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The dynamics of biofilm bacterial communities is driven by flow wax and wane in a temporary stream

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Abstract

Biofilm communities are exposed to long periods of desiccation in temporary streams. We investigated how water flow intermittency affected the bacterial community structure colonizing three different streambed compartments in a Mediterranean stream. Massive parallel sequencing revealed different bacterial communities in biofilms from sand sediments and cobbles. Bacterial communities were similar (62% of shared operational taxonomic units) in the epipsammic and hyporheic biofilms, and more diverse than those in the epilithic biofilms. The non-flow phase caused a decrease of bacterial diversity in the biofilms, when communities included only bacterial taxa assumed to be adapted to water stress. The most sensitive bacterial communities to flow intermittency were in the epilithic, where the exposure to physical stress was the highest. In sand sediments a wide group of bacterial taxa was tolerant to desiccation. During non-flow the proliferation of opportunistic taxa in the superficial compartments evidenced the biological link with the terrestrial environment. Bacterial communities better tolerate rewetting than desiccation, since a major number of taxa tolerant to rewetting occurred in all biofilms. Overall, bacterial communities in sandy compartments showed higher resistance to flow intermittency than those in epilithic biofilms.

Droughts are severely increasing in temperate regions worldwide and are usually associated with temporal and spatial increases of non-flow periods in streams and rivers. As a result, many systems become temporary, with unknown biogeochemical and ecological consequences for biodiversity and ecosystem functioning (Acuña et al. 2014). Particularly important are the non-flow and the flow recovery periods, when the system experiences desiccation and rewetting, respectively. These periods affect the biota survival and functioning in the stream and promote the selection of well-adapted communities.

In streambed sediments, microbial communities are directly affected by desiccation and rewetting (Amalfitano et al. 2008; Zoppini and Marxsen 2011; Timoner et al. 2012). Prokaryotes inhabiting these sediments play a pivotal role in the production and degradation of organic matter and nutrient cycling (Battin et al. 2003). Bacteria colonize all streambed substrata (mainly cobbles or sand sediments), and together with other microorganisms (archaea, algae, fungi, and protozoa) make part of biofilms. The bacterial community structure and functioning is related to environmental factors such as resource availability and temperature, but also to the water content in the sediments (Zeglin et al. 2011). Flow intermittency may therefore cause changes in the bacterial community composition of biofilms (Rees et al. 2006; Amalfitano et al. 2008; Febria et al. 2011), indicating that these communities respond to desiccation. Studies in soil and tidal sediments also show prominent changes in bacterial communities subjected to desiccation and rewetting (Fierer et al. 2003; McKew et al. 2011). During the non-flow phase, bacterial richness and diversity decrease, and only a few bacterial taxa surviving desiccation dominate (Rees et al. 2006; Febria et al. 2011). Changes in the community composition associated with desiccation could result both from a selection of the most resistant taxa (Evans and Wallenstein 2014), and from the colonization by immigrant and tolerant taxa (Fazi et al. 2008). Biofilm functioning, assessed through the ability of organic matter degradation (extracellular enzyme activities) decreases due to flow intermittency (Zoppini and Marxsen 2011) but does not disappear (Timoner et al. 2012). Further, the biofilm community structure and function rapidly recover after flow resumption (Rees et al. 2006; Zoppini and Marxsen 2011; Timoner et al. 2012). These are evidence that bacterial communities in temporary streams are able to thrive and function by adapting to the desiccation and rewetting cycles (Evans and Wallenstein 2014).

The present study aims to understand the mechanisms associated with the dynamics of bacterial communities during flow intermittency, by analyzing the specific responses of bacterial communities in the epilithic, epipsammic, and hyporheic biofilms. We conducted a detailed profiling of these bacterial communities through pyrotag sequencing of the small subunit of the ribosomal ribonucleic acid (rRNA) gene. We hypothesized that: (1) the effects of flow intermittency would be more pronounced and long-lasting on the epilithic bacterial community, because of the harsher environmental conditions (low water content, high light irradiances and air temperatures) occurring during the non-flow phase. (2) Water flow wane would drive a selection towards desiccation-resistant bacteria from the taxa already present during the flowing period. Accordingly, we would also expect that flow resumption would restore bacterial species prevalent before desiccation. (3) Bacterial taxa from the nearby terrestrial environment could perform as active colonizers in the more superficial streambed substrata, given the proximity between

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the aquatic and terrestrial compartments in small streams. The resulting bacterial community would therefore exhibit high resistance, expressed through the dominance of ecological strategies directed to thrive or persist under desiccation and rewetting cycles.

Methods

Study site and sample collection—Biofilm samples were collected between May and December 2009 from the Fuirosos, a Mediterranean third-order temporary stream located in northeast Spain. Three hydrological phases were analyzed: (1) a drying phase prior to flow cessation that extended from May until mid-July; (2) a non-flow phase that lasted for 112 d; and (3) a rewetting phase that started by the end of October. Sample collections were six during the drying phase (weeks 4, 6, 8, 9, 11, and 12), seven during the non-flow phase (weeks 13, 14, 15, 16, 17, 18, and 19), and four during the rewetting phase (weeks 20, 22, 27, and 29).

Samples were collected from the streambed following a randomized block design to account for the habitat heterogeneity in the study reach. Five biofilm samples from the epilithic, epipsammic, and hyporheic compartments were collected on each sampling date. Unglazed ceramic tiles (1 cm^2) were used as surrogate substrata for the epilithic substratum, glued onto flat bricks (100–150 tiles per brick), and randomly deployed along the study reach (20 bricks in total) until biofilm colonization (40 d). On each sampling date five bricks were chosen at random to collect the epilithic biofilm, while epipsammic and hyporheic biofilms were collected from the vicinity of the randomly chosen brick. Epipsammic and hyporheic materials were collected with a corer (2.6 cm in diameter), where the first 2 cm of sediment were considered epipsammic, the following 3 to 6 cm were discarded in order to avoid edge effects, and the last ones (from 7 to 10 cm) were considered hyporheic. Subsamples of 1 mL (1.2 cm in diameter) for bacterial community composition analysis were maintained in dark and cool conditions before freezing ($-20^{\circ}C$) within 3–6 h of sampling. Details concerning the study site, sampling strategy, biofilm functioning (extracellular enzyme activities), and environmental measurements are described in Timoner et al. (2012).

DNA extraction, 454-pyrosequencing, and phylogenetic analyses—Deoxyribonucleic acid (DNA) was extracted from biofilms using the Fast DNA Spin kit for soil (MP Biomedicals) according to the manufacturer's instructions. The extracted DNA was quantified using a Qubit® 2.0 Fluorometer (Invitrogen Molecular Probes). The samples for 454-pyrosequencing were obtained after pooling equal amounts of DNA extracts from the five replicates of each biofilm compartment and processed as composite samples. Three composite samples corresponding to each biofilm compartment for each sampling date (a total of 51 samples) were subsequently analyzed through tag-encoded FLX-Titanium amplicon pyrosequencing at the Research and Testing Laboratory (RTL). Briefly, genomic DNA from the biofilm communities was used as a template in polymerase chain reactions using primers 28F/519R targeting the V1–3 region of the bacterial 16S rRNA gene complemented with 454-adapters and sample-specific barcodes. Raw sequence data set was preprocessed at RTL facilities to reduce noise and sequencing artifacts as previously described (Dowd et al. 2008). Demultiplexing according to sample barcodes, sequence quality assessments, chimera detection, and downstream phylogenetic analyses were conducted using Quantitative Insights into Microbial Ecology (QIIME) software package (Caporaso et al. 2010) as described previously (Romaní et al. 2014). The denoised, quality-filtered, and chimera-free sequence data set (51 samples) consisted of 142,590 reads distributed in 2796 sequences per sample (minimum = 920, maximum = 6976, standard deviation = 1376.9), with an average length of 383 nucleotides (minimum = 223, maximum = 534). The relative abundance of sequences associated with different bacterial phyla across samples was corrected for the average number of rRNA operons present in their genomes to avoid interpretation biases. The average values used for this calculation were obtained from the Ribosomal RNA Database (http://rrndb.umms.med.umich.edu/; Lee et al. 2009). For community analysis, the number of sequences in each sample was normalized using a randomly selected subset of 900 sequences from each sample to standardize the sequencing effort across samples and minimize any bias due to a different number of total sequences. QIIME was used to calculate α-diversity indicators of richness (Chao1), diversity (Shannon–Wiener index), and Phylogenetic Diversity (Faith 1992) for each biofilm type and sampling date and to calculate the similarity between bacterial communities (β-diversity) using UniFrac distances (Lozupone and Knight 2005). The relative abundance of the most populated operational taxonomic units (OTUs; $>$ 100 members) for each biofilm type was calculated using the absolute abundance of each OTU across the samples of each biofilm type (OTU Heatmap in QIIME). Shared OTUs between biofilms and hydrological phases were graphically visualized in Venn diagrams constructed in Mothur (Schloss et al. 2009) using the corresponding OTU tables exported from QIIME. To determine whether bacterial species occurring during non-flow were derived from aquatic environments subjected to desiccation or alternatively from terrestrial environments, a representative sequence of the most populated OTUs ($>$ 100 members) was compared with the reference sequences of cultured microorganisms using the Basic Local Alignment Research Tool (Altschul et al. 1990).

Pyrosequencing data obtained from this study have been deposited in the National Center for Biotechnology Information database via the Biosample Submission Portal (http://www.ncbi.nlm.nih.gov/biosample/) under accession number SAMN02388893.

Ecological analysis—We performed a nestedness analysis to determine whether the bacterial communities observed during the non-flow phase were or were not subsets of those during the drying phase. The overall nestedness temperature (Atmar and Patterson 1993) was calculated for each biofilm type and compared with the nestedness temperatures occurring during each sampling date. The nestedness temperature (T) is analog of the community entropy, and provides an idea about the magnitude of

"disorder" in which species are gained or lost in communities. Within this context, low T (i.e., low disorder in species gain and loss) indicate nested patterns (i.e., subset of species from the preceding phase); and high T (i.e., high disorder in species gain and loss) indicate non-nested patterns (i.e., random occurrence). The statistical significance of T was calculated based on the means of fixed–fixed null model, comparing the observed nestedness temperature with that of 1000 simulated matrices (predicted nestedness temperature) using the Nestedness Temperature Calculator. In the fixed–fixed null model, both column (OTUs) and row (sampling dates) sums are fixed. This null model is used due to its restrictiveness and lower incidence of Type I errors (Almeida-Neto et al. 2008). Non-nested patterns occurring during sampling dates were attributed when the nestedness temperature value observed at a sampling date was higher than the overall nestedness temperature obtained for each biofilm type. Contrarily, nested patterns occurred when the nestedness temperature value observed at a sampling date was lower than the overall nestedness temperature obtained for each biofilm type. Moreover, the OTUs turnover between sampling dates during the non-flow phase was estimated using the Armstrong index. This index calculates the percentage of OTUs appearing and disappearing and considering the time elapsed between two consecutive sampling dates (Ruhí et al. 2012).

Biofilm bacterial communities were classified into ecological strategies using an approach similar to the one developed by Grime (1977) for plants under hydric stress. A similar approach has recently been applied to soil bacterial communities subjected to drying and rewetting (Evans and Wallenstein 2014). We calculated beforehand the relative abundances of the most populated OTUs ($>$ 100 members) for each biofilm type. An OTU was considered to be present during a hydrological phase when its relative abundance was > 0 over at least half of the sampling dates. Based on that, the relative abundance of each OTU was averaged for each hydrological phase and the percentage of change from drying to non-flow and from non-flow to rewetting was calculated. If an OTU increased $> 50\%$ from one phase to another it was considered opportunistic, if it decreased $> 50\%$ it was considered sensitive, and if changes were $< 50\%$ it was considered tolerant.

Statistical analysis—Differences in the bacterial community composition between biofilm types and throughout the different hydrological phases were outlined by means of a principal coordinate analysis (PCO). This analysis was performed using the weighted UniFrac distance matrix obtained in QIIME. The shifts observed in the PCO representation were assessed using a permutational multivariate analysis of variance (PERMANOVA) of two factors ("biofilm" and "phase"). The RELATE function (a Mantel-type test) was used to determine if there were significant correlations between the bacterial community structure (based on the weighted UniFrac distance matrix) and the measured environmental variables (percentage of water content, temperature, photosynthetic active radiation, and coarse benthic organic matter [CBOM]). A similarity matrix for the environmental variables was calculated from normalized Euclidean distances. Variables were log-transformed ($\log(x + 1)$) when necessary. The distance-based multivariate linear model (DistLM routine) selected (forward selection) the best combination of environmental variables to account for changes observed in the bacterial community composition. This method predicts the relative influence of each variable in the differences of the bacterial community structure. The significance level for all the analyses was $p < 0.05$. Statistical tests were performed using PRIMER-E and PERMANOVA software (PRIMER-E).

Results

Intermittency effects on the bacterial community composition—The biofilm bacterial communities changed their composition between streambed compartments as well as during the study period (Fig. 1). Sequences affiliated with *Cyanobacteria* and *Firmicutes* dominated in the epilithic biofilm, while *Actinobacteria* and *Proteobacteria* were more common in the epipsammic and hyporheic. In particular, cyanobacterial sequences abundant during the drying phase in the epilithic biofilm were replaced by *Firmicutes* during the non-flow phase, but recovered 2 weeks after flow resumption (Fig. 1A). Epipsammic and hyporheic biofilms showed minor variations in the relative abundance of sequences affiliated with *Actinobacteria*, *Alphaproteobacteria*, and *Firmicutes* during non-flow. *Actinobacteria* moderately increased during rewetting in the epipsammic and hyporheic (Fig. 1B,C).

Epipsammic and hyporheic biofilms shared 2048 OTUs, 62% of the total. The epilithic biofilm (Fig. 2A) shared a similar number of OTUs with the epipsammic (901 shared OTUs, 21%) and hyporheic biofilms (774 shared OTUs, 23%). Approximately 22% of the total OTUs (716 out of 3314) were shared between the three biofilm types studied (Fig. 2A). Up to 305 OTUs were characteristic of the non-flow phase, and the number of specific OTUs during drying and rewetting was lower (217 and 229 OTUs, respectively; Fig. 2B). The number of shared OTUs across all hydrological phases during the entire studied period was 1483 ($\sim$ 45%).

Prevailing OTUs in the epilithic during non-flow were affiliated with the genera *Exiguobacterium* (99% identity to *Exiguobacterium acetylicum* and *Exiguobacterium undae*) and *Chryseomicrobium* (98% identity to *Chryseomicrobium amylolyticum*). During the same period, OTUs affiliated with *Actinobacteria* (*Nocardioides jensenii*, 96% identity and *Nocardioides terrigena*, 94% identity) and *Alphaproteobacteria* (*Nitrobacter vulgaris*, 99% identity and *Sphingomonas suberifaciens*, 97% identity) prevailed in the epipsammic and hyporheic.

Intermittency effects on α *and* β *diversity*—Epipsammic and hyporheic biofilms had higher richness (Chao1) and diversity (Shannon–Wiener diversity index and Phylogenetic Diversity) than the epilithic biofilm (Fig. 3). The non-flow phase was associated with a decrease in the richness and diversity of all bacterial biofilm communities. The diversity decrease was more pronounced in the epilithic, and non-flow samples were segregated after clustering

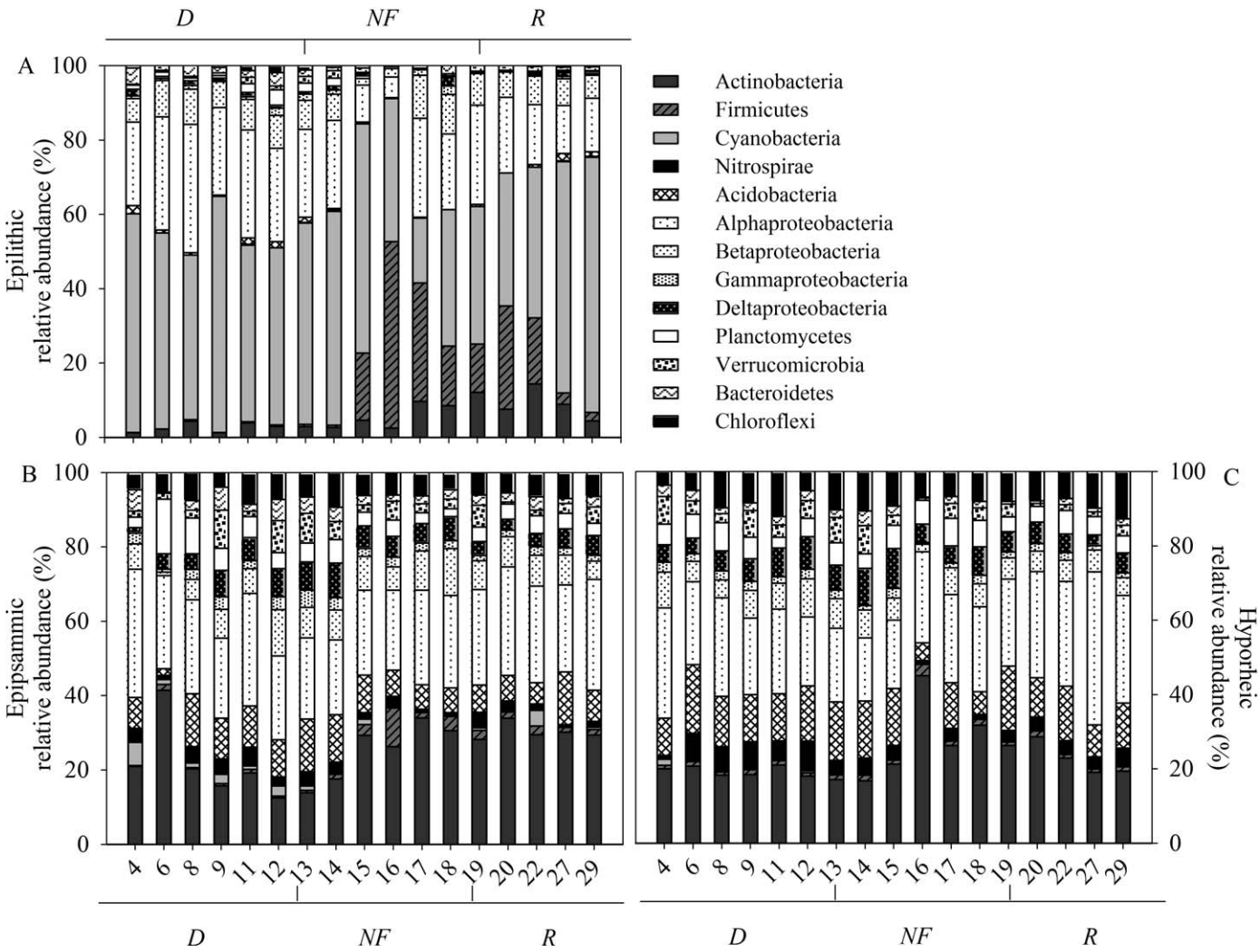

Fig. 1. Relative abundance of 16S rRNA gene sequences affiliated with the bacterial phyla (class for *Proteobacteria*) in each biofilm type after correction for the average rRNA operon copies in their genomes (*see* main text for details): (A) epilithic, (B) epipsammic, and (C) hyporheic. The labels at the top indicate the different hydrological phases: drying (*D*), non-flow (*NF*), and rewetting (*R*).

according to weighted UniFrac distances using PCO (Fig. 4). This analysis separated the epilithic samples from epipsammic and hyporheic ones along the first horizontal axis (51.3% of the variation), whereas the second vertical axis (20.4% of total variation) segregated the epilithic samples according to the different hydrological phases. The epipsammic and hyporheic samples overlapped in the first axis. The first epilithic samples after flow cessation (weeks 13 and 14) clustered with those during the drying phase (from week 4 to 12); the first samples after flow recovery (weeks 20 and 22) grouped with those from the non-flow phase (weeks 12 to 19); and those collected 2 months after flow recovery (weeks 27 and 29) grouped with those in the drying phase. These differences between biofilm communities were further identified through PERMANOVA analysis (biofilm: pseudo-$F_{2,42} = 30.758$; $p < 0.001$ and phase: pseudo-$F_{2,42} = 5.075$; $p < 0.001$).

Nestedness and OTUs turnover—Bacterial communities in the three different biofilms were highly structured, as indicated by the observed significant degree of nestedness. The overall observed nestedness temperature value (T_{obs}) for the epilithic ($T_{obs} = 34.85°C$ [P ($T < 34.85$) = 1.09×10^{-64}]) was significantly lower than the predicted value (T_{pred}) for randomly generated communities (Atmar and Paterson 1993; $T_{pred} = 56.10°C$). Epipsammic and hyporheic biofilms also exhibited lower nestedness temperatures ($T_{obs} = 35.45°C$ [P ($T < 35.45$) = 2.63×10^{-57}] and $T_{obs} = 42.01°C$ [P ($T < 42.01$) = 1.86×10^{-107}], respectively) than the randomly predicted values ($T_{pred} = 62.07°C$, $T_{pred} = 65.10°C$, respectively). T_{obs} values of the epilithic biofilm during the non-flow phase were relatively similar or even higher (week 16) than the overall observed nestedness temperature for the epilithic (Fig. 5A). However, the T_{obs} in the epipsammic and hyporheic during the non-flow phase were lower than their respective overall observed nestedness temperatures (Fig. 5B,C). This suggests that sandy biofilms during the non-flow phase were a subset of the taxa already present during the preceding drying phase. To be precise, the high T_{obs} of the epipsammic and

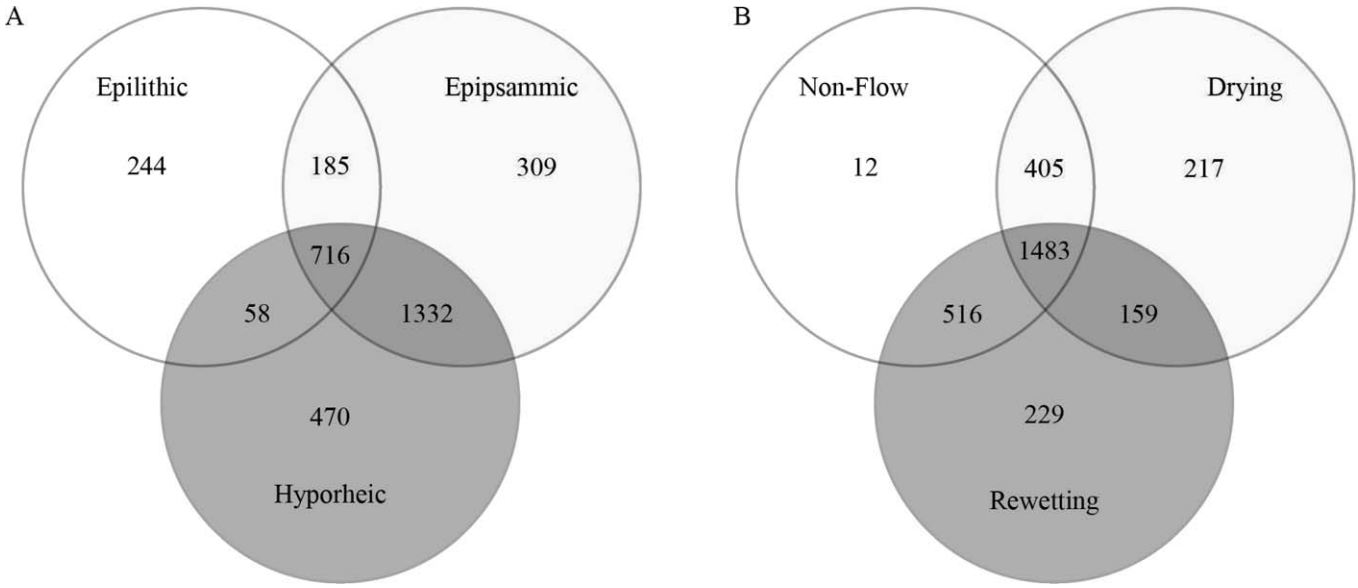

Fig. 2. Venn diagrams showing shared OTUs across (A) biofilms and (B) hydrological phases.

hyporheic biofilms during the drying phase indicate the occurrence of random taxa. Also, high T_{obs} characterized the flow resumption phase in all biofilms. The low T_{obs} during the rewetting phase indicated that the bacterial community was a subset of the preceding non-flow phase.

OTUs turnover increased during the transition from drying to non-flow and, in particular, from non-flow to rewetting phases (from week 19 to week 20; Table 1). A different trend occurred between biofilm compartments during non-flow, where OTUs turnover increased in the epilithic and decreased in the epipsammic and hyporheic (Table 1).

Ecological strategies of bacterial communities to desiccation and rewetting—The ecological strategies to desiccation and rewetting varied among dominant phyla and between the different biofilms. The epipsammic and hyporheic biofilms had a higher number of OTUs tolerant to desiccation (47% and 86%, respectively) than the epilithic (27%; Fig. 6A–C). OTUs sensitive to desiccation were abundant in epilithic biofilm (35%) and much lower in the epipsammic and hyporheic biofilms (15% and 2%, respectively). Opportunistic OTUs were higher during non-flow in the epilithic and epipsammic biofilms ($\sim$ 35%) than in the hyporheic (12%).

The number of OTUs tolerant to rewetting was higher in the epipsammic (60%) and hyporheic biofilms (72%) than in the epilithic biofilm (44%; Fig. 6D–F). A major proportion of OTUs were sensitive to rewetting in the epilithic and the epipsammic (23% and 19%, respectively) than in the hyporheic (12%). The opportunistic OTUs reached 33% in the epilithic, but only 21% in the epipsammic and 16% in the hyporheic.

Links between bacterial community composition and environmental variables—Several environmental variables (Mantel tests, $\rho = 0.229$, $p = 0.001$) accounted for the bacterial community structure in the different biofilms and hydrological phases. Water content, CBOM, and temperature accounted for 37.5% of the total variation in the bacterial community composition (Table 2). Water content explained the greatest proportion of variation (21.9%), followed by CBOM and temperature, which accounted for 9.2% and 6.4%, respectively.

Discussion

Changes in the community composition of biofilm bacteria were tightly associated to flow intermittency, but $\sim$ 45% of the taxonomic units occurred in all three hydrological phases. Changes were not uniform for all stream compartments colonized by biofilms. The bacterial community in epilithic biofilms suffered the most abrupt composition changes during the non-flow phase, probably as

Table 1. OTUs turnover assessed using the Armstrong index between consecutive sampling dates at each biofilm type.

Sampling date (weeks)		Epilithic	Epipsammic	Hyporheic
Drying	4–6	0.000021	0.000006	0.000004
	6–8	0.000004	0.000008	0.000006
	8–9	0.000027	0.000019	0.000014
	9–11	0.000011	0.000008	0.000005
	11–12	0.000016	0.000016	0.000009
Non-flow	12–13	0.000024	0.000015	0.000009
	13–14	0.000017	0.000013	0.000009
	14–15	0.000017	0.000003	0.000003
	15–16	0.000019	0.000005	0.000004
	16–17	0.000032	0.000007	0.000007
	17–18	0.000023	0.000005	0.000007
	18–19	0.000023	0.000008	0.000007
Rewetting	19–20	0.000190	0.000128	0.000137
	20–22	0.000035	0.000017	0.000023
	22–27	0.000010	0.000003	0.000002
	27–29	0.000005	0.000003	0.000001

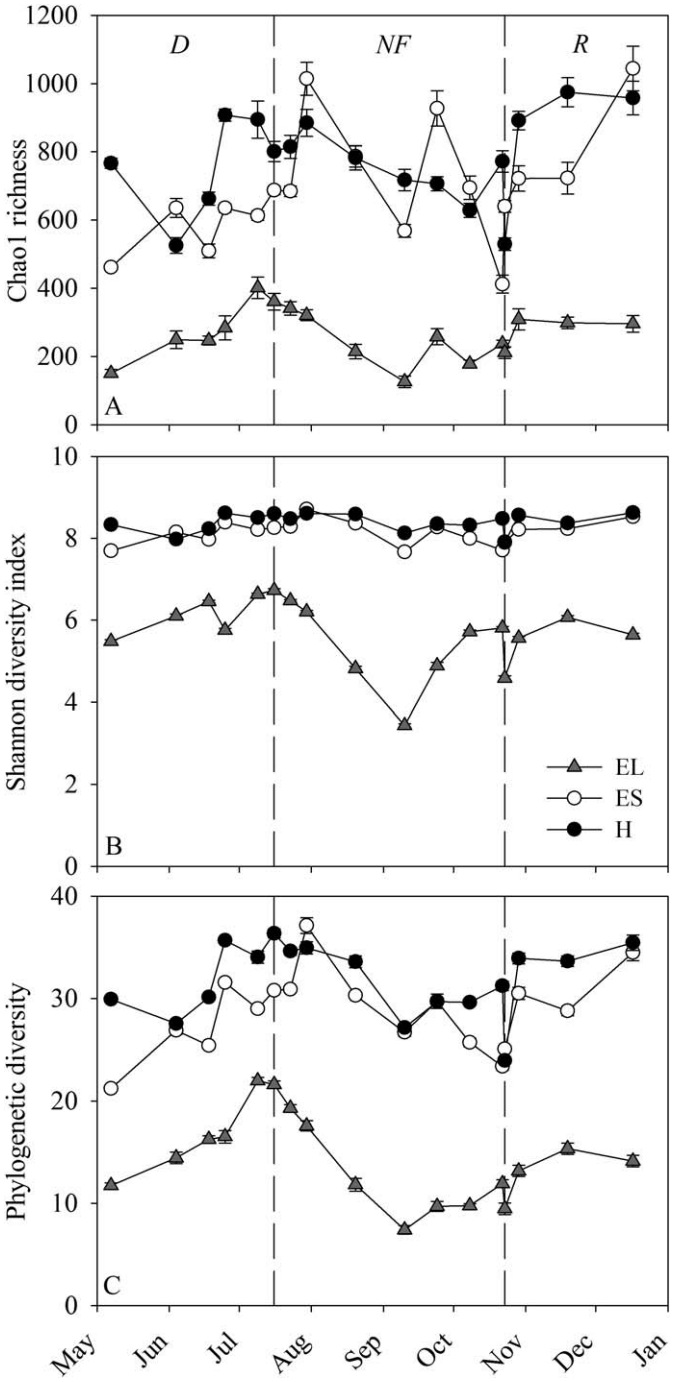

Fig. 3. Dynamics of (A) Chao 1 richness, (B) Shannon diversity index, and (C) Phylogenetic Diversity for the three biofilms during the study period. The symbols represent the different biofilms: epilithic (EL), epipsammic (ES), and hyporheic (H). The vertical dashed bars indicate the different hydrological phases: drying (*D*), non-flow (*NF*), and rewetting (*R*).

a result of their major exposure to desiccation, higher air temperatures and major influence of airborne bacteria reaching the streambed, probably arriving from the surrounding terrestrial environment (Hervas and Casamayor 2009; Langenheder and Székely 2011). The bacterial communities on sand compartments (epipsammic and hyporheic) showed smaller changes, likely because their physical configuration provided higher protection from desiccation and temperature variations. The non-flow period generally reduced the richness and diversity of bacterial communities, and more importantly when the streambed desiccation was the highest. This period also provided new opportunities for bacterial taxa involved in ecosystem functions such as dry organic matter mineralization.

Table 2. Relationship of the bacterial community composition (based on weighted UniFrac distances) between environmental variables (percentage of water content, coarse benthic organic matter [CBOM], temperature, and photosynthetic active radiation [PAR]), analyzed using a forward selection procedure in DistLM. The *p*-values were obtained using 9999 permutations of residuals under a reduced model.

Variable	R^2 (cumulative)	Sum of Squares (trace)	Pseudo-*F*	*p*
% water content	0.219	0.52	13.728	0.001
CBOM	0.311	0.22	6.451	0.001
Temperature	0.375	0.15	4.743	0.005
PAR	0.400	0.06	1.964	0.100

Epilithic bacterial communities were the most singular in the stream (< 25% of shared OTUs with other biofilm communities), while those on epipsammic and hyporheic were similar to each other (62% of shared OTUs). These two biofilms had OTUs affiliated to *Actinobacteria* and *Proteobacteria* throughout the study period. These are taxa common in soils and river sediments (Tamames et al. 2010), and their members play an important role in the decomposition of organic matter. In sand sediments both particulate and dissolved organic materials are retained and processed by their associated bacterial communities, which are responsible for up to 80% of the carbon mineralization in streams (Marxsen 2001). Epilithic biofilms had *Cyanobacteria* and *Firmicutes* as the most abundant bacterial groups. *Cyanobacteria* were predominantly observed during the drying and rewetting phases when these phototrophic organisms have suitable water availability and light conditions, whereas members of *Firmicutes* were prevalent during the non-flow phase. The bacterial richness (Chao1) and diversity (Shannon–Wiener and Phylogenetic Diversity) were higher in the epipsammic and hyporheic biofilms, likely because of the high number of niches provided by sand particles. The bacterial communities colonizing rocks and cobbles are generally limited by the availability of dissolved carbon in the flowing water and by that of the endogenous carbon derived from the biofilm activity (Freeman and Lock 1995). However, the bacterial communities in sand sediments can obtain additional resources from entrained particulate and dissolved carbon (Battin et al. 2003). Thus, lower diversity and richness in the epilithic biofilm might result from the dominance of phototrophic organisms, the lower number of niches, and the lower resource availability.

A major change in the composition of the bacterial community of epilithic biofilms occurred 14 d after flow

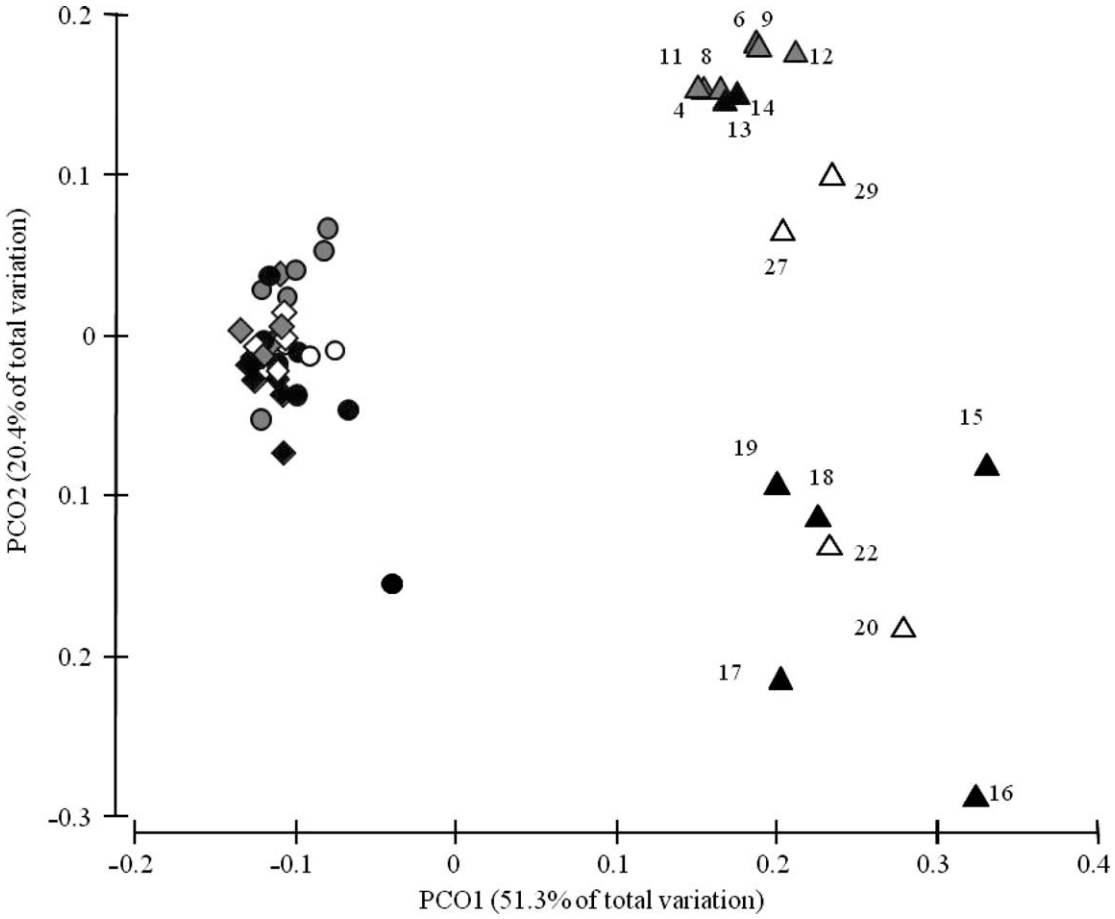

Fig. 4. Principal coordinates analysis (PCO) of the UniFrac pairwise dissimilarity of the bacterial communities for the three biofilms and during the study period. The numbers indicate sampling weeks, the symbols indicate the different biofilm (epilithic [triangles], epipsammic [circles], and hyporheic [diamonds]), and the colors indicate the different hydrological phases: drying (gray), non-flow (black), and rewetting (white).

cessation. This apparent resistance to change might be associated with the protective effect of the growth form of epilithic biofilms, which are tightly attached to the substratum, resembling a ''crust'' (Sabater 2000; Belnap et al. 2004). The proliferation of *Firmicutes* during the non-flow phase is probably associated with the characteristics of this group, which includes fast-growing species in favorable conditions. Members of this group are adapted to thrive under extreme conditions such as desiccation (Vishnivetskaya et al. 2009). In addition, the multiplicity of rRNA operons in the genomes of *Firmicutes* (seven on average) can also be considered key to the competitive success of these bacteria (Klappenbach et al. 2000). *Firmicutes* were also observed in the epipsammic during the period of maximum desiccation (weeks 16 to 18). Representative sequences of the two dominant OTUs affiliated with this phylum were closely related to extremophilic members of the genera *Exiguobacterium* and *Chryseomicrobium* (OTU-0, -1, -1003, and -86; Vishnivetskaya et al. 2009). OTUs affiliated with *Firmicutes* were classified as tolerant and opportunistic with regard to desiccation. During the non-flow phase, the most populated OTUs in the epipsammic and the hyporheic were affiliated with *Solirubrobacter* and *Nocardioides* (*Actinobacteria*, OTU-9, -10, -16, and -21), and with *Nitrobacter* and *Sphingomonas* (*Alphaproteobacteria*, OTU-3 and OTU-29), common genera from soils showing tolerance to desiccation and in some cases capacity to grow under non-flow conditions (opportunistic, OTU-16, OTU-21, and OTU-29) as observed after classifying them into the different ecological strategies. In fact, the number of living bacterial cells increased during the non-flow phase in all streambed compartments (Timoner et al. 2012). *Firmicutes* and *Actinobacteria* have gram-positive cell wall type (Fierer et al. 2003; Schimel et al. 2007), and this probably offers to these groups a high resistance to desiccation and rewetting in temporary streams (Marxsen et al. 2010) and soils frequently exposed to hydrological variability (Fierer et al. 2007; Schimel et al. 2007). There was, therefore, a wide group of bacterial taxa adapted to flow intermittency (45% of OTUs shared between the three hydrological phases), which may be crucial in maintaining the ecosystem processes. Similarly, a high number of ubiquitous taxa were observed in floodplain soils highly subjected to wet and dry phases (Baldwin et al. 2013).

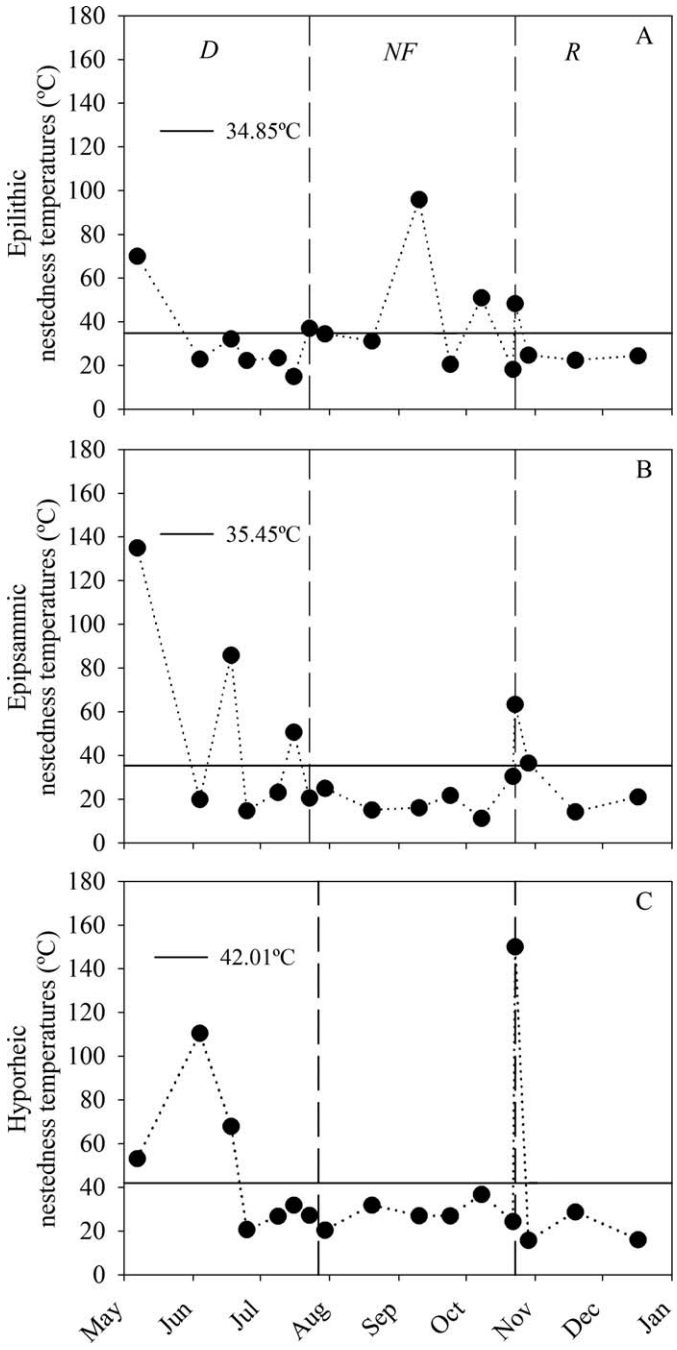

Fig. 5. Nested temperatures calculated at each sampling date for the (A) epilithic, (B) epipsammic, and (C) hyporheic. The vertical dashed bars indicate the different hydrological phases: drying (*D*), non-flow (*NF*), and rewetting (*R*). The observed nestedness temperature for each biofilm type is indicated with a horizontal solid line.

The observed decrease in richness and diversity during the non-flow phase was probably a reflection of the replacement of many sensitive species by a few other dominant species favored by the new environmental conditions. This decrease in richness and phylogenetic diversity occurred mostly by species sorting through the selection of species tolerant to desiccation. The bacterial community shifted to a narrower phylogenetic diversity, where only specialized taxa survived (Febria et al. 2011). The low nestedness temperatures in the epipsammic and hyporheic biofilms during the non-flow phase indicated that bacterial communities derived from the species pool of the preceding wetted phase (the drying phase), where dormancy could play a key role in the recovery of bacterial diversity (Zeglin et al. 2011). However, the high nested temperatures in the epilithic during the same phase were indicative of a non-nested pattern, and suggested a poor adaptation of indigenous members of the community to desiccation. This was confirmed by the high occurring proportion of sensitive taxa to desiccation in that biofilm. The occurrence of immigrant bacteria ($\sim$ 36% were opportunistic to desiccation) in the epilithic biofilm probably derived from airborne bacteria coming from the nearby terrestrial sources (e.g., dust deposition and leaf accumulation). The Armstrong index also shows an increase in the OTUs turnover in epilithic biofilms associated with the occurrence of new taxa. The occurrence of immigrant microorganisms from external sources is an expression of the biological link between terrestrial and aquatic ecosystems during the non-flow phase. The high nestedness temperatures also occurring during the drying phase might be associated with the streambed patchiness during water flow contraction, where a wide range of habitats could be used for versatile bacteria (Febria et al. 2011).

Flow recovery promoted changes in the bacterial community composition, primarily in the epilithic compartment. The nestedness temperatures and the Armstrong index indicated that the transition from the non-flow phase to the rewetting phase severely affected biofilm bacterial communities. While desiccation was a slow process and enabled the gradual community response, water flow return occurred in few hours and drastically affected those taxa unable to resist inundation (sensitive taxa). Our results show, however, that epilithic and epipsammic bacterial communities better tolerated the rewetting than the desiccation. The increase in the nestedness temperature immediately after flow recovery might be due to the occurrence of opportunistic taxa in the stream following washout (Fazi et al. 2008). The epilithic and epipsammic biofilms showed the highest proportion of sensitive and opportunistic OTUs to rewetting and indicated their tight association with flow intermittency. Those bacterial communities in the hyporheic had a more stable composition through the study period, with a major number of tolerant OTUs to both desiccation and rewetting ($\sim$ 80%). The epilithic biofilm required $>$ 2 weeks to recover to a composition similar to that before desiccation, and this recovery was shorter in the sandy compartments. One week after flow resumption bacterial richness and diversity in all biofilms recovered to values similar to those before desiccation. This certainly stresses the high resistance of bacterial communities (and especially of those in subsuperficial substrata) to cope with flow discontinuities in temporary streams. This recovery in community composition coincided with that in the biofilm activity. The β-glucosidase and phosphatase enzymatic activities were higher during the rewetting phase than before flow cessation (Timoner et al. 2012), when the heterotrophic

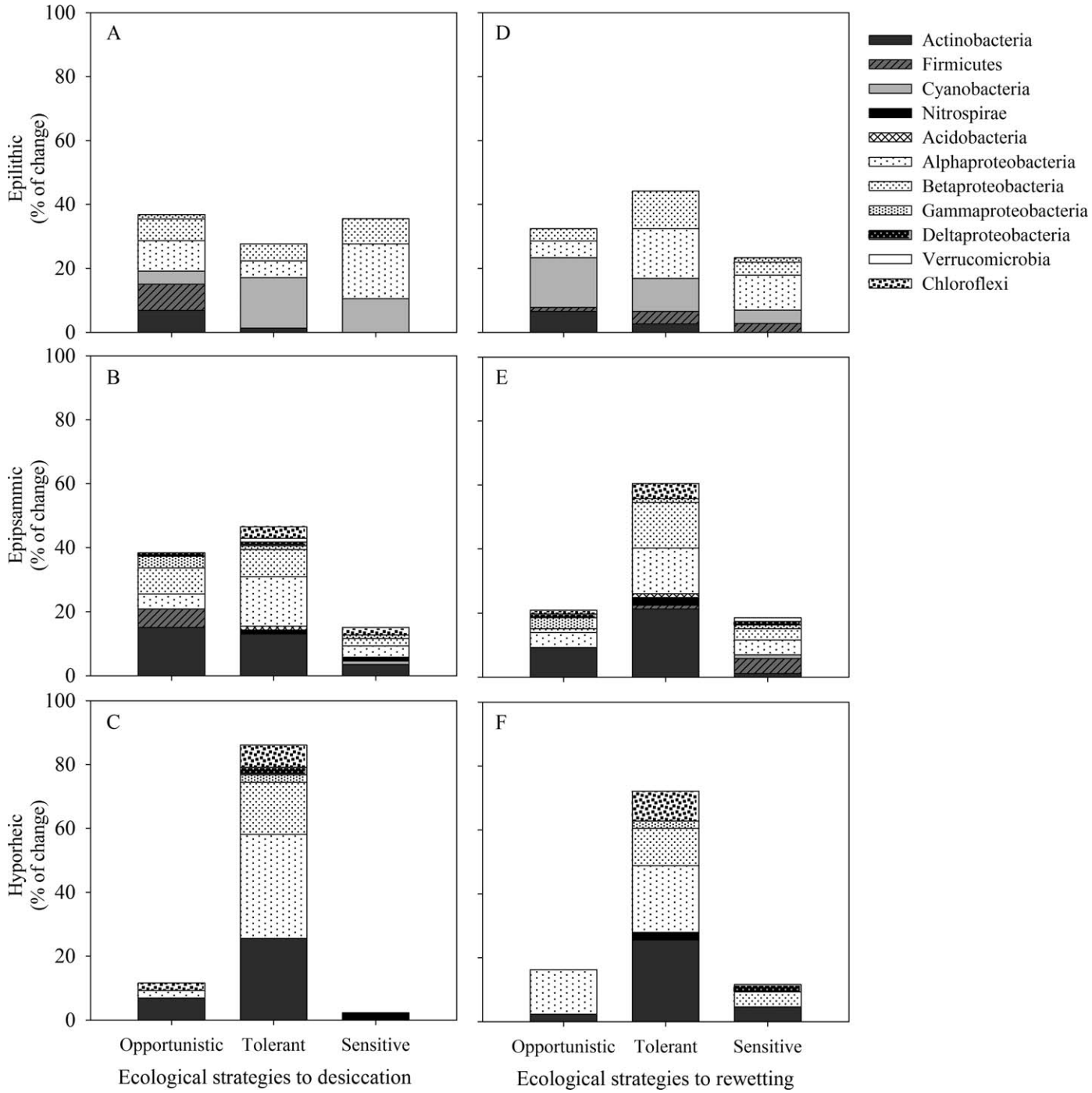

Fig. 6. Ecological strategies of biofilm bacterial communities to desiccation on the (A) epilithic, (B) epipsammic, and (C) hyporheic. Ecological strategies of biofilm bacterial communities to rewetting on the (D) epilithic, (E) epipsammic, and (F) hyporheic.

processes were prevalent. The return of flow rehydrated the leaf litter accumulated during non-flow and activated the liberation of phosphorus and nitrogen (Ylla et al. 2010; Timoner et al. 2012), triggering the bacterial activity of a community well adapted to the new situation.

Streams in temperate regions are under risk of recurrent flow intermittency, implying extended interactions between terrestrial and aquatic ecosystems, and potential effects on biogeochemical cycles and ecosystem functioning. Global change is accelerating the rate at which such changes occur, and potentially impair the capacity of microbial communities to cope with drastic fluctuations of environmental conditions. However, the natural selection of biofilm bacterial communities in temporary streams, which show a high adaptability to flow intermittency, might mitigate to some extent these effects, specifically when these are

compared to other systems with lower intra-annual water flow variability. Assuming the crucial role of streambed microbial communities in biogeochemical cycles and ecosystem functioning, the understanding of how these communities respond and adapt to such environmental changes may help to anticipate potential consequences at local and global scales.

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