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## The effects of sediment depth and oxygen concentration on the use of organic matter: An experimental study using an infiltration sediment tank

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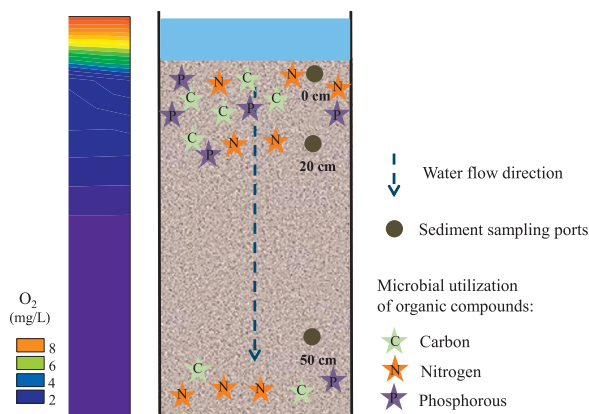
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### HIGHLIGHTS

- Microbial activity occurs at all depths of the sediment column.
- Organic C compounds are better used at surface while N compounds in depth.
- Anoxia inhibits phosphatase and peptidase activities.
- Microbial community becomes more specialized in time and depth.
- Oxic and anoxic metabolism coexists, showing specific metabolic fingerprints.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Water flowing through hyporheic river sediments or artificial recharge facilities promotes the development of microbial communities with sediment depth. We performed an 83-day mesocosm infiltration experiment, to study how microbial functions (e.g., extracellular enzyme activities and carbon substrate utilization) are affected by sediment depth (up to 50 cm) and different oxygen concentrations. Results indicated that surface sediment layers were mainly colonized by microorganisms capable of using a wide range of substrates (although they preferred to degrade carbon polymeric compounds, as indicated by the higher  $\beta$ -glucosidase activity). In contrast, at a depth of 50 cm, the microbial community became specialized in using fewer carbon substrates, showing decreased functional richness and diversity. At this depth, microorganisms picked nitrogenous compounds, including amino acids and carboxyl acids. After the 83-day experiment, the sediment at the bottom of the tank became anoxic, inhibiting phosphatase activity. Coexistence of aerobic and anaerobic communities, promoted by greater physicochemical heterogeneity, was also observed in deeper sediments. The presence of specific metabolic fingerprints under oxic and anoxic conditions indicated that the microbial community was adapted to use organic

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matter under different oxygen conditions. Overall the heterogeneity of oxygen concentrations with depth and in time would influence organic matter metabolism in the sediment tank.

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## 1. Introduction

The connection between surface water and groundwater and the processes occurring in this interface (i.e., the hyporheic sediment) are important for river ecosystem metabolism (Brunke and Gonser, 1997; Nogaro et al., 2013). The hyporheic zone promotes the exchange of water, nutrients, and biota between alluvial groundwater and stream water (Boulton et al., 1998). This exchange, in turn, influences stream water quality (Sobczak and Findlay, 2002). Microbial communities in sediments are principally composed of heterotrophic microorganisms including bacteria, fungi, and small metazoans which are attached to sand grains and assembled in a polymeric matrix (Pusch et al., 1998) that plays key roles in biogeochemical processes (Findlay et al., 1993; Mermillod-Blondin et al., 2005). Microbial communities are responsible for most of the metabolic activity in hyporheic sediments (Storey et al., 1999), including the degradation of organic matter and the reduction of electron acceptors (e.g., oxygen, nitrate and sulfate) (Ghiorse and Wilson, 1988; Hedin et al., 1998). These processes act as water purification processes, ultimately impacting the water quality of river and aquifer systems. Similarly, when water flows through sediments in the vadose zone, microbial activity enhances the quality of surface water as in slow infiltration bed or in managed artificial recharge facilities (Greskowiak et al., 2005) removing organic carbon content, nutrients and trace organic chemicals (Li et al., 2012; Regnery et al., 2015).

Decomposition of organic matter is one of the main metabolic roles of microorganisms in soils and sediments. Extracellular enzymes released by microbes promote organic carbon cycling, by transforming polymeric material into soluble monomers that can be assimilated by microbes. These actions constitute a limiting step in the entrance of organic matter to the food web (Allison et al., 2007; Romani et al., 2012). Although many studies have analyzed enzyme activities in surface sediments (Romani and Sabater, 2001), much less is known about how these activities change according to depth. For instance, in the upper 12 cm of river sediment, extracellular enzyme activities involved in the degradation of cellulose, hemicellulose, and organic phosphorus compounds decreased together with bacterial density (Romani et al., 1998). Changes in the utilization of organic matter at different sediment depths may be linked to microbial colonization. Indeed, microorganisms are found in largest quantities at the soil surface, and their abundance declines rapidly with increasing depth (Taylor et al., 2002). Microbially active zones are often limited to the top sediment layer (<60 cm) where bacterial biomass and exchange rates between the river and the hyporheic zone are the highest (Taylor et al., 2002). Bacteria in deeper sediments are more sensitive to physical and chemical changes compared to those in surface layers (Fierer et al., 2003) due to the relatively more stable conditions (Fischer et al., 2005). In deeper sediments, organic matter use may be further affected by physical and chemical changes in oxygen, pH, temperature and nutrient availability (Douterelo et al., 2011). Moreover, the sediment biofilm structure reduces the water infiltration capacity by pore clogging (e.g. Or et al., 2007), also decreasing the soil porosity, stream bed permeability, and thus the water exchange between river and vadose zone (Brunke and Gonser, 1997; Descloux et al., 2010).

Physicochemical conditions appear to be highly heterogeneous at different sediment depths (Storey et al., 1999), and this heterogeneity promotes the coexistence of aerobic and anaerobic microbial communities in sediments (Harvey et al., 1995; Storey et al., 1999). Previous publications showed vertical oxygen consumption in sediments (Glud et al., 2005; Revsbech et al., 1986), but they did not focus on how to link this

oxygen gradient to the decomposition of organic matter along the sediment's profile. This is in spite of oxygen and organic matter being known to play key roles in nutrient cycles (Hedin et al., 1998; Nogaro et al., 2013; Rubol et al., 2012). Low oxygen content and redox potential in deeper sediments may cause shifts in microbial metabolism. Indeed, decomposition of organic matter is more rapid and efficient in oxygenic conditions (Storey et al., 1999) and some extracellular enzymatic activities are inhibited in anoxic conditions (Goel et al., 1998).

The objective of this study was to analyze changes in microbial organic matter use at different sediment depths under continuous infiltration conditions. We hypothesized that microbial activity and biomass would be higher at the sediment surface and decline with depth. At that deeper layer gradients would be more pronounced at the end of the experiment consistent with a vertical oxygen gradient. Specifically, the experiment aims at: i) analyzing organic matter decomposition capabilities and microbial functional diversity of the community developed in depth as a result of a colonization sequence; and ii) investigating the vertical changes of organic matter use due to different oxygenic conditions.

To reach these objectives, a 1-meter sediment tank with continuous infiltration of synthetic water was used to monitor several physical and chemical parameters, including oxygen, temperature, conductivity, inorganic nutrients, dissolved organic carbon, and microbial metabolism. Activities of  $\beta$ -glucosidase, leucine-aminopeptidase and phosphatase were assessed to monitor the hydrolysis of organic compounds containing carbon, nitrogen, and phosphorus (Romani et al., 2012). Functional diversity and functional fingerprints of sediment microbial communities were analyzed on the community-level using Biolog Ecoplates (Salomo et al., 2009). A meso-scale was chosen to produce biogeochemical and microbial parameters under controlled interstitial flow conditions, similar to those experimental studies using sediment columns (Battin et al., 1999; Mermillod-Blondin et al., 2005) or a sediment tank (Weber and Legge, 2011).

## 2. Material and methods

### 2.1. Experimental design

An infiltration (flow-through) experiment was conducted in a vertical intermediate-scale tank reconstructed with a heterogeneous sediment porous media. The dimensions of the sediment tank were 1.20 m high  $\times$  0.45 m long  $\times$  0.15 m wide. The base of the tank was filled with a 15 cm layer of silicic sand (0.7 to 1.8 mm diameter, supplied by Triturados Barcelona, Inc.) covered with a permeable geo-synthetic fabric membrane to prevent soil flowing through. Sediments were collected from a managed aquifer recharge facility site located in the Llobregat River near Barcelona (UTM coordinates 418,446.63 N, 4,581,658.18 E). Dry sediments were sieved at 0.5 cm and packed in the tank by a repeating series of wetting and drying cycles, see Rubol et al. (2014) for details. The top 20 cm of the tank were left free of sediment to allow ponding. A concentrated synthetic solution of 10 L mixture of inorganic and organic compounds was prepared in a carboy. This concentrated solution was diluted with deionized water prior to its injection into the infiltration pond of the tank. The carboy solution was continuously mixed with a magnetic stirrer (AREX 230v/50 Hz, VELP Scientific) and supplied at the surface of the tank with no recirculation. The carboy was replaced every 4–7 days (depending on water consumption). The chemical composition of the mixture mimics the typical Llobregat River water reported by Fernández-Turiel et al. (2003) which is characterized by high

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