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Small freshwater ecosystems with dissimilar microbial communities exhibit 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

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

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

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

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

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

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

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

### *Statistical analyses*

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

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

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

$$\frac{-\sum_i p_i \times \ln(p_i)}{\ln(S)}$$

with  $p_i$  the frequency of OTU and  $S$  the observed number of OTUs in the

sample. Diversity was estimated according to the Gini-Simpson index (Gini, 1912; Lee, Buckley, Etienne, & Lear, 2013; Simpson, 1949) calculated as follows using the ‘diversity’ function of the ‘vegan’ R-package (Oksanen et al., 2018):  $S = 1 - \sum_i p_i^2$ .

Microbial community structuring was investigated in the different ecosystems. We drawn Venn diagrams to show shared OTUs between ecosystems, using the ‘gplots’ R-package (Warnes et al., 2015). Hierarchical cluster analysis was build using Bray-Curtis dissimilarities and the Ward2 algorithm (Murtagh & Legendre, 2014) in the ‘hclust’ function of the ‘stats’ R-package to determine clusters of microbial communities based on the distances between every samples of the five ecosystems. Patterns in the temporal dynamics of prokaryotes and eukaryotes were explored at intra- (seasons) and inter-annual (between years) scales. Pairwise Bray-Curtis dissimilarities were computed between samples collected the first year and samples

from the same system the second year over increasing time-lags (from one-month to 12-months) to characterize the temporal dynamics of microbial communities at the year-scale. Time-lag analyses (TLA, Collins, Micheli, & Hartt, 2000) between sampling dates over increasing time-lags (from one-month to 23-months) were computed using Bray-Curtis dissimilarities for microbial communities and Euclidean distances for environmental parameters to determine their variability over consecutive years. We computed polynomial regressions (fitting better than sinusoidal regressions) of Bray-Curtis dissimilarities over time to estimate the frequency of any putative temporal cyclic pattern and linear regressions over time to estimate global shifts in the microbial community compositions and environmental parameters over the two-year survey using the ‘lm’ function of the ‘stats’ R-package. The best fitted polynomial model was determined based on the Akaike Information Criterion (AIC, Akaike, 1973). In TLA, the slope of the linear regression indicates the rate and direction of temporal changes. To test for the significance of slopes for each ecosystem, a randomization procedure was applied with 5 000 permutations using the ‘lperm’ function of the ‘lmPerm’ R-package (Wheeler & Torchiano, 2016). A null distribution was estimated from each slope that was calculated on randomized data. The observed slopes were then compared to the distribution of *F*-values obtained under the null hypothesis. OTU turnovers were calculated between seasons or between years of the two-year survey, using the ‘codyn’ R-package based on the following formula (Diamond, 1969; Hallett et al., 2016; MacArthur & Wilson, 1963):

$$Turnover = \frac{Speciesgained + Specieslost}{TotalSpeciesobservedinbothtimepoints}.$$

Canonical correspondence analyses were conducted to determine the contribution of each environmental factor to the variation of microbial community composition.

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

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

## Results

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

### *Prokaryotic community composition*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

If predation is one of the main recognized biotic interactions at the base of trophic webs, symbioses (including interactions going from parasitism to mutualism) are widespread among microbial communities and can also play key roles (Harding et al., 2018; Hudson, Dobson, & Lafferty, 2006). We observed a large proportion of probable symbiotic taxa among the microbial eukaryotes and prokaryotes identified 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

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

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

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

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#### Conflict of Interest

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.

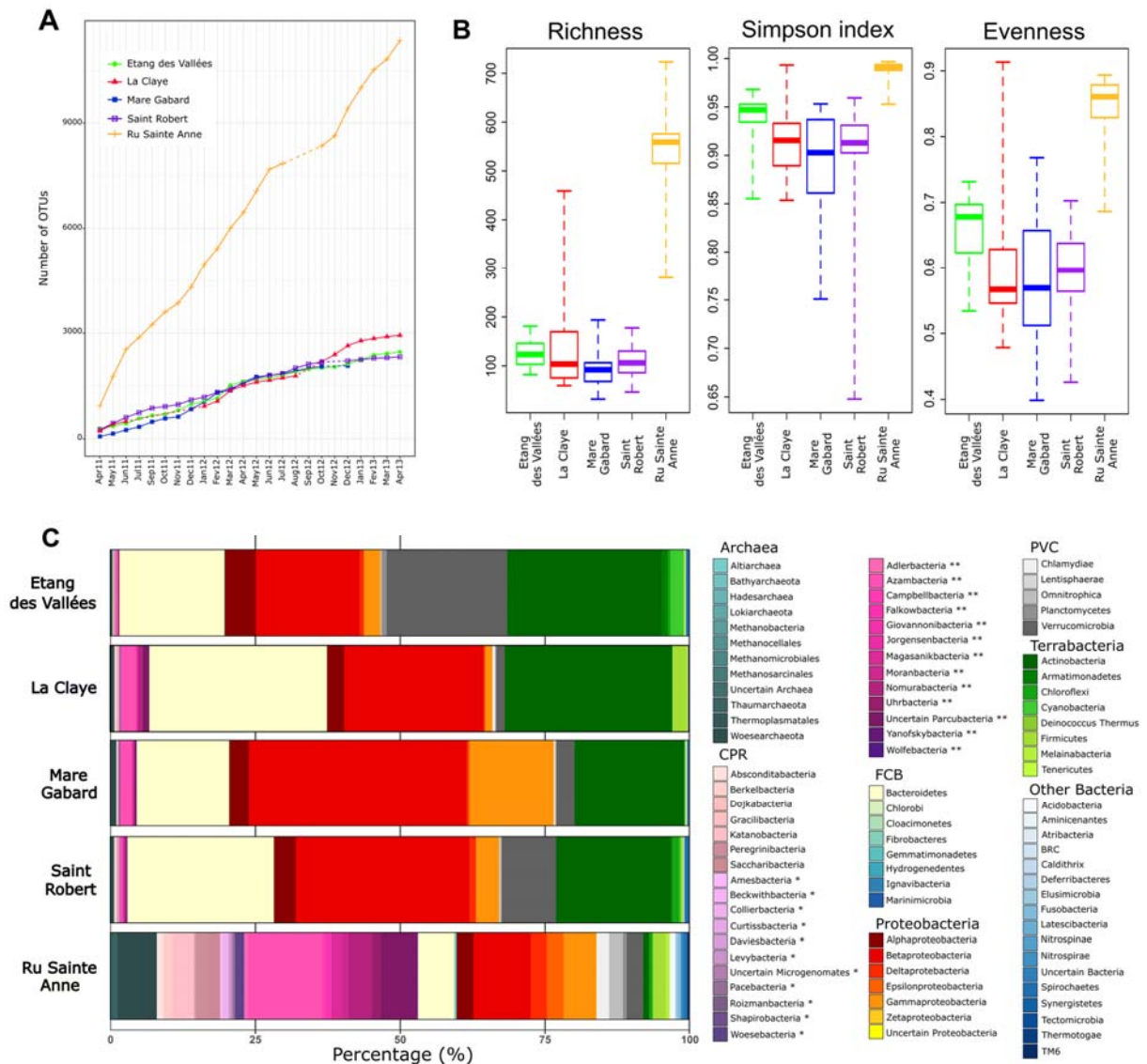
1045 **Author contribution**

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.

**Table 1:** Permutational multivariate analyses of variance (PERMANOVA) partitioning of the whole microbial community, prokaryotes and microbial eukaryotes, based on Bray-Curtis dissimilarities calculated using Wisconsin standardized OTU relative abundances among hierarchical-defined clusters 1 and 2 (see Figure S6), location (ecosystem), season and year.

|                           | 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                |
| Whole microbial community | 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> |
| Prokaryotes               | 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> |
| Microbial eukaryotes      | 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> |

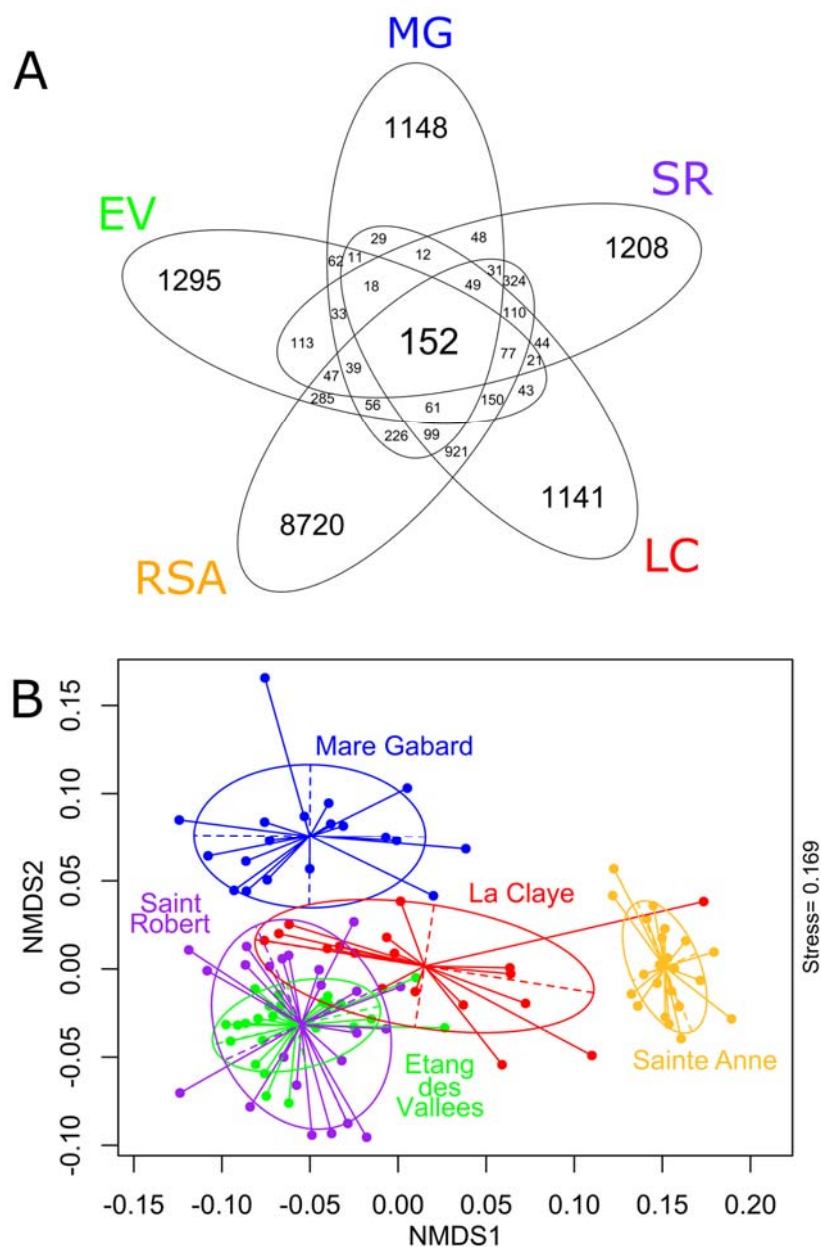
**Table 1: David et al.**



**Figure 1: David et al.**

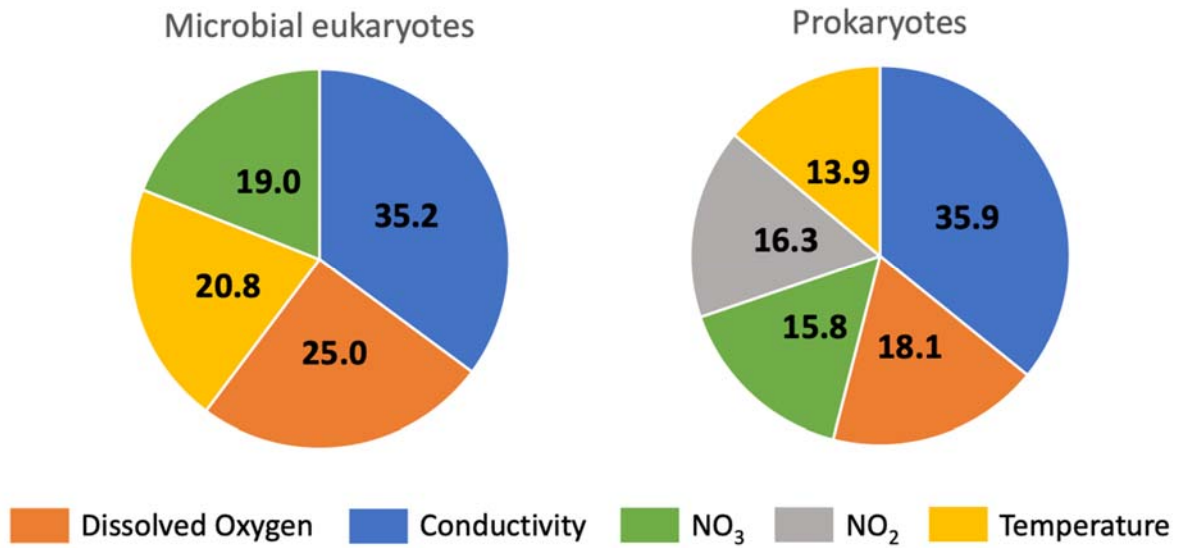
**Figure 1: (a)** Cumulative curves showing the total number of prokaryotic OTUs detected in each ecosystem from April 2011 until April 2013 during the temporal survey in the five ecosystems (Etang des Vallées, La Claye, Mare Gabard, Saint Robert and Ru Sainte Anne). Dashed lines indicate missing data 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

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



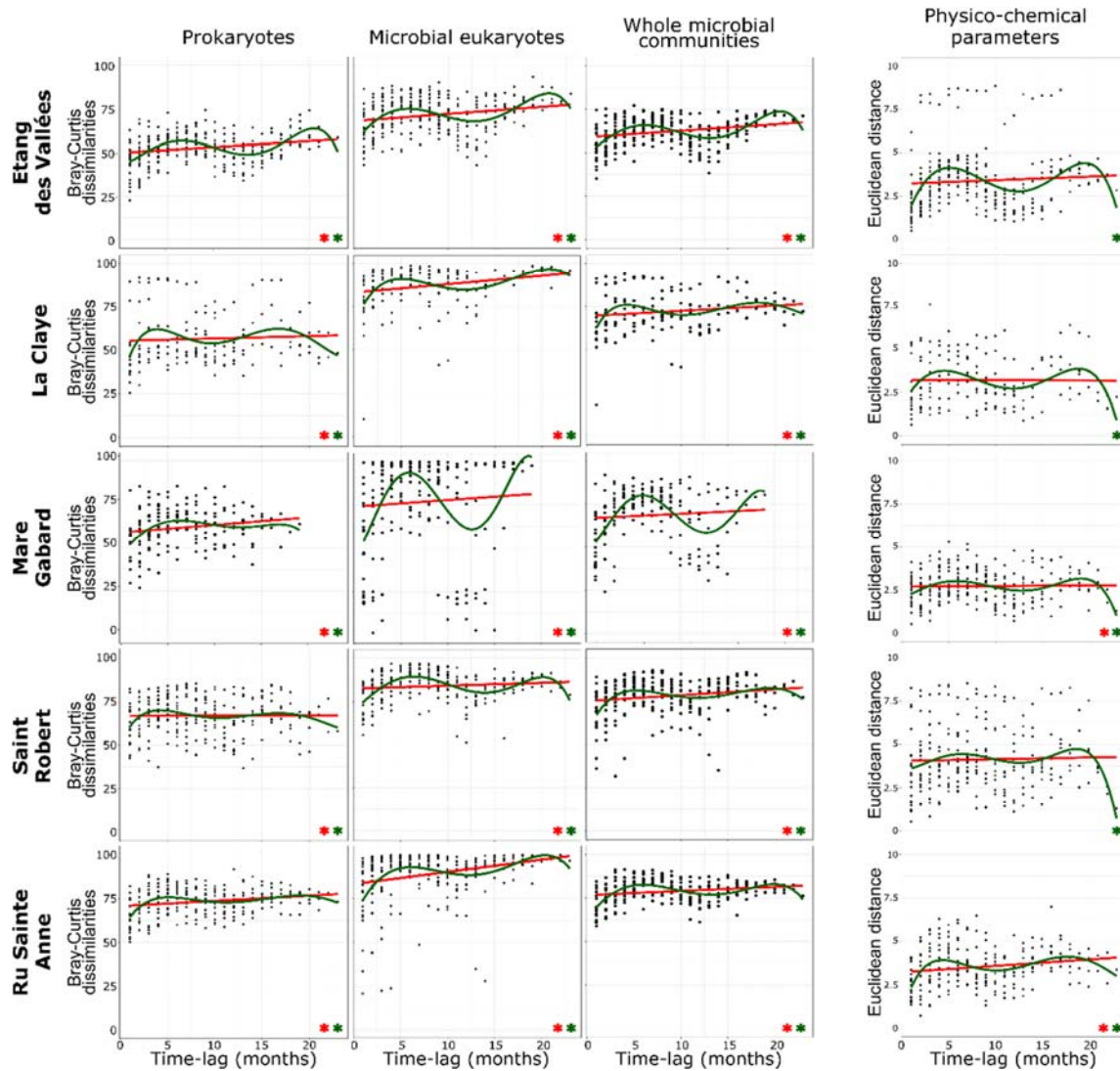
**Figure 2: David et al.**

**Figure 2: (a)** Venn diagram showing the number of OTUs shared by several ecosystems or specific to an ecosystem. All samples from the two-year survey are pooled for each ecosystem. **(b)** Non-metric multidimensional scaling plot, built on Wisconsin-standardized Bray–Curtis dissimilarities between all samples. Stress value is displayed on the right border of the plot.



**Figure 3: David et al.**

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



**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).

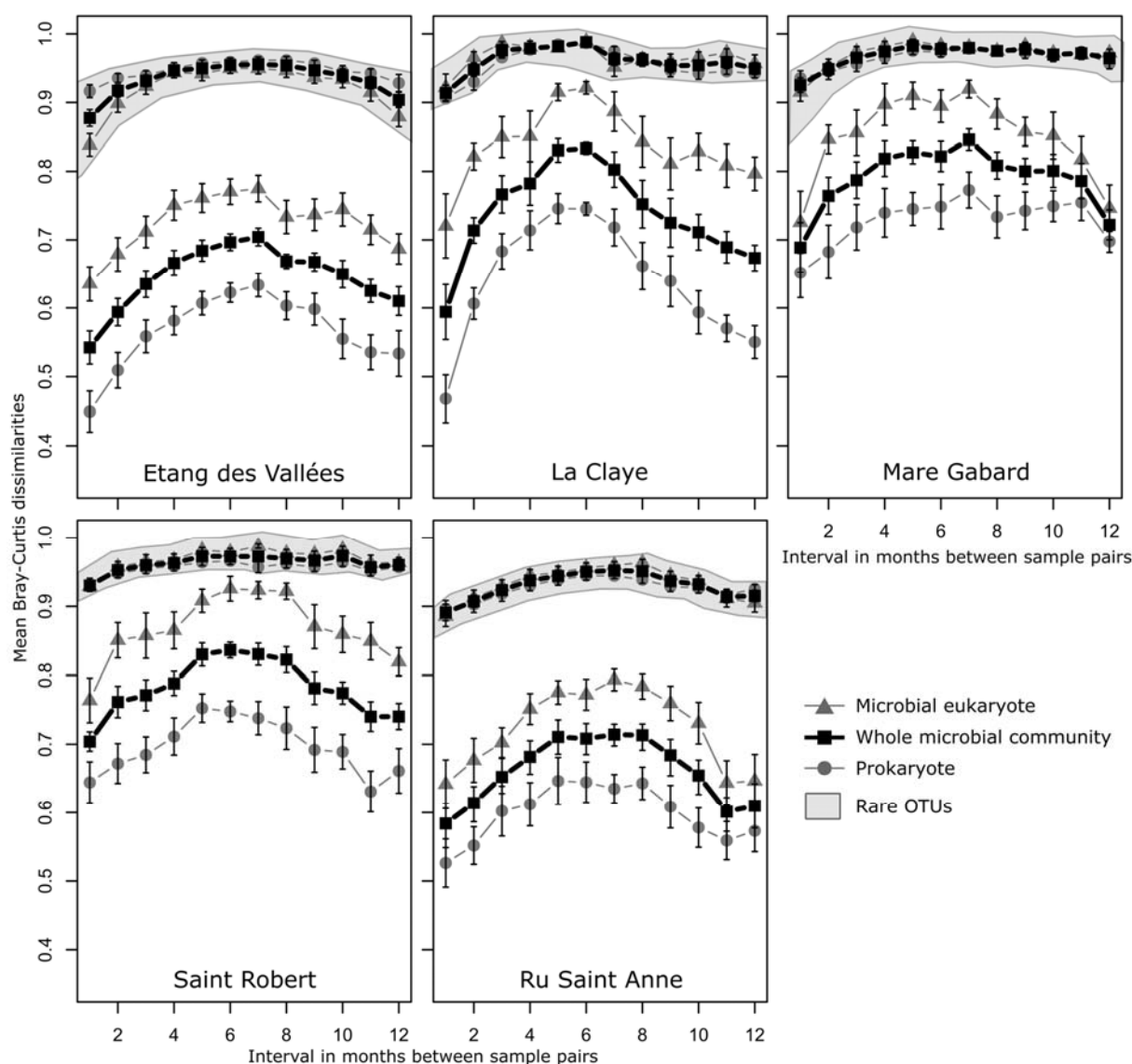


Figure 5: David et al.

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