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Two-dimensional microfluidic nutrient patches for direct visualization of microbial resource cycling

Hanbang Zou[1], Pelle Ohlsson[2], and Edith Hammer[1]
[1]Department of Microbial Ecology, Lund University, Lund, Sweden
[2]Department of Biomedical Engineering, Lund University, Lund, Sweden

A better understanding of soil carbon sequestration is important as the rapid elevation of carbon emission has significantly impacted our climate. The soil carbon dynamic process is strongly associated with trophic interactions in the soil communities. However, direct investigation of microbes and trophic interactions at microscale has been a major challenge due to the opacity of soil and the exceedingly complex pore spaces that limit direct in situ observation of trophic interactions and their variations with soil structure. As such, we decide to use microfluidic technique to create a two dimensional heterogeneous porous microenvironment containing nutrient patches of different quantity and quality to mimic the complex soil pore network and its heterogenous distribution of resources. This allows us to directly visualize and investigate the ability of organisms to access spaces starting from an initial ecophysiological precondition to changes of spatial accessibility mediated by interactions with the microbial community.

Microfluidics is a multidisciplinary platform that integrates micro fabrication, physical chemistry analysis, automation and microscopy. It has been widely used in life science and chemistry as it allows precise liquid manipulation, rapid measurements, real-time visualization at microscale, which is especially of interest and benefit to microbial studies. We created a complex pore network mimicking different soil environments – earlier considered impossible to achieve experimentally. The microfluidic channel contains a random distribution of cylindrical pillars of different sizes so as to mimic the pore space variations found in real soil. The randomness in the design creates various spatial availability for microbes. The nutrient patches within the pore space are achieved via capillary force trapping or UV curing hydrogel. In the former method, the patches are created by trapping nutrients in the predesign pore space due to interfacial tension between air and nutrient liquid. However, displacement instability during air injection makes it hard to control the formation of the patches. The latter enables better and more precise patches size and location manipulation but hydrogel biocompatibility with various fungal species remains a major challenge and hydrogel curing requires special facilities. The microfluidic nutrient patches provide spatial heterogeneities of resource distribution, which allows us to have a better understanding of the influence of spatial accessibility on the microbial community. The chip has five different nutrient distribution levels, ranging from centralized – a single large nutrient patch- to increasingly dispersed nutrient distributions - sets of up to 49 loosely distributed nutrient patches of equal total volume. The experiments will be carried out using sterile cultures of fluorescent bacteria and

fungi, synthetic communities of combinations of these, or a whole soil community inoculum. We will quantify the consumption of organic matter from the different areas via fluorescent substrates, and measure the bio- and necromass produced. We hypothesise that denser distribution will increase the net decomposition of organic matter as a centralized nutrient location increases the interactions within the microbial communities and individuals present in that area, which induces higher competition stress in the different communities.