:e0194946.
554 doi:10.1371/journal.pone.0194946.
- 555 26. Lerch TZ, Chenu C, Dignac MF, Barriuso E, Mariotti A. Biofilm vs. Planktonic
556 Lifestyle: Consequences for Pesticide 2,4-D Metabolism by Cupriavidus necator
557 JMP134. Front Microbiol 2017;8:1–11. doi:10.3389/fmicb.2017.00904.
- 558 27. Zhao K, Li Y, Yue B, Wu M. Genes as early responders regulate quorum-sensing
559 and control bacterial cooperation in Pseudomonas aeruginosa. PLoS One
560 2014;9:1–8. doi:10.1371/journal.pone.0101887.

- 561 28. Angly FE, Heath C, Morgan TC, Tonin H, Rich V, Schaffelke B, et al. Marine
562 microbial communities of the Great Barrier Reef lagoon are influenced by
563 riverine floodwaters and seasonal weather events. *PeerJ* 2016;4:e1511.
564 doi:10.7717/peerj.1511.
- 565 29. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al.
566 Ultra-high-throughput microbial community analysis on the Illumina HiSeq and
567 MiSeq platforms. *ISME J* 2012;6:1621–4. doi:10.1038/ismej.2012.8.
- 568 30. Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, et al. Systematic
569 improvement of amplicon marker gene methods for increased accuracy in
570 microbiome studies. *Nat Biotechnol* 2016;34:942–9. doi:10.1038/nbt.3601.
- 571 31. Aronesty E. Comparison of Sequencing Utility Programs. *Open Bioinforma J*
572 2013;7:1–8. doi:10.2174/1875036201307010001.
- 573 32. Staley C, Gould TJ, Wang P, Phillips J, Cotner JB, Sadowsky MJ. Evaluation of
574 water sampling methodologies for amplicon-based characterization of bacterial
575 community structure. *J Microbiol Methods* 2015;114:43–50.
576 doi:10.1016/j.mimet.2015.05.003.
- 577 33. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig WG, Peplies J, et al. SILVA:
578 a comprehensive online resource for quality checked and aligned ribosomal RNA
579 sequence data compatible with ARB. *Nucleic Acids Res* 2007;35:7188–96.
- 580 34. Huse SM, Welch DM, Morrison HG, Sogin ML. Ironing out the wrinkles in the
581 rare biosphere through improved OTU clustering. *Environ Microbiol*
582 2010;12:1889–98. doi:10.1111/j.1462-2920.2010.02193.x.

- 583 35. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves
584 sensitivity and speed of chimera detection. *Bioinformatics* 2011;27:2194–200.
- 585 36. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid
586 assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ*
587 *Microbiol* 2007;73:5261–7. doi:10.1128/AEM.00062-07.
- 588 37. Langille MGI, Clemente JC, Knight R, Burkepile DE, Caporaso JG, McDonald
589 D, et al. Predictive functional profiling of microbial communities using 16S
590 rRNA marker gene sequences. *Nat Biotechnol* 2013;31:814–21.
591 doi:10.1038/nbt.2676.
- 592 38. R Foundation for Statistical Computing. R: A language and environment for
593 statistical computing. vol. 2. 2007.
- 594 39. World Health Organization. Guidelines for Drinking-water Quality 4th ed.,
595 WHO, Geneva, p. 340. World Heal Organ 2011. doi:10.1016/S1462-
596 0758(00)00006-6.
- 597 40. Gholizadeh MH, Melesse AM, Reddi L. A comprehensive review on water
598 quality parameters estimation using remote sensing techniques. *Sensors*
599 (Switzerland) 2016;16. doi:10.3390/s16081298.
- 600 41. Mende DR, Bryant JA, Aylward FO, Eppley JM, Nielsen T, Karl DM, et al.
601 Environmental drivers of a microbial genomic transition zone in the ocean's
602 interior. *Nat Microbiol* 2017;2:1367–73. doi:10.1038/s41564-017-0008-3.
- 603 42. Thompson LR, Williams GJ, Haroon MF, Shibl A, Larsen P, Shorenstein J, et al.
604 Metagenomic covariation along densely sampled environmental gradients in the

- 605 Red Sea. ISME J 2017;11:138–51. doi:10.1038/ismej.2016.99.
- 606 43. Godoy-Lozano EE, Escobar-Zepeda A, Raggi L, Merino E, Gutierrez-Rios RM,
607 Juarez K, et al. Bacterial Diversity and the Geochemical Landscape in the
608 Southwestern Gulf of Mexico. Front Microbiol 2018;9:1–15.
609 doi:10.3389/fmicb.2018.02528.
- 610 44. Tekere M, Lötter A, Olivier J, Jonker N, Venter S. Metagenomic analysis of
611 bacterial diversity of Siloam hot water spring, Limpopo, South Africa. African J
612 Biotechnol 2011;10:18005–12. doi:10.5897/AJB11.899.
- 613 45. Desta AF, Assefa F, Leta S, Stomeo F, Wamalwa M, Njahira M, et al. Microbial
614 community structure and diversity in an integrated system of anaerobic-aerobic
615 reactors and a constructed wetland for the treatment of tannery wastewater in
616 Modjo, Ethiopia. PLoS One 2014;9:1–22. doi:10.1371/journal.pone.0115576.
- 617 46. Jang J, Di DYW, Lee A, Unno T, Sadowsky MJ, Hur HG. Seasonal and
618 genotypic changes in Escherichia coli phylogenetic groups in the Yeongsan River
619 basin of South Korea. PLoS One 2014;9:5–12.
620 doi:10.1371/journal.pone.0100585.
- 621 47. Lewenza S, Abboud J, Poon K, Kobryn M, Humplik I, Bell JR, et al.
622 Pseudomonas aeruginosa displays a dormancy phenotype during long-term
623 survival in water 2018:1–19.
- 624 48. Hallatschek O, Hersen P, Ramanathan S, Nelson DR. Genetic drift at expanding
625 frontiers promotes gene segregation. Proc Natl Acad Sci 2007;104:19926–30.
626 doi:10.1073/pnas.0710150104.

- 627 49. Herschend J, Damho ZB V, Marquard AM, Sven B, Sørensen SJ, Hä P, et al. A
628 meta-proteomics approach to study the interspecies interactions affecting
629 microbial biofilm development in a model community 2017:1–13.
630 doi:10.1038/s41598-017-16633-6.
- 631 50. Berlanga M, Guerrero R. Living together in biofilms□: the microbial cell factory
632 and its biotechnological implications. Microb Cell Fact 2016:1–11.
633 doi:10.1186/s12934-016-0569-5.
- 634 51. Stephens WZ, Wiles TJ, Martinez ES, Jemielita M, Burns AR, Parthasarathy R,
635 et al. Identification of population bottlenecks and colonization factors during
636 assembly of bacterial communities within the zebrafish intestine. MBio
637 2015;6:1–11. doi:10.1128/mBio.01163-15.
- 638 52. Łukasik P, Nazario K, Van Leuven JT, Campbell MA, Meyer M, Michalik A, et
639 al. Multiple origins of interdependent endosymbiotic complexes in a genus of
640 cicadas. Proc Natl Acad Sci 2018;115:E226–35. doi:10.1073/pnas.1712321115.
- 641 53. Chen X, He S, Liang Z, Li QX, Yan H, Hu J, et al. Biodegradation of
642 pyraclostrobin by two microbial communities from Hawaiian soils and metabolic
643 mechanism. J Hazard Mater 2018;354:225–30.
644 doi:10.1016/j.jhazmat.2018.04.067.
- 645 54. Satapute P, Kaliwal B. Biodegradation of propiconazole by newly isolated
646 Burkholderia sp . strain BBK _ 9. 3 Biotech 2016;6:1–10. doi:10.1007/s13205-
647 016-0429-3.
- 648 55. Rana S, Jindal V, Mandal K, Kaur G, Gupta VK. Thiamethoxam degradation by
649 Pseudomonas and Bacillus strains isolated from agricultural soils. Environ Monit

- Assess 2015;187. doi:10.1007/s10661-015-4532-4.
56. Ibrahim GGA, Amin MK, Hassan AA, El-Sheikh EA. IDENTIFICATION OF PESTICIDES DEGRADING BACTERIA ISOLATED FROM EGYPTIAN SOIL Chemicals and Reagents. Zagazig J Genet Biotechnol 2015;42:1129–43.
57. Popp N, Schlömann M, Mau M. Bacterial diversity in the active stage of a bioremediation system for mineral oil hydrocarbon-contaminated soils. Microbiology 2006;152:3291–304. doi:10.1099/mic.0.29054-0.
58. Roller M, Lucić V, Nagy I, Perica T, Vlahoviček K. Environmental shaping of codon usage and functional adaptation across microbial communities. Nucleic Acids Res 2013;41:8842–52. doi:10.1093/nar/gkt673.
59. Brooks AN, Beer KD, Turkarslan S, Baliga NS, Li FY. Adaptation of cells to new environments. Wiley Interdiscip Rev Syst Biol Med 2011;3:544–561. doi:10.1002/wsbm.136.Adaptation.
60. Komarova NL. Spatial interactions and cooperation can change the speed of evolution of complex phenotypes. Proc Natl Acad Sci 2014;111:10789–95. doi:10.1073/pnas.1400828111.
61. Flemming HC, Wuertz S. Bacteria and archaea on Earth and their abundance in biofilms. Nat Rev Microbiol 2019;17:247–60. doi:10.1038/s41579-019-0158-9.

Supporting information

S1 Fig: Number of sequences assigned by community and by taxonomic category and their respective percentages within each community. M1: preheating planktonic

671 community; M2: postheating planktonic community; B1: preheating biofilm
 672 community; B2: postheating biofilm community; G1: preheating container biofilm
 673 community preheating; G2: postheating container biofilm community.

674 **S1 Table:** Agrochemicals whose containers were washed and the water used in the
 675 process to obtain the samples evaluated in this study.

676 **S2 Table:** Correlations of the functional characteristics identified in our analyses of the
 677 studied communities. M1: preheating planktonic community; M2: postheating
 678 planktonic community; B2: postheating biofilm community; r2: coefficient of
 679 determination; p: coefficient of significance.

680

Pesticide containers

Tank with water used to clean pesticide containers

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Biofilm collection from water previously heated

Biofilm and planktonic
amplicon sequencing

High-diversity planktonic communities

Low-diversity biofilm communities

High potential for degradation and biofilm pathways in biofilm communities

Genetic Drift