

1        **Small freshwater ecosystems with dissimilar microbial communities exhibit**  
2                                **similar temporal patterns**

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## 20 Abstract

21 Despite small freshwater ecosystems are biodiversity reservoirs and significantly  
22 contribute to greenhouse fluxes, their microbial communities remain largely  
23 understudied. Yet, microorganisms intervene in biogeochemical cycling and impact  
24 water quality. Because of their small size, these ecosystems are in principle more  
25 sensitive to disturbances, seasonal variation and pluri-annual climate change. However,  
26 how microbial community composition varies over space and time, and whether  
27 prokaryotic and eukaryotic communities behave similarly, remain fully open questions.  
28 Here, we aim at unravelling the composition and intra/inter-annual temporal dynamic  
29 patterns for both prokaryotes and microbial eukaryotes in a set of small freshwater  
30 ecosystems. We monitored prokaryote composition during 24 consecutive months in four  
31 ponds and one brook from North-Western France by 16S rDNA amplicon sequencing  
32 (microbial eukaryotes were previously investigated for the same systems). Unexpectedly  
33 for oxic environments, bacterial Candidate Phyla Radiation (CPR) were highly diverse  
34 and locally abundant. Our results suggest that microbial community structure is mainly  
35 driven by environmental conditions acting over space (ecosystems) and time (seasons).  
36 A low proportion of OTUs (<1%) was shared by the five ecosystems despite their  
37 geographical proximity, making microbial communities almost unique in each ecosystem  
38 and highlighting the strong selective influence of local environmental conditions. Marked  
39 and similar seasonality patterns were observed for prokaryotes and microbial eukaryotes  
40 in all ecosystems despite strong turnovers of rare OTUs. Over the two-year survey,  
41 microbial community composition varied despite relatively stable environmental  
42 parameters. This suggests that biotic associations play an important role in inter-annual  
43 community assembly.

44

45

## 46 Introduction

47 Small freshwater ecosystems such as ponds or shallow lakes remain largely  
48 unexplored, despite being widespread and numerically very important ( $3.2 \times 10^9$   
49 natural ponds on Earth of  $0.0001$ - $0.001$  km<sup>2</sup> in size) and, covering altogether a large  
50 area ( $0.8$  billion km<sup>2</sup>) (Downing, 2010). Collectively, they contribute up to 15% of CO<sub>2</sub>  
51 and, especially, 40% of CH<sub>4</sub> emissions (Holgerson & Raymond, 2016; Yvon-Durocher,  
52 Hulatt, Woodward, & Trimmer, 2017). They exhibit highly diverse physicochemical  
53 conditions and are reservoirs of an important biodiversity in terms of small  
54 invertebrates and microorganisms as identified by classical observation (Dudgeon et  
55 al., 2006) and a few molecular diversity studies (e.g. Liu et al., 2020; Qin et al.,  
56 2016; Simon et al., 2014, 2015). Because of their small volume, small aquatic  
57 ecosystems have lower buffering capacity toward environmental variation at short  
58 i.e. seasons and longer, multiple-year time scales. Consequently, their communities  
59 and functioning are in principle more reactive to environmental changes than those  
60 of larger ecosystems (Downing, 2010; Downing et al., 2006; McKee et al., 2003).  
61 Understanding the temporal dynamics of organisms inhabiting these overlooked  
62 ecosystems is one of a first step to understand and predict their functioning in the  
63 long run.

64 Indeed, because their fundamental roles in ecosystem functioning (Azam et al.,  
65 1983; Bar-On, Phillips, & Milo, 2018; Cho & Azam, 1988), changes in microbial  
66 community composition can have profound impacts on ecosystem processes due to  
67 changes in their functional capacities (Bell, Newman, Silverman, Turner, & Lilley,  
68 2005). However, most studies of microbial communities in freshwater systems have  
69 focused on their composition and spatial distribution (Debroas et al., 2017;  
70 Grossmann et al., 2016; Richards & Bass, 2005; Richards et al., 2015; Shade, Gregory

71 Caporaso, Handelsman, Knight, & Fierer, 2013; Zhong et al., 2016; Zwirgmaier et  
72 al., 2008). Temporal surveys in aquatic systems have been only occasionally carried  
73 out, mostly at relatively short scales (days to months) (Bunse & Pinhassi, 2017;  
74 Fuhrman, Cram, & Needham, 2015; Scherer et al., 2017). However, longer-term  
75 studies eventually reflecting seasonal patterns are still very rare (Gilbert et al.,  
76 2012; Karl & Church, 2014; Linz et al., 2017). Furthermore, most microbial diversity  
77 studies, including temporal surveys, focus on either prokaryotic or eukaryotic  
78 communities, but an integrated view of how the two change with time, eventually  
79 revealing underlying biotic interactions, are extremely scarce and restricted to large  
80 environments such as oceans and lakes (Biller et al., 2018; Obertegger, Pindo, &  
81 Flaim, 2019; Steele et al., 2011). Yet, differences in overall metabolic capabilities,  
82 physiology, trophic features, generation time and dispersal might reflect in distinct  
83 temporal patterns of prokaryotes (bacteria, archaea) versus microbial eukaryotes.  
84 For instance, microbial eukaryotes have much more restricted metabolic capabilities  
85 compared to prokaryotes that have widely diverse energy and carbon metabolisms.  
86 In contrast, many microbial eukaryotes are predators, including some microalgae  
87 (Bjorbækmo, Evenstad, Røsæg, Krabberød, & Logares, 2020; Sherr & Sherr, 1994;  
88 Zubkov & Tarran, 2008), while predation is exceptional in prokaryotes (Shiratori,  
89 Suzuki, Kakizawa, & Ishida, 2019).

90 Here, we investigate the bacterial and archaeal community composition during 24  
91 consecutive months in four shallow ponds and one brook located in the natural  
92 regional park of the Haute Vallée de Chevreuse (France) using 16S rDNA amplicon  
93 sequencing. We subsequently compare the temporal patterns of prokaryotic  
94 communities to those of microbial eukaryotes previously analyzed for the same  
95 ecosystems (Simon, Jardillier, et al., 2015), analyzing them as a function of several

96 environmental parameters using diverse metrics and multivariate statistical  
97 analyses. Although the five ecosystems harbour specific microbial communities, their  
98 temporal patterns were similar. Interestingly, both the prokaryotic and eukaryotic  
99 communities also show similar inter-annual temporal dynamics.

## 100 Material and methods

### 101 *Sampling and measurement of physicochemical parameters*

102 We sampled monthly at the deepest site surface waters from five  
103 representative small and shallow freshwater ecosystems (the four ponds Etang des  
104 Vallées (EV), La Claye (LC), Mare Gabard (MG), Saint Robert (SR), and the brook Ru  
105 Sainte Anne (RSA)), previously described (Simon et al., 2014), between April 2011  
106 and April 2013 (24 sampling dates) using sterilized plastic carboys. A total of 106  
107 samples were collected (LC and RSA dried out occasionally). Single samples were  
108 analysed at each time point of this study since our protocol ensured very similar  
109 replicates (Simon et al., 2014). The five ecosystems are located in the Natural  
110 Regional Park of the Chevreuse Valley (Ile-de-France, France), less than 10 km apart.  
111 During our two-year survey, the Ru Sainte Anne and La Claye experienced several  
112 dry periods, from August to September 2012 for Ru Sainte Anne, and from July to  
113 December 2011 and in September 2012 for La Claye. These five ecosystems differ in  
114 their environmental conditions (Simon, López-García, et al., 2015). Water  
115 temperature (T), dissolved oxygen concentration (O<sub>2</sub>), pH and conductivity (Cond)  
116 were measured *in situ* using a multiparameter probe Multi 350i (WTW, Weilheim,  
117 Germany). Concentrations of dissolved nutrients (total phosphorus (P<sub>tot</sub>),  
118 orthophosphate (PO<sub>4</sub><sup>3-</sup>), total nitrogen (N<sub>tot</sub>), nitrite (NO<sub>2</sub><sup>-</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and  
119 ammonia (NH<sub>3</sub>)) were measured on the sampling day using colorimetric tests

120 following manufacturer instructions (Hach-Lange, Düsseldorf, Germany). Samples  
121 filtered onto 0.2µm pore-size Nuclepore membranes (Whatman) were immediately  
122 frozen at -20°C until measurement (Rochelle-Newall, Hulot, Janeau, & Merroune,  
123 2014) of dissolved organic carbon (DOC) using a TOC VCSH analyzer (Shimadzu,  
124 Tokyo, Japan). Water samples were transported back to the laboratory and  
125 immediately serially filtered through 100, 30, 5 and 0.2-µm pore-size Nuclepore  
126 filters (Whatman). Filters were immediately stored frozen at -80°C until DNA  
127 extraction.

128

#### 129 *DNA extraction, amplification and sequencing of 16S and 18S rDNA fragments*

130 DNA was extracted from cells collected in the smallest size-fraction (0.2–5  
131 µm) using the PowerSoil DNA extraction kit (Qiagen, Hilden, Germany) as previously  
132 described (Simon et al. 2014). 16S rDNA fragments of ca. 290 bp, encompassing the  
133 V4 hypervariable region, were PCR-amplified using the universal primers U515F (5'-  
134 GTGCCAGCMGCCGCGTAA-3') and 806R (5'-GGACTACVSGGTATCTAAT-3') targeting  
135 both bacteria and archaea (Baker, Smith, & Cowan, 2003; Takai, Horikoshi, & Takai,  
136 2000). These primers were tagged with different 10 bp Molecular IDentifiers (MIDs)  
137 to allow differentiation of PCR products from 20 distinct samples pooled together for  
138 multiplex sequencing. PCR amplification was conducted in a total volume of 25 µl  
139 using 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.3 µM primers, 0.3-3 µl of DNA sample and 0.5  
140 U HotStart Taq polymerase (Taq Platinum, Invitrogen, Carlsbad, CA, USA). Twenty-  
141 five amplification cycles (94°C for 30 s, 58°C for 45 s and 72°C for 90 s) were  
142 preceded by a 3-min denaturation step at 94°C and ended by a 10-min for final  
143 extension at 72°C. The products of five PCR reactions were pooled together for each

144 sample. These pools were then purified with the QIAquick PCR purification kit  
145 (Qiagen, Hilden, Germany). Equivalent amounts of purified amplicons of 20 different  
146 samples were pooled and pyrosequenced using the 454 GS-FLX Titanium technology  
147 from Roche (Beckman Coulter Genomics, Danvers, MA, USA). In the case of  
148 eukaryotes, 18S rDNA fragments of ca. 550 bp, encompassing the V4 hypervariable  
149 region, were PCR amplified (Simon et al., 2014) with primers EK-565F and 18S-EUK-  
150 1134-R–UnonMet (5'-TTTAAGTTTCAGCCTTGCG-5') biased against Metazoa (Bower et  
151 al., 2004).

152

### 153 *Analysis of 16S and 18S rDNA 454-pyrosequences*

154 A total of 1 705 835 16S rDNA amplicon reads was obtained. Reads were filtered  
155 through an in-house pipeline to remove spurious sequences. Briefly, pyrosequences  
156 without the two flanking primer sequences or containing errors in primer/MIDs  
157 sequences or positions with undetermined bases were discarded. To further  
158 eliminate PCR and pyrosequencing errors, pairwise flowgram alignments were  
159 clustered and treated with the PyroNoise program of AmpliconNoise (Quince,  
160 Lanzen, Davenport, & Turnbaugh, 2011). Filtered reads were then clustered into  
161 Operational Taxonomic Units (OTUs) with a 97% similarity cut-off (Stackebrandt &  
162 Goebel, 1994) using CD-hit (Fu, Niu, Zhu, Wu, & Li, 2012) and SWARM (Mahé, Rognes,  
163 Quince, de Vargas, & Dunthorn, 2015). Singletons (OTUs composed of only one single  
164 sequence) were excluded. Each OTU was represented by its most abundant  
165 sequence, used as reference. Reference sequences were assigned to taxonomic  
166 groups using 'vsearch' (Rognes et al., 2016), based on similarity with sequences of  
167 the SILVA 128 database (<http://www.arb-silva.de>). Reads affiliated to mitochondria  
168 and chloroplast were excluded from the dataset. At the end, 1 131 900 high-quality

169 reads were retained and assigned to 16 573 (CD-HIT) and 32 802 (SWARM) OTUs.  
170 Sequences from all OTUs were then attributed to samples according to their MIDs.  
171 OTU sequences of 0.2-5  $\mu\text{m}$  microbial eukaryotes were obtained following a similar  
172 procedure to that followed for 18S rDNA amplicons (Simon, López-García, et al.,  
173 2015). The number of OTUs affiliated to microbial eukaryotes was 3 742 (CD-HIT) and  
174 9 278 (SWARM). Sequencing data for the last four months of the temporal series in  
175 Mare Gabard could unfortunately not be generated. Prokaryotic sequences have been  
176 deposited at GenBank under Bioproject number PRJNA539931.

177

### 178 *Statistical analyses*

179 All statistical analyses were conducted with the R software ([http://cran.r-](http://cran.r-project.org)  
180 [project.org](http://cran.r-project.org)) (R Development Core Team 2019). Missing physico-chemical values (13%  
181 of the physico-chemical dataset) were replaced by the overall average value of the  
182 parameter and nutrient concentrations below kit detection limits (19% of the  
183 physico-chemical dataset) were set as the limit value minus a small random number  
184 (normal distribution  $\mu = 0$ ;  $\sigma = 10^{-6}$ ) for all statistical analyses. To take into account  
185 the measured environmental factors altogether in the diverse statistical analyses,  
186 their values were normalized ( $\mu = 0$ ,  $\sigma = 1$ ). OTU datasets based on CD-hit and SWARM  
187 yielded similar statistical analysis results. For clarity, we therefore discuss only  
188 results based on CD-hit sequence clustering. Relative OTU abundances were obtained  
189 by dividing the number of reads for each OTU by the total number of reads retrieved  
190 in the sample. OTUs were divided into two groups based on their relative abundance  
191 and temporal frequencies as in Simon et al. (2015): abundant OTUs, with a mean  
192 relative abundance superior to 0.1% and detected in at least 25% of the samples  
193 within one ecosystem, and rare OTUs, represented by all the remaining OTUs. All



194 subsequent statistical analyses based on Bray-Curtis dissimilarities (Bray & Curtis,  
195 1957) were conducted on Wisconsin-standardized OTU relative frequencies to  
196 balance the weight between abundant OTUs and rare OTUs. For global microbial  
197 community analyses, i.e. considering eukaryotic and prokaryotic datasets together,  
198 tables of Wisconsin-standardized OTU frequencies were pooled into a single table  
199 without changing the initial relative OTU frequencies.

200 In each ecosystem, richness, evenness and diversity index were calculated. Richness  
201 was evaluated by rarefaction analyses as the estimated number of OTUs in a random  
202 subsample of each sequence library, which is of the same size as the smallest one  
203 (Hurlbert, 1971). Evenness,  $e$ , was calculated according to Pielou (1966):  $e =$

204  $\frac{-\sum_i p_i \times \ln(p_i)}{\ln(S)}$  with  $p_i$  the frequency of OTU and  $S$  the observed number of OTUs in the

205 sample. Diversity was estimated according to the Gini-Simpson index (Gini, 1912;  
206 Lee, Buckley, Etienne, & Lear, 2013; Simpson, 1949) calculated as follows using the

207 'diversity' function of the 'vegan' R-package (Oksanen et al., 2018):  $S = 1 -$

208  $\sum_i p_i^2$ .

209 Microbial community structuring was investigated in the different ecosystems. We

210 drawn Venn diagrams to show shared OTUs between ecosystems, using the 'gplots'

211 R-package (Warnes et al., 2015). Hierarchical cluster analysis was build using Bray-

212 Curtis dissimilarities and the Ward2 algorithm (Murtagh & Legendre, 2014) in the

213 'hclust' function of the 'stats' R-package to determine clusters of microbial

214 communities based on the distances between every samples of the five ecosystems.

215 Patterns in the temporal dynamics of prokaryotes and eukaryotes were explored at

216 intra- (seasons) and inter-annual (between years) scales. Pairwise Bray-Curtis

217 dissimilarities were computed between samples collected the first year and samples

218 from the same system the second year over increasing time-lags (from one-month to  
 219 12-months) to characterize the temporal dynamics of microbial communities at the  
 220 year-scale. Time-lag analyses (TLA, Collins, Micheli, & Hartt, 2000) between  
 221 sampling dates over increasing time-lags (from one-month to 23-months) were  
 222 computed using Bray-Curtis dissimilarities for microbial communities and Euclidean  
 223 distances for environmental parameters to determine their variability over  
 224 consecutive years. We computed polynomial regressions (fitting better than  
 225 sinusoidal regressions) of Bray-Curtis dissimilarities over time to estimate the  
 226 frequency of any putative temporal cyclic pattern and linear regressions over time  
 227 to estimate global shifts in the microbial community compositions and environmental  
 228 parameters over the two-year survey using the 'lm' function of the 'stats' R-package.  
 229 The best fitted polynomial model was determined based on the Akaike Information  
 230 Criterion (AIC, Akaike, 1973). In TLA, the slope of the linear regression indicates the  
 231 rate and direction of temporal changes. To test for the significance of slopes for  
 232 each ecosystem, a randomization procedure was applied with 5 000 permutations  
 233 using the 'lperm' function of the 'lmPerm' R-package (Wheeler & Torchiano, 2016).  
 234 A null distribution was estimated from each slope that was calculated on randomized  
 235 data. The observed slopes were then compared to the distribution of *F*-values  
 236 obtained under the null hypothesis. OTU turnovers were calculated between seasons  
 237 or between years of the two-year survey, using the 'codyn' R-package based on the  
 238 following formula (Diamond, 1969; Hallett et al., 2016; MacArthur & Wilson, 1963):  
 239 
$$Turnover = \frac{Speciesgained + Specieslost}{TotalSpeciesobservedinbothtimepoints}.$$
 Canonical correspondence  
 240 analyses were conducted to determine the contribution of each environmental factor  
 241 to the variation of microbial community composition.

242 Microbial community assembly was investigated considering the potential interplay  
243 between habitats (i.e. ecosystems) and time. To assess how close are microbial  
244 community compositions between sampling sites or seasons, non-metric  
245 multidimensional scaling (NMDS) analyses were performed based on Bray-Curtis  
246 dissimilarities using the ‘vegan’ R-package. To quantify the role played by the  
247 ecosystem clusters (i.e. groups of ecosystems defined in the hierarchical cluster  
248 analysis), the habitat (i.e. ecosystems), the time (i.e. year of sampling or the  
249 sampling season) in the structuring of the microbial communities, we conducted  
250 permutational multivariate analyses of variance (PERMANOVA) using Bray-Curtis  
251 dissimilarities calculated on OTU relative frequencies using the ‘adonis’ function of  
252 the ‘vegan’ R-package.

253 We explored biotic interactions by inferring how prokaryotes and microbial  
254 eukaryotes may influence each other assembly over time in each ecosystem. To do  
255 so, putative multicollinearity between environmental parameters was checked by  
256 computing the variance inflation factors (VIF) with the function `vif.cca` of the ‘vegan’  
257 R-package. A VIF value above 10 indicated a strong multicollinearity. Environmental  
258 factors were excluded from the analysis if they were characterized by  
259 multicollinearity. Forward selection was then used to sort environmental parameters  
260 explaining significantly community composition variation for CCA model (Monte Carlo  
261 permutation tests, 999 permutations). The synchrony of microbial eukaryotes and  
262 prokaryotes was assessed using the correlation between multivariate vectors (RV  
263 index) that represents the variability explained by the two communities. This index  
264 was calculated using co-inertia analysis based on 999 permutations (Dolédec &  
265 Chessel, 1994). CCA combined to forward selection were conducted to determine  
266 how prokaryotes explain the variance of microbial eukaryotes and inversely.

267

## 268 Results

269 We generated prokaryotic 16S rDNA amplicon data (Supplementary Table S1) for  
270 plankton samples (106 samples; 0.2-5  $\mu\text{m}$  size-fraction) collected monthly for two  
271 years from five shallow freshwater ecosystems for which protist communities had  
272 been previously studied (Simon, López-García, et al., 2015). These spatially close  
273 ecosystems varied in their physico-chemical conditions. The main differences were  
274 lower pH (6.2 on average) and nitrite concentrations ( $0.0086 \text{ mg l}^{-1}$  on average) in  
275 Mare Gabard, lower ammonia concentrations ( $0.07 \text{ mg l}^{-1}$  on average) in La Claye,  
276 variable ammonia inputs ( $<0.1$  to  $2.65 \text{ mg l}^{-1}$ ) in Saint Robert, higher oxygen (79.2%  
277 on average) and lower orthophosphate ( $0.05 \text{ mg l}^{-1}$ ) concentrations in Etang des  
278 Vallées, and higher conductivity ( $657.3 \mu\text{S cm}^{-1}$ ) in the stream Ru Sainte Anne. All  
279 the ecosystems were characterized by strong temporal variations in water  
280 temperature (thermal amplitude of  $19.4^\circ\text{C} \pm 2.3$  on average, for the five ecosystems)  
281 whereas the other environmental parameters had no apparent temporal variability  
282 (Simon, López-García, et al., 2015).

### 283 *Prokaryotic community composition*

284 From the total number of retained high-quality 16S rRNA gene reads (1 131  
285 900), we determined a total of 16 573 prokaryotic OTUs (14 701 bacterial and 1 872  
286 archaeal). The total number of OTUs in the Ru Sainte Anne brook was higher than in  
287 the four ponds, where similar amounts of OTUs were detected (Figure 1A), an  
288 observation consistent with that of eukaryotic diversity (Simon, López-García, et al.,  
289 2015). Consequently, richness, evenness and diversity (Simpson index) were similar  
290 in the four ponds and reached higher values (mean richness of 546, Simpson index of

291 0.99 and evenness of 0.85) in the Ru Sainte Anne (Figure 1B). The spread of the  
292 quartiles of the box plots suggested that these indices varied strongly over time and  
293 differently among ecosystems (Figure 1B). Overall, only 271 OTUs (considering only  
294 one occurrence for OTUs detected in several ecosystems; 1.6% of all OTUs) were  
295 abundant (i.e. detected in each ecosystem in at least 25% of samples and with  $\geq 0.1\%$   
296 relative abundance per sample) but represented 84.0% of the total number of reads  
297 (Supplementary Table S2). The vast majority of the prokaryotic diversity was thus  
298 composed of rare OTUs (Supplementary Figure S1A and S1B).

299 Prokaryotic OTUs affiliated to 78 different phyla, which we classified into 8  
300 super-groups of different taxonomic levels: Archaea (not further subdivided in taxa  
301 owing to their lower relative diversity and abundance in our samples), Fibrobacteres-  
302 Chlorobi-Bacteroidetes (FCB), Microgenomates, Parcubacteria, Proteobacteria,  
303 Planctomycetes-Verrucomicrobia-Chlamydiae (PVC), Terrabacteria and Other  
304 Bacteria (Figure 1C). The highest number of phyla (71 against 64 on average for the  
305 other ecosystems) was found in Ru Sainte Anne. Bacteria clearly dominated (98.0%  
306 of all reads) the prokaryotic community in the five ecosystems. Archaea were  
307 globally rare at the exception of Ru Sainte Anne, where they reached their higher  
308 abundances (8.1% of all reads). Woesearchaeota prevailed within Archaea (1.7% of  
309 all reads), whereas Bacteria were dominated in the five ecosystems by four phyla:  
310 Proteobacteria (on average, 34.1% of reads; including, by decreasing frequency  
311 order, Beta-, Gamma-, Alpha-, Delta- and Epsilonproteobacteria with respectively  
312 23.4, 5.24, 3.87, 0.97, 0.60 % of the reads on average), Actinobacteria (20.0%),  
313 Bacteroidetes (19.3%), and Verrucomicrobia (9.0%). Although Proteobacteria was the  
314 most abundant phylum in all ecosystems, the other phyla contributed differently to  
315 the structure of the prokaryotic communities in the five ecosystems (Supplementary

316 Figure S1C). The bacterial Candidate Phyla Radiation (CPR) was represented in our  
317 samples by 27 phyla that accounted for 9.9% of all reads. They corresponded mainly  
318 to the super-phyla Parcubacteria (67.2% of CPR-affiliated reads), Microgenomates  
319 (8.5%) and to the phyla Gracilibacteria (8.1%). In contrast with its relatively small  
320 frequency in the ponds, Parcubacteria was the most abundant group in Ru Sainte  
321 Anne (28.4% of all reads).

322 The five ecosystems harboured very diverse prokaryotic communities at the  
323 OTU level. Only 152 (CD-hit) and 91 (SWARM) OTUs were shared by all the ecosystems  
324 (Figure 2A and Supplementary Figure 2A). Although they represented less than 1% of  
325 all OTUs, they contributed to 61.0% of all reads in the five ecosystems. Shared OTUs  
326 were affiliated to 23 bacterial phyla and only to 2 archaeal phyla (Woesearchaeota  
327 and Methanobacteria). Abundant OTUs represented 59.9% of the shared OTUs and  
328 belonged to the most frequent phyla (i.e. Proteobacteria, Bacteroidetes,  
329 Actinobacteria, Verrucomicrobia). Among the rest of shared OTUs, i.e. the rare ones,  
330 Archaea were prominent along with Parcubacteria, Proteobacteria and  
331 Bacteroidetes. Prokaryotic communities were mainly composed of shared OTUs in La  
332 Claye (5.2% OTUs and 80.8% reads) and non-shared ones in Ru Sainte Anne (98.7%  
333 OTUs and 68.4% reads). We used NMDS analysis based on OTU frequencies (CD-hit  
334 and SWARM based clustering) to highlight the dissimilarities between the prokaryotic  
335 communities of the five ecosystems (Figure 2B and Supplementary Figure 2B). These  
336 communities seemed to be more similar in Etang des Vallées and Saint Robert, where  
337 Proteobacteria, Actinobacteria, Bacteroidetes and Verrucomicrobia were the most  
338 abundant phyla (Supplementary Figure S3). Mare Gabard harboured the highest  
339 abundance of Proteobacteria, which represented more than half of the prokaryotic  
340 community in this ecosystem (56% of all reads, divided mostly between

341 betaproteobacteria and gammaproteobacteria with represented respectively 37.6  
342 and 14.5% of the reads). Ru Sainte Anne had a more homogenous prokaryotic  
343 community structure at the phylum level and hosted the highest proportions of  
344 Azambacteria and Woeseearchaeota.

345         The temporal dynamics of the prokaryotic phyla was characterized by small  
346 changes at the month scale in the five ecosystems (Supplementary Figure S3). The  
347 highest abundances of Archaea were recorded in Ru Saint Anne in November 2011  
348 (29.1% of all reads), July 2012 (25.2%) and, to a lower extent, in November 2012  
349 (18.6%) and December 2012 (20.9%), and were mainly driven by Woeseearchaeota  
350 (Supplementary Figure S4). Proteobacteria, Actinobacteria and Bacteroidetes  
351 showed very different temporal dynamics between ecosystems. Proteobacteria  
352 reached their highest abundances in June 2012 in Mare Gabard (81.8% of all reads,  
353 including gamma- and betaproteobacteria with respectively 45.7 and 34.1%) and  
354 Saint Robert (60.4%, mostly represented by betaproteobacteria with 50.8%) and in  
355 October 2011 in Ru Sainte Anne (49.8%, mostly represented by epsilon- and  
356 betaproteobacteria with respectively 21.1 and 18.0%). Actinobacteria peaked in  
357 Etang des Vallées in March 2013 (50.3%) and at similar levels in June and November  
358 2012 in La Claye (46.7% and 45.0%, respectively). The highest abundances of  
359 Bacteroidetes were recorded in Saint Robert in March 2013 (63.7%) and in January  
360 2012 in Saint Robert and La Claye (54.4% and 46.6%, respectively). Bacteria belonging  
361 to the CPR varied also greatly over time and between ecosystems. They dominated  
362 in June 2011 and June 2012 (62.9% and 63.5%, respectively) in Ru Sainte Anne and  
363 represented up to 25.2% of reads in October 2012 in La Claye. Parcubacteria and  
364 Microgenomates exhibited similar temporal patterns. By contrast, Cyanobacteria

365 were low abundant in the five ecosystems all along our survey, even in summer,  
366 representing on average 0.7% of all reads.

### 367 *Comparison of eukaryotic and prokaryotic community structures*

368 After analyzing prokaryotic diversity alone, we incorporated data of the small (0.2-  
369 5  $\mu\text{m}$ ) eukaryotes present in the same samples (Simon, López-García, et al., 2015).  
370 NMDS analysis of pooled prokaryotic and eukaryotic OTU frequencies revealed  
371 marked differences in microbial community composition among the five ecosystems  
372 (Supplementary Figure S5). Hierarchical clustering analysis based on Bray-Curtis  
373 dissimilarities of OTU frequencies supported these differences between the  
374 ecosystems. As in the case of the prokaryotic community, the Ru Sainte Anne global  
375 microbial community was set apart of the cluster composed of the four ponds  
376 (Supplementary Figure S6). Interestingly, the cluster of Ru Sainte Anne samples also  
377 contained a sample collected in La Claye just after a drought event (LC37, October  
378 2012).

379 To determine the effect of the environment on the microbial community  
380 composition, we conducted CCA on either prokaryotic or eukaryotic OTUs and the  
381 significant physico-chemical parameters sorted out by a forward analysis.  
382 Conductivity, water temperature, concentrations of nitrite and dissolved oxygen  
383 were the only parameters that had a significant effect on the microbial communities  
384 (10.0 and 6.9% of variance for prokaryotes and eukaryotes, respectively) (Figure 3).  
385 In addition, the influence of nitrate concentration appeared to be significant only  
386 for prokaryotes. Conductivity, a proxy of the total ion concentration, explained a  
387 third of the variance of prokaryotes and microbial eukaryotes on average. The  
388 remaining four significant abiotic factors contributed similarly to explain the



389 community structure. To further explore how the environment controls the most  
390 abundant microbial taxa, we carried out CCA between various physicochemical  
391 parameters and the most abundant OTUs (Supplementary Figure S7). Prokaryotes and  
392 microbial eukaryotes in Etang des Vallées were highly related to pH, concentrations  
393 of dissolved oxygen, nitrite and nitrate. In Ru Sainte Anne, microbial communities  
394 appeared to be only linked to conductivity. There was no apparent correlation  
395 between the measured environmental parameters and the abundant prokaryotes and  
396 microbial eukaryotes in the other ecosystems. To assess putative biotic interactions  
397 between prokaryotes and microbial eukaryotes, we computed the RV index.  
398 Considering all communities in the five ecosystems together, co-inertia analysis  
399 shown that the dynamics of prokaryote and protist communities were highly and  
400 significantly similar with  $RV = 0.745$  ( $p = 0.001$ ), suggesting co-variations as a result  
401 of similar environmental triggers or biotic interactions between the two  
402 communities. At the ecosystem level, a significant temporal co-variation of microbial  
403 communities was observed only in Etang des Vallées and Saint Robert (Supplementary  
404 Table S3). CCA analysis based on OTUs grouped by phyla showed that eukaryotes  
405 explained as much as 38.6% of the variance of the prokaryotic community, mainly by  
406 four eukaryotic phyla (Cryptomycota, Oomyceta, Marine Stramenopiles,  
407 Chrysophyta) for which the co-inertia was significant. Inversely, five prokaryotic  
408 phyla explained 21.2% of the eukaryotic variance (Proteobacteria including beta-,  
409 gamma- and epsilonproteobacteria, Actinobacteria, Bacteroidetes, Amesbacteria  
410 and Woesebacteria).

411 A PERMANOVA analysis was used to quantify the role played by habitat (i.e.  
412 ecosystem) and time (season and year) in the microbial community structuring at the  
413 OTU level. Habitat and time had similar influences on the composition of the global

414 microbial, prokaryotic and eukaryotic communities. Differences in the global  
415 microbial communities between the Ru Sainte Anne and the other ecosystems were  
416 greater (F.model value of 4.739) than between these four remaining ecosystems  
417 (F.model value of 2.9607) (Table 1). To a lower extent, microbial communities  
418 shifted over time at seasonal and year scales (F.model values of 1.4079 and 1.2424,  
419 respectively). Similar results were obtained when considering OTUs defined with the  
420 SWARM clustering method (Supplementary Table 4).

421 *Intra- and inter-annual dynamics of abiotic parameters and microbial*  
422 *community composition*

423 We conducted time-lag analyses based on linear and polynomial regressions of  
424 Bray-Curtis distances over time on environmental factors and microbial community  
425 composition to assess the temporal patterns at intra- and inter-annual scales. The  
426 slopes of the linear regressions of abiotic parameters were either not significant  
427 (Etang des Vallées, La Claye and Saint Robert) or only slightly marked (Mare Gabard  
428 and Ru Sainte Anne, linear regression slopes of 0.025 and 0.036, respectively) (Figure  
429 4 and Supplementary Table S5). By contrast, those for prokaryotes, microbial  
430 eukaryotes and all microbes considered together were characterized by significant  
431 and positive linear regressions. All the slopes were stronger for the microbial  
432 communities than for the abiotic parameters (from 0.091 for La Claye to 0.192 for  
433 Mare Gabard). These results suggest no or weak temporal evolution of abiotic  
434 parameters when microbial communities diverged increasingly over time. These  
435 effects were visible and significant even when considering similar numbers of  
436 pairwise Bray-Curtis dissimilarity comparisons for all time-lag periods (data not  
437 shown). The model that best fitted the distribution of the time-lag analyses for  
438 prokaryotes, eukaryotes and physico-chemical parameters was a fifth order

439 polynomial regression that revealed marked changes in dissimilarities at a 6-month  
440 frequency. The SWARM-based dataset yielded similar results, at the exception of the  
441 linear regression slopes of microbial eukaryotic communities that presented lower  
442 values (Supplementary table 6). This analysis revealed significant seasonal patterns  
443 for microbial communities and the environmental conditions in the five ecosystems.  
444 The dispersion of the data was better explained for the biotic than for the abiotic  
445 factors.

446 Pairwise Bray-Curtis dissimilarities calculated on rare and abundant OTU  
447 frequencies between the two years of sampling revealed seasonality for prokaryotes,  
448 microbial eukaryotes and both considered together. Globally, the temporal dynamics  
449 of the abundant OTUs was characterized by seasonality, with higher dissimilarities  
450 for samples separated by 5 to 6 months (i.e. opposite seasons, 0.79 average Bray-  
451 Curtis dissimilarity) than for temporally contiguous samples (consecutive months,  
452 0.62 average Bray-Curtis dissimilarity) (Figure 5). Rare OTUs showed, to a lower  
453 extent, similar patterns (average Bray-Curtis dissimilarity of 0.94 and 0.86 for  
454 opposite seasons and consecutive months, respectively). The seasonality of abundant  
455 OTUs was most pronounced in La Claye whereas there was no difference in the  
456 seasonality of rare OTUs among the five ecosystems. NMDS analysis based on OTU  
457 frequencies highlighted that the strongest shifts in community composition occurred  
458 between winter and summer (Supplementary Figure S8), in agreement with previous  
459 similar observation for microbial eukaryotes (Simon et al., 2015). Turnover values  
460 for the abundant prokaryotic and eukaryotic OTUs were more marked between years  
461 (19.6 and 31.4% on average, respectively) than between seasons (10.9 and 22.7% on  
462 average, respectively). Turnover indices calculated for each couple of seasons were  
463 higher between summer and winter, at the exception of La Claye. Rare OTUs, both

464 prokaryotic and eukaryotic ones, were characterized by higher OTU turnover  
465 between seasons and between years than abundant OTUs (Supplementary Figure S9).

466

## 467 Discussion

### 468 *Local environmental selection drives different community compositions in* 469 *small freshwater ecosystems*

470 In order to study the diversity and temporal dynamics of planktonic  
471 microorganisms (0.2-5  $\mu\text{m}$  size-fraction) of the three domains of life in small  
472 freshwater systems, we generated 16S rDNA amplicon sequences for samples  
473 collected over 24 months from 4 ponds and one brook for which we previously  
474 generated 18S rDNA datasets from the same original samples (Simon, López-García,  
475 et al., 2015). These systems were characterized by differences in their physico-  
476 chemical conditions (Simon, López-García, et al., 2015). Ru Sainte Anne and La Claye  
477 experienced drought events that lasted up to five months. We retrieved a total  
478 number of 16 573 OTUs affiliated to prokaryotes (14 701 bacterial OTUs and 1 872  
479 archaeal OTUs) for a total of 3 583 OTUs affiliated to microbial eukaryotes. These  
480 numbers are higher than those usually retrieved in other freshwater ecosystems  
481 (Bock, Salcher, Jensen, Pandey, & Boenigk, 2018; Llíros et al., 2014) even after our  
482 very stringent analysis of the sequence data (see Methods; Supplementary Table S1  
483 and S2). The higher diversity actually reflects the cumulative effect of observed  
484 OTUs along time series and highlights the importance of integrating temporal scales  
485 to better depict global microbial biodiversity (Simon, López-García, et al., 2015).  
486 Not surprisingly, microbial communities were mainly composed of rare OTUs  
487 (Supplementary Table 2), for both prokaryotes and microbial eukaryotes. Rare OTUs

488 were affiliated to all the 78 detected prokaryotic phyla whereas abundant OTUs  
489 ( $\geq 0.1\%$ ) were restricted to 32 phyla.

490 Most prokaryotic phyla were widespread in the five ecosystems; only a few low-  
491 abundant ones were not detected in all of them. On average, the prokaryotic OTU  
492 richness was about five times higher than that of eukaryotes at each sampling date  
493 (Figure 1B), which is in agreement with the global higher diversity of prokaryotes  
494 (e.g. Hug et al., 2016). Proteobacteria (mostly alpha-, beta- and  
495 gammaproteobacteria), Bacteroidetes, Actinobacteria and Verrucomicrobia  
496 dominated (Supplementary Figure S1), as often observed in freshwater lentic  
497 ecosystems (Newton, Jones, Eiler, McMahon, & Bertilsson, 2011; Riemann et al.,  
498 2008; Zwart, Crump, Kamst-van Agterveld, Hagen, & Han, 2002). Cyanobacteria were  
499 detected at low abundances, likely because we focused on the small size-fraction  
500 (0.2-5  $\mu\text{m}$ ) whereas freshwater Cyanobacteria are usually of larger size or  
501 filamentous. CPR bacteria and the Diapherotrites-Parvarchaeota-Aenigmarchaeota-  
502 Nanoarchaeota-Nanohaloarchaeota (DPANN) supergroups, which have few cultured  
503 representatives and are likely composed of symbionts (Castelle et al., 2018), were  
504 observed in the five ecosystems. In particular, Woesearchaeota was the most  
505 abundant archaeal phylum (X. Liu et al., 2018) (Figure 1C, Supplementary Figure S4).

506 Despite these overall similarities and despite high OTU turnover rates at intra-  
507 and inter-annual scales (Supplementary Figure S9), each ecosystem harboured  
508 specific prokaryotic communities mainly composed of non-shared OTUs (Figure 2 and  
509 Supplementary Figure S6) as observed in other freshwater environments, including  
510 lakes (Bock et al., 2018; Linz et al., 2017). Each of the five ecosystems appears to  
511 represent a specific habitat for diverse prokaryotic communities because of  
512 particular physico-chemical conditions, nutrient composition, availability as

513 previously described for these ecosystems (Simon, López-García, et al., 2015), and  
514 for other freshwater environments (Fisher, Klug, Lauster, Newton, & Triplett, 2000;  
515 Peura et al., 2012). In clear contrast with the four ponds analyzed, the prokaryotic  
516 community in the Ru Sainte Anne brook was very different. This lotic ecosystem  
517 harboured a prokaryotic community that was characterized by the highest richness  
518 and evenness as well as by the highest abundances of Archaea and CPR bacteria  
519 (Figure 1A-C and 2B, Supplementary Figure S6). This higher richness might be related  
520 to a higher input on allochthonous microbes (e.g. from soil or upstream waters) into  
521 the system (e.g. Crump, Amaral-Zettler, & Kling, 2012; Teachey, McDonald, &  
522 Ottesen, 2019).

523 In addition to this major difference in community composition between the brook  
524 and the four ponds, we also observed differences among all the five small freshwater  
525 ecosystems (Figure 2, Supplementary Figure S5 and S6) despite their spatial  
526 proximity. These differences may be the result of a combination of diverse ecological  
527 processes (Goldford et al., 2018) that can explain the high OTU turnover rates  
528 observed (Supplementary Figure S9). Many microbes exhibit a high dispersion  
529 capacity promoted by natural vectors such as wind, rain, and animals (birds and  
530 mammals). However, the capacity to colonize a new environment faces diverse biotic  
531 and abiotic filters (e.g. competition, chemistry of the ecosystem, hydrology). Among  
532 these factors, competition between autochthonous and dispersing microbes is known  
533 to be crucial to limit successful immigration (Comte, Berga, Severin, Logue, &  
534 Lindström, 2017; Logue & Lindström, 2010). In this regard, we detected 62.7 and  
535 39.6% of prokaryotic and eukaryotic OTUs, respectively, that occurred only once in  
536 all ecosystems over the two-year survey. In addition to their very sporadic  
537 occurrence, the abundance of these OTUs was very low, with less than 15 reads on

538 average. These OTUs likely represent dispersing cells that did not succeed to colonize  
539 our ecosystems and were no longer detected at subsequent dates. However, this was  
540 not the case for all rare OTUs, since some of them reached high abundances  
541 punctually. For instance, a rare OTU affiliated to Firmicutes accounted for 2 403  
542 (30.2%) reads in La Claye in July 2012. We can hypothesize that these rare OTUs  
543 come from a local seed bank and may bloom when very particular environmental  
544 conditions occur. The fact that two of our ecosystems (La Claye and Ru Sainte Anne)  
545 experienced drought events that lasted 1 to 5 months and that, each time, microbial  
546 communities recovered their original structure within a month after water refill  
547 (Supplementary Figure S3) also strongly advocates for the existence of seed banks in  
548 these ecosystems for both the abundant and part of the rare OTUs (Shade et al.,  
549 2012; Simon et al., 2016).

#### 550 *Prokaryotes and microbial eukaryotes share similar temporal patterns*

551 Our multivariate statistical analyses showed that the microbial community  
552 composition in the five freshwater ecosystems was not only driven by the particular  
553 environmental conditions of each system but also by their temporal variations at  
554 different time scales (Table 1). Microbial communities can exhibit non-seasonal  
555 dynamics due to marked and fast shifts in environmental conditions. These shifts can  
556 easily take place in small freshwater ecosystems because their shallow depths and  
557 small volumes make them sensitive to local and short-time scale climate variations  
558 (Bock et al., 2018; Downing, 2010; Simon, López-García, et al., 2015). In fact, even  
559 if rapid changes can occur in larger ecosystems such as lakes (Mangot et al., 2013),  
560 climate has usually a lower impact at short-time scales on lakes and oceans, where  
561 microbial seasonal successions are well documented (Fuhrman et al., 2015; Tammert  
562 et al., 2015). Nevertheless, despite punctual marked shifts in environmental

563 conditions, we observed global seasonal patterns in both the environmental  
564 parameters (Figure 4), and the prokaryotic and eukaryotic microbial communities  
565 (Table 1 and Supplementary Table S5, Figures 4-5 and Supplementary Figure S8).  
566 These communities were more differentiated at summer and winter, possibly  
567 because of higher OTU turnover rates occurring at these two opposed seasons.  
568 Concomitantly, communities also experienced composition changes at a monthly  
569 scale, more pronounced for rare taxa than for abundant taxa (Supplementary Figure  
570 S9). Interestingly, at a much longer time scale, we also observed constant directional  
571 shift (increasing dissimilarity over time) of both the prokaryotic and eukaryotic  
572 communities, and the whole microbial community structures over the two-year  
573 survey (Figure 4, Supplementary Table S5), a phenomenon that has already been  
574 observed in larger lakes and in marine systems (Chow et al., 2013; L. Liu, Yang, Yu,  
575 & Wilkinson, 2015).

576 As mentioned above, diverse simultaneous processes affecting community  
577 assembly can explain this marked microbial dynamics, including immigration, the  
578 presence of a seed bank, and genetic or ecological drift (Chase, 2007; Evans, Martiny,  
579 & Allison, 2016; Logares et al., 2018). The temporal community composition changes  
580 observed in our ecosystems occurred in environmental conditions that were  
581 relatively stable at the inter-annual scale (Figure 4). This may explain partly why  
582 seasonal changes of both prokaryotes and microbial eukaryotes were slightly driven  
583 (<10% of variance explained) by a restricted subset of measured physico-chemical  
584 parameters (temperature, total concentration of ions (conductivity), dissolved  
585 oxygen and nitrate concentrations) (Figure 3 and Supplementary Figure S7). In  
586 addition to possible abiotic factors not measured during this study (e.g. light quantity  
587 and quality, wind, rainfall, specific hydrochemistry), biotic interactions were most



588 likely also involved in determining the microbial community assembly as is assumed  
589 for other ecosystems (Lindström & Langenheder, 2012; Yannarell & Triplett, 2005).  
590 Many microbial eukaryotes prey on prokaryotes (Boenigk & Arndt, 2002; Pernthaler,  
591 2005). Likewise, diverse symbioses (from mutualistic to parasitic ones) are  
592 established between microorganisms of the three domains of life (González-Olalla,  
593 Medina-Sánchez, Lozano, Villar-Argaiz, & Carrillo, 2018; Zehr, Shilova, Farnelid,  
594 Muñoz-Maríncarmen, & Turk-Kubo, 2016) and influencing community composition. In  
595 return, the composition of prey and host communities may affect that of their  
596 respective predators and symbionts (Šimek et al., 2013). Time series with adapted  
597 time scale and sampling frequency are crucial to resolve these biotic interactions  
598 between microorganisms (Kelly et al., 2019; Moreira & López-García, 2019). Although  
599 our monthly sampling was likely too sparse to accurately assess potential synchrony  
600 between prokaryotes and microbial eukaryotes (that can often experience rapid  
601 changes on daily or weekly basis), we were able to identify an overall marked  
602 correlation between the changes in prokaryote community composition and those of  
603 microbial eukaryotes (Supplementary Table 3). This observation supports, beyond  
604 physicochemical change, the occurrence of top-down processes between  
605 heterotrophic eukaryotes and prokaryotes (Massana & Logares, 2013).

606 *Symbiosis may be a major process in small freshwater microbial ecosystems*

607 If predation is one of the main recognized biotic interactions at the base of  
608 trophic webs, symbioses (including interactions going from parasitism to mutualism)  
609 are widespread among microbial communities and can also play key roles (Harding  
610 et al., 2018; Hudson, Dobson, & Lafferty, 2006). We observed a large proportion of  
611 probable symbiotic taxa among the microbial eukaryotes and prokaryotes identified  
612 in our ecosystems. For example, based on the very limited biosynthetic capacities

613 encoded in their genomes, CPR bacteria are inferred to live in symbiosis with other  
614 prokaryotes (Castelle & Banfield, 2018). Overall, in the five ecosystems, the most  
615 abundant CPR phyla were Azambacteria (3.14%), Nomurabacteria (0.92%),  
616 Gracilibacteria (0.80%), Saccharibacteria (0.52%) and Peregrinibacteria (0.43%).  
617 Interestingly, CPR bacteria have been found mostly in oxygen-depleted, sulfide-rich  
618 environments (Castelle et al., 2018). Our sampled ecosystems are somewhat oxygen-  
619 depleted but display oxic conditions on the surface. We recorded the highest  
620 proportions (44.9% of reads, 46.3% of OTUs) in Ru Sainte Anne (47.2% [O<sub>2</sub>] on average)  
621 and low proportions (2.3% of reads, 24.3% of OTUS) in Mare Saint Robert (38.6% [O<sub>2</sub>]  
622 on average). We cannot rule out the hypothesis that CPR bacteria were more  
623 abundant in the sediments or in the suboxic deeper waters of our ecosystems, and  
624 that those that we detected were cells suspended again from the bottom to the  
625 surface since these ecosystems are characterized by frequent water column mixing.  
626 Nevertheless, we did retrieve in our samples very low numbers of sequences from  
627 anaerobic prokaryotes typical of sediments, such as methanogenic archaea (0.14% of  
628 all reads in all ecosystems) or sulfate-reducing Desulfobacteraceae (0.068%). This  
629 suggests a moderate input of cells from the sediment, so that the much larger  
630 amounts of CPR bacteria that we detected most likely correspond to bona fide  
631 members of the planktonic community and not cells suspended again from the  
632 sediment. Nonetheless, methanogenesis and sulfate-reduction are active local  
633 processes associated to the degradation of abundant organic matter (bubbling and  
634 strong H<sub>2</sub>S smell during sampling are observed in some systems, e.g. Mare Gabard,  
635 especially in warmer seasons). Therefore, CPR and their hosts might be more linked  
636 to sulfide availability than to the absence of dissolved oxygen per se. In addition,  
637 CPR bacteria were consistently retrieved from the five ecosystems even if their

638 presumed size (Luef et al., 2015) is below the 0.2  $\mu\text{m}$  pore-size of the membranes  
639 that we used to collect cells. Therefore, their relative proportion might have been  
640 underestimated in our study, since many cells unattached to their hosts might have  
641 escaped detection. At any rate, the relatively high abundance of CPR bacteria in  
642 these small water bodies opens intriguing questions about their ecological role.  
643 Despite their likely dependence on other organisms, CPR bacteria exhibit a wide  
644 range of metabolic activities. For example, among the CPR lineages detected in this  
645 study, Microgenomates have the potential to degrade a large range of carbon  
646 substrates (Danczak et al., 2017), Parcubacteria can be involved in the nitrogen cycle  
647 through denitrification and Saccharibacteria (TM7) and Dojkabacteria (WS6) have  
648 been reported as parasites (Castelle & Banfield, 2018). The same situation might  
649 apply to the potentially parasitic Woesearchaeota (X. Liu et al., 2018), which reach  
650 relative high abundance in some of our shallow freshwater ecosystems. The  
651 importance of parasitism in aquatic plankton communities is well known (Hudson et  
652 al., 2006; Jephcott et al., 2016; Lafferty, Dobson, & Kuris, 2006). In particular, since  
653 parasites have complex life cycles and are known to infect organisms at various  
654 trophic levels, they increase the complexity of planktonic trophic networks by  
655 extending the food chains (Grami et al., 2011) and enhancing the level of biodiversity  
656 (Hudson et al., 2006). This, in turn, may result in the increase of both the  
657 connectivity and efficiency of carbon fluxes, although these potential consequences  
658 are still subject to debate (Grami et al., 2011).

659 In summary, our study revealed a high diversity of prokaryotes in addition to that  
660 previously observed for microbial eukaryotes in small freshwater ecosystems.  
661 Despite a microbial community structure characterized by a predominance of rare  
662 taxa and a marked OTU turnover rate, we observed marked seasonal patterns and

663 community drift over the two-year survey for both prokaryotes and microbial  
664 eukaryotes of five different microbial communities. These observations suggest that  
665 diverse prokaryote and eukaryote communities react similarly to environmental  
666 changes over time (seasonal to multiple-year scale). These changes were only  
667 partially driven by measured environmental conditions. By contrast, biotic  
668 interactions, such as symbiosis, are suspected to play more significant roles. The  
669 abundance of CPR bacteria in some of these ecosystems suggests that they may be  
670 important ecological players also in oxic environments.

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#### 679 Conflict of Interest

680 The authors declare no conflict of interest.

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1042 **Data Accessibility**

1043 DNA sequences: BioSample accessions SAMN11525783 - SAMN11525888; NCBI SRA:  
1044 SRR11218723 - SRR11218819.

1045 **Author contribution**

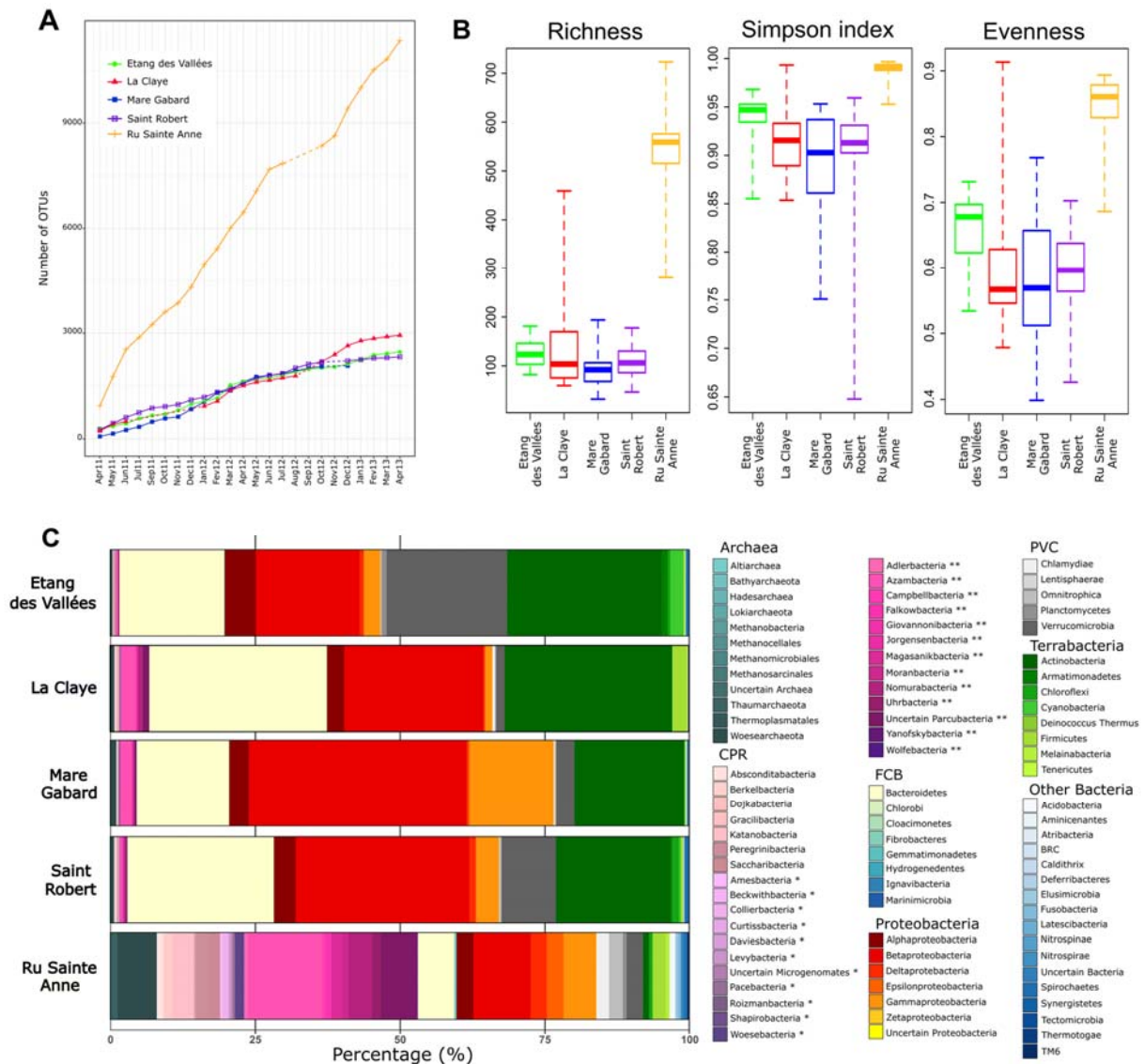
1046 Gwendoline M. David, Purificación López-García, David Moreira and Ludwig Jardillier  
1047 contributed substantially to the study conception, data acquisition and analysis as  
1048 well as drafting the manuscript. Benjamin Alric, Philippe Deschamps, Gwendal  
1049 Restoux and Elisa Thébault contributed substantially to data analysis and drafting  
1050 the manuscript. Marianne Simon and Emma Rochelle-Newall contributed  
1051 substantially to data acquisition and drafting the manuscript. Paola Bertolino  
1052 contributed substantially to data acquisition. All authors approved the final  
1053 submitted manuscript.

1054 **Table 1:** Permutational multivariate analyses of variance (PERMANOVA) partitioning of the whole  
 1055 microbial community, prokaryotes and microbial eukaryotes, based on Bray-Curtis dissimilarities  
 1056 calculated using Wisconsin standardized OTU relative abundances among hierarchical-defined clusters  
 1057 1 and 2 (see Figure S6), location (ecosystem), season and year.  
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	Cluster				Location   Cluster				Season				Year			
	Df	F. model	r <sup>2</sup>	P value	Df	F. model	r <sup>2</sup>	P value	Df	F. model	r <sup>2</sup>	P value	Df	F. model	r <sup>2</sup>	P value
<b>Whole microbial community</b>	1	4.74	4.1 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	2.96	7.7 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	1.41	3.6 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	1	1.24	1.1 × 10 <sup>-2</sup>	1.5 × 10 <sup>-2</sup>
<b>Prokaryotes</b>	1	4.51	3.9 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	2.65	6.9 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	1.31	3.4 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	1	1.26	1.1 × 10 <sup>-2</sup>	9.0 × 10 <sup>-3</sup>
<b>Microbial eukaryotes</b>	1	4.95	4.1 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	3.30	8.3 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	3	1.48	3.7 × 10 <sup>-2</sup>	5.0 × 10 <sup>-4</sup>	1	1.25	1.0 × 10 <sup>-2</sup>	1.5 × 10 <sup>-2</sup>

**Table 1: David et al.**

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**Figure 1: David et al.**

**Figure 1: (a)** Cumulative curves showing the total number of prokaryotic OTUs detected in each ecosystem from April 2011 until April 2013 during the temporal survey in the five ecosystems (Etang des Vallées, La Claye, Mare Gabard, Saint Robert and Ru Sainte Anne). Dashed lines indicate missing data due to the drought: Ru Sainte Anne was dried in August and September 2012, and La Claye from the end of July to December 2011 and in September 2012. **(b)** Richness and diversity estimators computed for all the samples. Richness corresponds to the expected number of OTUs in random subsamples of the size of the smallest sequence library (1309 reads in Mare Gabard in May 2011). The thickest line inside each box represents the median on the distribution, bottom and top borders of boxes

1086 correspond to the first and third quartiles and whiskers extend to minimal and maximal distances. (c)  
1087 Histograms showing the relative abundance of 16S rDNA amplicon reads assigned to high-rank taxa  
1088 averaged over the two-year survey, from the five ecosystems (CPR: Candidate Phyla Radiation, FCB:  
1089 Fibrobacteres-Chlorobi-Bacteroidetes, PVC: Planctomycetes-Verrucomicrobia-Chlamydiae). One star  
1090 (\*) indicates Microgenomates and two stars (\*\*) shows Parcubacteria.

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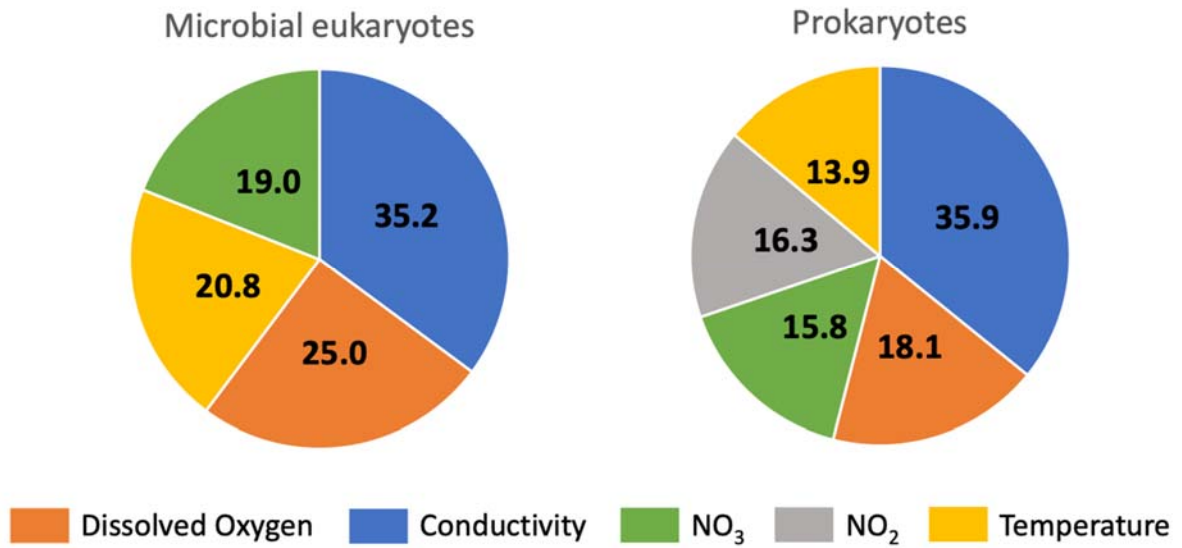
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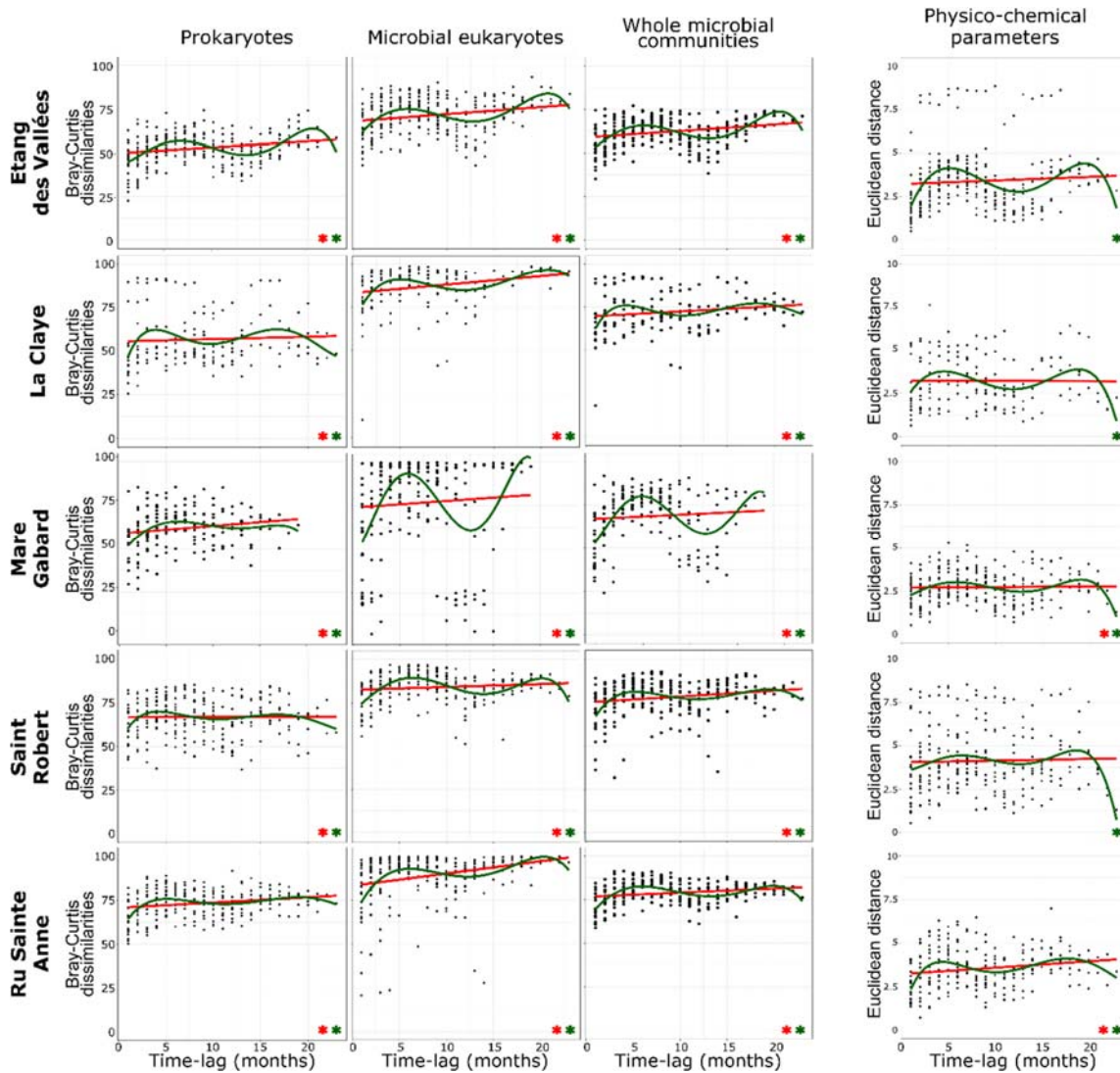




**Figure 3: David et al.**

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 1125 **Figure 3:** Canonical correspondence analyses conducted on all OTUs affiliated either to microbial  
 1126 eukaryotes or prokaryotes retrieved in the five ecosystems over the two-year survey. OTU proportion  
 1127 were Wisconsin standardized. Among all measured physico-chemical parameters, only conductivity,  
 1128 temperature, concentrations of dissolved oxygen nitrate and nitrite were explained significantly the  
 1129 variance of the OTUs ( $P < 0.05$ ). Considered together, these abiotic parameters explained 6.95 and 9.98%  
 1130 of the global variance of the OTUs belonging to microbial eukaryotes and prokaryotes, respectively.  
 1131 Values within each section of the pie charts indicate the proportion of variance explained by each  
 1132 parameter of the global variance of the OTUs.

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**Figure 4: David et al.**

**Figure 4:** Time-lag analyses (TLA) were computed for each ecosystem between sampling dates over increasing time-lags over the two-year survey (from one-month to 23-months). Wisconsin standardized Bray-Curtis dissimilarities were used with all OTUs of prokaryotes, microbial eukaryotes and whole microbial communities while Euclidean distances were applied with environmental parameters. Linear regressions, in red, and polynomial regressions of the 5th order, in green were calculated for each TLA dataset. Significant regressions ( $P < 0.05$ ) are indicated with a green star (linear regressions) or a red star (polynomial regressions).

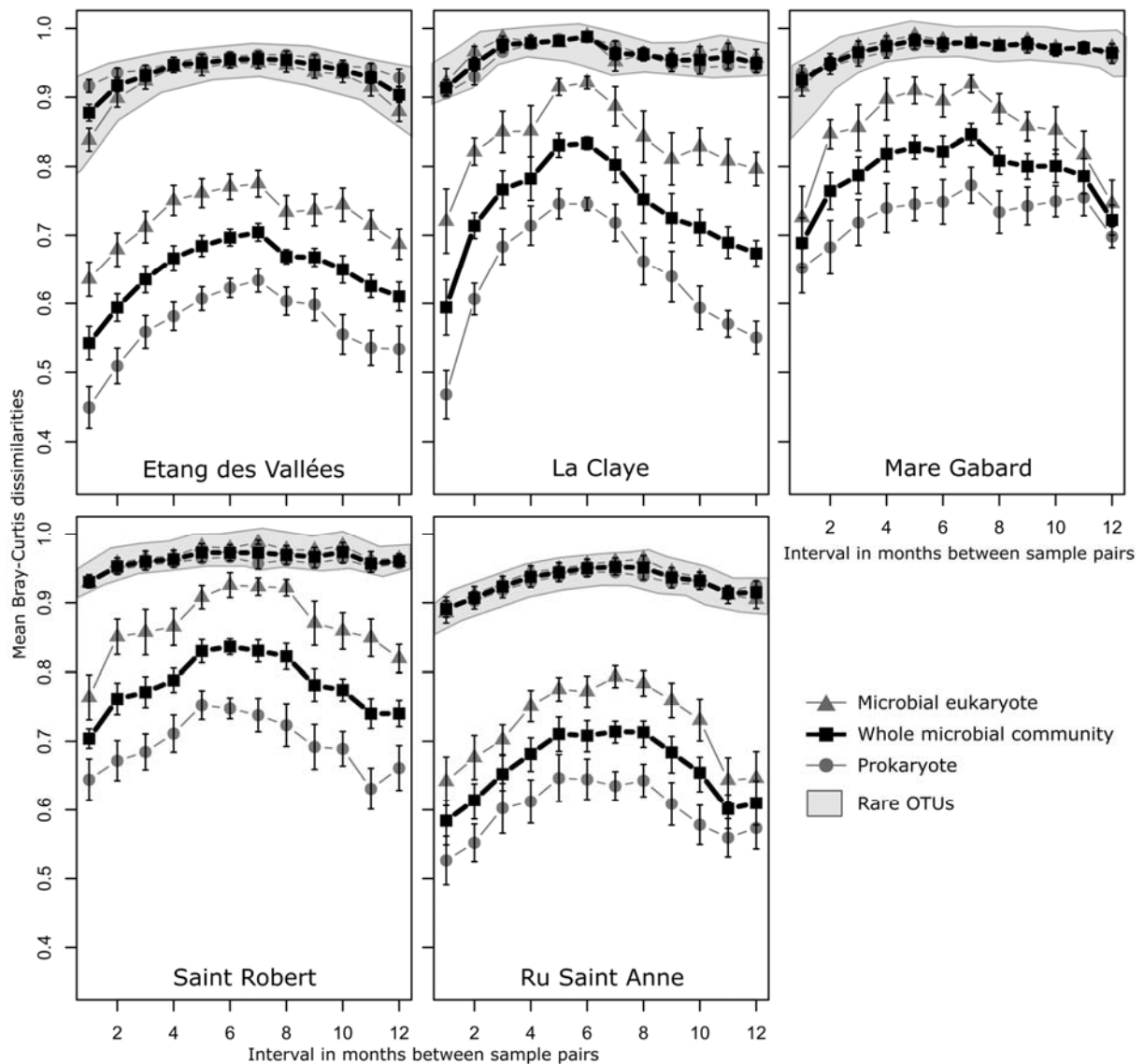


Figure 5: David et al.

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 1157 **Figure 5:** Wisconsin standardized pairwise Bray-Curtis dissimilarities were calculated between samples  
 1158 separated from 1 to 12 months for abundant (detected in each ecosystem in at least 25% of samples, and  
 1159 with >0.1% mean relative abundance per sample ) and rare OTUs (detected in each ecosystem in less  
 1160 than 25% of samples, and with  $\leq 0.1\%$  mean relative abundance per sample; curves highlighted with a  
 1161 grey shade) belonging to microbial eukaryotes (triangles), prokaryotes (circles) and whole microbial  
 1162 community (squares) detected in the five ecosystems over the two-year survey. Each point represents  
 1163 mean value of pairwise Bray-Curtis dissimilarities and is plotted with standard errors.