Functional diversity in a Mediterranean river: a new analysis of Carbon substrate utilization profiles through compositional methodology

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A common used technique in Ecology to study the metabolic fingerprint of microbial communities inhabiting soils, sediments and freshwater environments is the measurement of carbon substrate utilization profiles by using *Biolog Ecoplates*. This technique is based on the incubation of a microbial extract from an environmental sample in a 96-well plate which contains 31 different carbon substrates (in triplicate). Plates are incubated for 3-10 days and absorbance is measured every 24 hours until reaching saturated absorbance values. Increasing absorbance values indicate the potential capacity of the community to degrade each specific carbon substrate. Taking into account all the absorbance values measured along the incubation period at each well is possible to configure the metabolic fingerprint of the microbial community that was incubated. Through this information, functional differences between samples/study factors could be detected.

However, *Biolog Ecoplates* technique has some drawbacks related to the large amount of data generated and the fact of being an incubation technique with its own dynamics. Usually data are analysed by selecting a single incubation time or a single average well colour development (AWCD) and performing a classical multivariate analysis (i.e., MDS or PCA) in order to analyse similarities between samples. This cross-sectional study is not completely informative because the information may change depending on the incubation time, so the results of the analysis can be different. The primary aim consists of using a method to analyse the information generated by the 31 substrates during all the incubation times. Moreover, environmental studies typically include additional factors such as the season, the location and the depth where the samples were collected. Because the metabolic fingerprint of the microbial community is in essence relative information, an analysis through compositional methodology will provide new insight about functional diversity. Using this methodology we will integrate the microbial community dynamics of substrate decomposition during all the incubation times.

In this work we present the results obtained in the preliminary analyses that include: zero pre-processing, basic descriptive analyses, repeated measures MANOVA tests, and canonical variates analysis. The results show that incorporating all the data from *Biolog Ecoplates* by CoDa analysis we are able to distinguish microbial metabolic fingerprint from fluvial sediment communities between different seasons in study points presenting different water regimes. Future developments embracing compositional 3-way analysis and functional analyses are explored as well as an extension to the measures of functional diversity.