



Study of soil pore space influence on organic matter turnover using microfluidics

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Soils contain more organic carbon than the atmosphere and the global vegetation together. Emergent perspectives where stabilization of soil organic matter (SOM) is attributed to accessibility of microorganisms to a potential substrate have been proposed to explain carbon accumulation in soils. Studying the distribution of soil pore space is therefore necessary for understanding how such protection occurs in soils.

An approach to study the soil pore space is the use of model systems, such as controlled assembly of different soil materials or different types of transparent materials. A particularly promising system is the use of microengineered or microfluidic chips.

Microfluidics are defined as the manipulation of fluids within structures at micrometer scale. The advantages of using microfluidics are due to the unique characterization it provides of phenomena occurring in the nano-scale, and the systematical and controlled simulation of patterns found in soil pores to study the effects of soil physical characteristics on soil microbial communities.

In the present study we have developed a soil chip to study the effect that tortuosity and channel angles have on the growth and activity of soil bacteria. Tortuosity is, in our case, the ratio of the length of a channel between two points to the straight-line distance between those points. We hypothesized that high tortuosity channels will have a negative effect on bacterial growth while a positive effect would be produced by high angle values. Thus, channels with high tortuosity will have less bacteria inside compared to the less tortuous channels, and proteolytic activity will be lower. On the other hand, channels with high angle value would have a higher proteolytic activity than channels with lower angles.

The channel-chip was designed to simulate nine types of micro channels with different tortuosity and angle values. Bacterial growth and activity were measured using epifluorescence microscopy of a Green Fluorescent Protein tagged strain of *Pseudomonas putida* and of a fluorogenic BSA substrate quenched with Texas Red X (DQ Red BSA), respectively. DQ Red BSA is a fluorogenic substrate that becomes fluorescent when the primary structure of the BSA is cleaved. In this way we have managed to measure the rate of growth of bacteria inside channels of different tortuosity and angles and the proteolytic activity inside them. We measured preferential accumulation of bacterial colonies in response to the channel shapes that led to strong differences in clogging and thus the further colonization of the channels. Tortuosity and channel angles affected in a different way the bacterial growth and activity. Also, the results suggest that other parameters in the channels beside the ones analyzed might play an important role in bacterial colonization and activity.