I Specialized Conference on Ecology, Management and River Restoration: Practices and Experiences. 2015

Lisbon University/FLUVIO Programme – Federal University of Bahia/MAASA

Salvador, Brazil, 27-28 July 2015

Assessing the effect of multiple-stressors on stream biofilms - responses to flow variability and organic loads

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Abstract

This study will focus on the responses of key component of stream ecosystems (biofilm: algae, fungi and bacteria; and organic matter decomposition) to the simultaneous effect of hydrological disturbances (flow velocity reduction) and organic load pressures (provided by different oxygen concentrations). Microbiota responses will be determined at a mesocosm scale in terms of core functions (e.g. enzymatic activity) and abundance/composition changes.

Keywords: Multiple-pressures, stream biofilms, water scarcity, oxygen depletion, microbiota composition

Introduction, scope and main objectives

Aquatic ecosystems are susceptible to multiple stressors, including climate change, organic and inorganic pollutants, physical habitat alterations, water abstraction, pathogens and invasive species. (Arce *et al*. 2014, Fausch *et al*. 2010). These pressures interact together generating changes on water availability and also on ecological and chemical quality (Ormerod *et al*. 2010). Such effects on biodiversity, ecological processes, and ultimately in ecosystem functioning, are often complex and difficult to predict because of synergies, feedback and cross-amplification among stressors and need to be disentangled. In particular, Mediterranean rivers are characterized by frequent hydrological disturbances including reduced connectivity often translated in standing water pools and dewatering of river beds due to prolonged summer droughts, followed by flood events during winter and spring (Gasith and Resh 1999). Additionally, most streams within Mediterranean climate are densely populated and are prone to water contamination from diffuse and point sources, which include inputs of municipal, industrial and agricultural sectors. In addition, slow-flowing and standing waters during droughts may promote higher water temperature, reduced dilution and increased residence time which might be particularly relevant for ecological impacts due to organic contamination and inherent deoxygenation.

Biofilms are assemblages of bacteria, algae, fungi, and other microbial organisms and detritus embedded in a polymeric matrix in aquatic environments (Wetzel 1983). Biofilms are one of main communities in lotic systems to interact with organic matter, nutrients and other chemical compounds (e.g. Paule *et al*. 2009, Rodrigues et al. 2010, Martins *et al*. 2012). Therefore, several studies have been used and supported biofilms as a reliable tool for bioassessment in freshwater systems (e.g. Ancion et al.

2010, Battin *et al*. 2003, Lear and Lewis 2009). In fact, biofilms may be used to detect the early effects these simultaneous pressures might have on the ecosystem, through measuring changes in their function (e.g. decay in enzymatic activity; decline in litter decomposition) and shifts in community structure and composition (Liess *et al*. 2009, Romani *et al*. 2012, Sabater *et al*. 2007). Is known that biofilm cell detachment from substrate increase with fluid velocity (Trulear and Charackis 1982) and external fluid velocity affects internal mass transfer (Brito and Melo 1999) and accordingly river flow significantly affect biofilm development, yielding higher biomass under slower flows (Battin *et al*. 2003). Biofilm microbial composition is influenced by different concentrations of humic substances (main constituent of organic matter and DOC in streams) in addition of flow velocities (Rodrigues *et al*. 2010). However, limited studies had addressed the effect of drought in biofilm community structure and composition. Romani *et al*. (2012) found that the drying process induced a decrease of biofilm functions (e.g. bacterial production and chlorophyll biomass), however both autotrophs and heterotrophs assemblages recovered quickly with the rewetting progress highlighting the biofilm resilience and ability for withstand periods of desiccation. Nevertheless, stream biofilm heterotrophs have shown to be more resistant to water depletion than autotrophic community towards the dewatering of streambed (Timoner *et al*. 2012). Recently, Corcoll and co-workers (2015), found that flow intermittency modulated the effects of chemicals on biofilm algae and bacteria: while algae revealed cumulative effects between the two stressors, bacteria indicated a co-tolerance effect. Reduced flow also enhanced the negative impact of sediment addition on aquatic biota (Matthaei *et al*. 2010).

Microbiota also are responsible for major riverine processes such as decomposition of organic matter, which releases nutrients into the water and could enhance biofilm growth. The decomposition of leaf litter entering streams is influenced by physical abrasion but is mainly a biological process involving macroinvertebrates detritivores, fungi and bacteria (Gessner *et al*. 1999). In fact, aquatic heterotrophic microorganisms (fungi and bacteria) are crucial for the mineralization of leaf litter and also render it more palatable for leaf shredding by invertebrates (Barlocher 1985, Graça 2001, Suberkropp 1992). Consequently, fungi and bacteria are important intermediaries in energy flow in lotic ecosystems (Suberkropp and Klug 1976).

Riverine biofilm composition and functions are expected to change under the effect of multiple stressors, in particular, organic load and flow velocity. Therefore, the aim of the doctoral study is to breakthrough on the knowledge regarding the response of stream biofilm to multiple stressors in Mediterranean rivers and to identify novel indicators of ecological status based on the use of biofilms. In that regard, the research objective will evaluate riverine biofilms response to flow velocity and organic load combined pressures, namely on the following aspects:

1. Composition, diversity and function of heterotrophic and autotrophic biofilm communities in terms of fungi and algae populations.
2. Composition and diversity of biofilm bacterial functional groups.
3. Comparative responses in terms of composition and diversity of algae, fungi and bacteria within the biofilm.
4. Riverine functional processes indicator, such as leaf litter decomposition.

Methodology/approach

This study will be carry out at a mesocosm scale. A mesocosm system (Fig. 1) will be installed at ISA (School of Agronomy, University of Lisbon) field facilities, composed by 6 independent stainless-steel-lined channels (width 0.4 m X length 4 m X depth 0.2 m). Water will be collected from a nearby natural source to a central container (3000L), and then redistributed to smaller

deposits in each artificial channel. Each channel will be equipped with a pump to allow for recirculation and maintenance of same conditions independently of the source container.

In order to assess the impact of multiple stressors in stream microbial biofilm community, the mesocosm will be used to test the microorganism's responses to the combination of low flow velocity and different organic load contamination.

Experiments will test the microbial communities' structural and functional responses towards two hydrological scenarios: a) High flow velocity (Hf; ≈ 1 m/s); and b) No flow velocity (Nf; ≈ 0 m/s) mimicking water scarcity with disconnected pools during summer. These hydrological treatments will be combined with two different levels of dissolved oxygen in the water (Low $[O_2]$ ≈ 10% and High $[O_2]$ ≈ 90%) resulting in 4 different treatments (2 flow velocities X 2 $[O_2]$ = Nf10; Hf10; Nf90; Hf90).

Oxygen low concentration in the water will be induced by adding a known oxygen scavenger agent (sodium sulphite) to the water in the necessary amount to maintain the planned oxygen concentration.

Biofilm response

Biofilm will be obtained from unglazed ceramic tiles previously left to colonize for 4 weeks in the same natural water source supplying the mesocosm system. After colonization, biofilm tiles will be placed in each artificial channel in ISA mesocosm and submitted to 4 treatments (Nf10; Hf10; Nf90; Hf90) during 14 days. Each treatment will have 3 replicates. Tiles will be collected and preserved for laboratory work throughout the experiment on days 0, 3, 9 and 14 to obtain a gradient response. Also water of all artificial channels will be collected at the same sampling dates to characterize the main physical and chemical parameters ([BOD], pH, dissolved oxygen, conductivity, and temperature). Water nutrient concentrations ($P\text{-}PO_4$; $N\text{-}NH_4$; $N\text{-}NO_3$; $N\text{-}NO_2$; SO_4^{-2}; Cl) will be determined at the beginning and at the end of the experiment.

All biofilm community will be characterized and analysed as following:

- Extracellular enzymatic activity of the enzymes phosphatase and glycosidase measured by means of fluorescent-linked substrates (methylumbelliferyl - MUF).
- Biofilm mass losses (measured in terms of ash free dry mass) will be determined by scraping a known area of a biofilm tile to a known volume of distilled water that posteriorly will be filter through a GF/C filter (previously weighted). The filters with the samples will be placed in an oven at 70 °C for 72 h, weighed, ashed at 550 °C for 4 h and reweighed to calculate AFDM.
- Algae will be characterized in terms of total biomass by determination of chlorophyll-a concentration contents and major groups' composition (i.e. diatoms, green algae and cyanobacteria) through pigment composition analysis. Also molecular analysis will be performed (PCR sequencing and DGGE)
- Fungi will be characterized in terms of biomass (ergosterol concentration contents), heterotrophic plate counts (CFU) and diversity by molecular analysis (DGGE and PCR sequencing).
- Bacteria will be characterized in terms of total heterotrophic plate counts (CFU) and diversity by molecular analysis (DGGE and PCR sequencing).

Decomposition rates response

Simultaneously, organic matter decomposition rates will be tested using leaf litter mesh bags with alder leaves.

Corse mesh bags (10 mm) with dry alder leaves will be previously colonize in stream for 3 weeks, and then placed in mesocosm and submitted to the different treatments for 14 days. Each treatment will have 3 replicates. Alder leaves will be collected for analyses on days 0, 9 and 14.

Decomposition rates will be determined in terms of AFDM, fungal biomass (ergosterol concentration), bacterial abundance (total counts), leaves C:N:P estoiquiometry (C, N and P total contents analysis) and diversity and composition of macroinvertebrate colonization.

Predictive Models construction on local responses of microbial communities to multiple-stressors

The main goal of this task, is to forecast microbial communities local responses to the studied stressors by creating a predictive model with the data obtained previously (see above Biofilm Response section). In order to modelling ecological interactions with stressors will be employed a simulation software (STELLA) to assess the identifiability and to estimate the values of model parameters (using measured data), and also estimate prediction uncertainty.

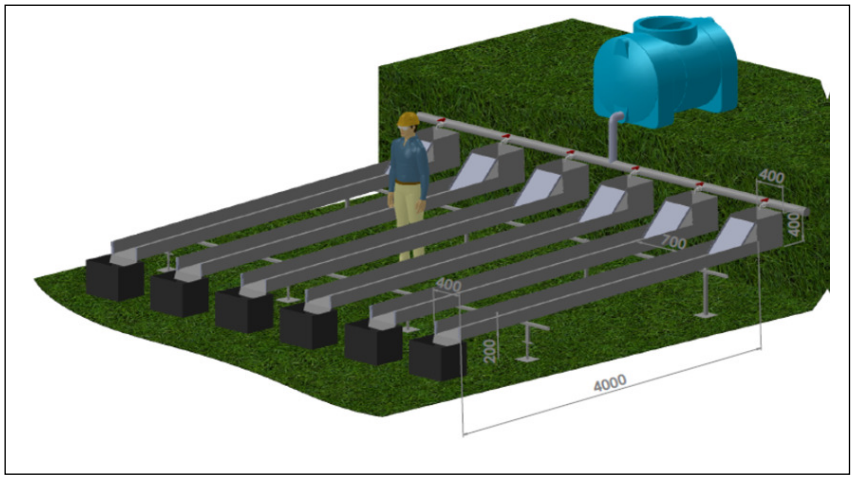

Fig. 1: Schematic construction drawing of ISA Mesocosm that will be use in the experiments

Acknowledgements

The fist author is part of the FLUVIO doctoral program supported by a grant from the Fundação para a Ciência e Tecnologia (SFRH/BD/52510/2014).
We acknowledge LAIST (Laboratório de Análises do Instituto Superior Técnico, ULisboa) and Ricardo Santos; WasserCluster Lunz (Biologische Station GmbH) and Elisabeth Bondar; Teresa Ferreira, Paulo Branco and José Maria Santos.

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