



## **Patterns of biofilm formation in intermittent and permanent streams: analysis of biofilm structure and metabolism**

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The development and functioning of benthic microbial communities in streams is largely dependent on the hydrological conditions. Climate change projections predict that the hydrological characteristics will probably be affected because of the rainfall regime. Hence, rivers from the Mediterranean region will become more similar to those draining arid or desert regions, while temperate streams will suffer of higher water flow fluctuations. In this study, we compared the process of biofilm formation between an intermittent (the Fuirosos, Mediterranean) and a permanent (the Walzbach, Central European) stream. Specifically, we analyzed the succession of bacterial and algal populations in the biofilm through bacterial rDNA sequences analysis (16S rDNA and 16S-23S intergenic sequence) and diatom taxa identification over a 60-days colonization experiment. Moreover, changes in biofilm structural (microbial biomass and extracellular polysaccharide content) and metabolic (extracellular enzyme activities) parameters were also analyzed. The successional patterns of microbial populations in the Fuirosos showed clear discontinuities coinciding with flood episodes while at the Walzbach the time sequence was more gradual. Although both study sites were forested, greater microbial biomass standing stock (algal and bacterial) and greater species biodiversity was detected during biofilm development at the Mediterranean site. The higher bacterial biodiversity may be related to the potential effect of flooding episodes in reducing biological interactions in complex microbial communities, such as the competitive exclusion of species. Moreover, the presence of rapid colonizing diatom species might be an adaptation to hydrological changes. In contrast, species competition could define the more stable environments, such as that observed in the Central European stream. Overall, the historical evolutionary pressure from the different bioclimatic regions could be also affecting the microbial community composition. Further, the analysis of the whole biofilm colonization sequence showed a greater EPS development and a higher potential extracellular enzyme activity rates in the Mediterranean stream. The higher EPS reflected a functional response of the biofilms to avoid detachment during high flow episodes and the higher enzyme activities were according to the low water nutrient concentration available for microbes. However, the higher nutrient conditions (higher nitrate and phosphate concentrations) at the Central European stream was not reflected on higher biomass standing stock in biofilms. Likely, the biomass accrual of biofilms was related to the differences in climatic conditions between study sites (e. g. daily insolation, temperature oscillation). The results suggest that microbial community development and functioning is primarily related to the physical characteristics of the different sites (specially the stream hydrology, but also the light regime).