MicroScapesX

Design and Systems Biology of Functional Microbial Landscapes

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Background

Microbial multispecies assemblies have crucial roles in both natural and engineered ecosystems. They form largely spontaneously, exhibit a highly dynamic nature, and may have both detrimental consequences (e.g., material destruction, disease) as well as provide large benefits (e.g., biofuel production, waste management, microbiome-related health). Astonishingly, the basic principles that promote and control microbial multispecies assemblies remain largely unknown and underexplored. This knowledge gap limits our capacity to engineer and manage multispecies assemblies to fulfill or restore functions far more complex than can be achieved by manipulating single species. The primary overarching objective of this project is thus to **study the biology of microbial multispecies assemblies at a systems level, and obtain quantitative and predictive models for their formation and behavior upon manipulation**.

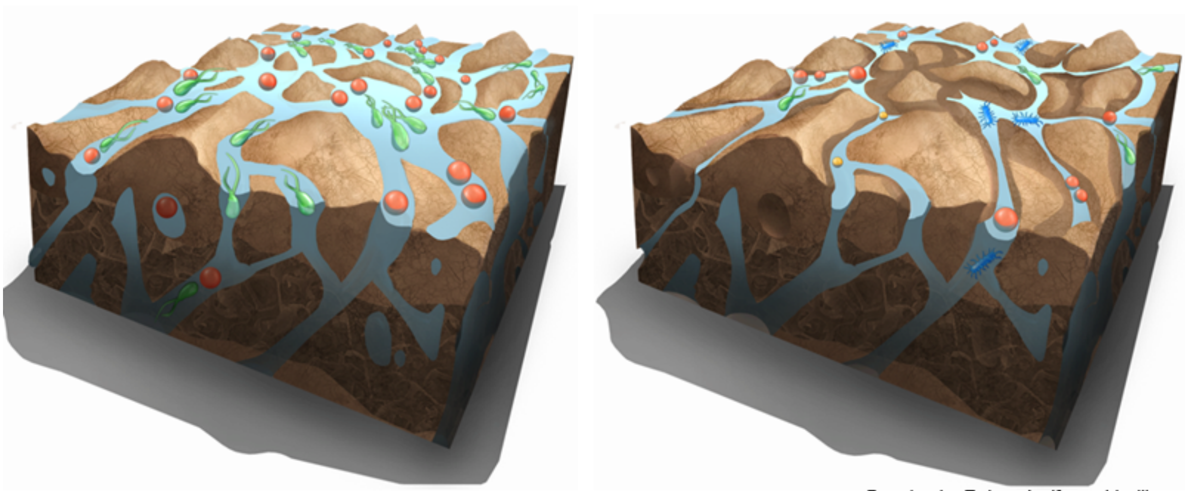
The key innovation proposed in this project is the systematic use of a **synthetic and systems biology approach** to **elucidate** and **model the basic principles** that govern and shape **assembly and functionalities** of microbial communities in a spatial context. This enabling knowledge is then essential for a second step: can we actually reproduce or manipulate the structure of microbial communities in a manner that prevents mal-functioning (e.g., disease) or helps to restore optimal functionality (e.g., pollution degradation or chemical production)?

Given the possibilities for experimental manipulation of microbial populations, and with recent conceptual advances in synthetic biology (i.e., understanding through assembly from building blocks), we expect that through the controlled design of microbial multispecies assemblies we can learn many of the basic rules that control and create functional microbial landscapes in unsaturated media such as soil. Therefore, our main goals are:

- Quantify the mechanisms giving rise to stable spatial patterns of microbial consortia and link these to ecological functions.
- Control of environmental conditions that promote desired configurations and select for target consortia members.

In the study of soil bacterial communities the hydration status of the environment is essential since the aqueous phase is indispensable to bacterial life and mediates the microbial interactions. The unsaturated conditions present in the soil lead to the fragmentation of the aqueous habitat which promotes the creation of ecological niches. This results in the unparalleled bacterial diversity found in soil.

Schematic representation of microbial diversity in soil as a function of hydration conditions



Drawing by Raluca Iosifescu-Vasiliu

Saturated conditions lead to a fully connected environment that can support a higher microbial concentration but less diversity

Unsaturated conditions lead to a fragmented environment that can support a lower bacterial concentration but a higher diversity

Funding and Project Partners

MicroScapesX is an initiative funded by SystemX (http://www.systemsx.ch/) that brings together five research groups from several institutes:

- Microbial Community Assembly Group, Department of Environmental Microbiology, Swiss Federal Institute of Technology (ETHZ and EAWAG)
- Soil and Terrestrial Environmental Physics, Institute of Terrestrial Ecosystems, Swiss Federal Institute of Technology (ETHZ)
- Laboratory of Environmental and Evolutionary Microbiology, Department of Fundamental Microbiology, Université de Lausanne (UNIL)
- Laboratory of Computational Systems Biotechnology, Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL)
- Centre romand des brûlés, Centre Hospitalier Universitaire Vaudois (CHUV), University Hospital of Lausanne

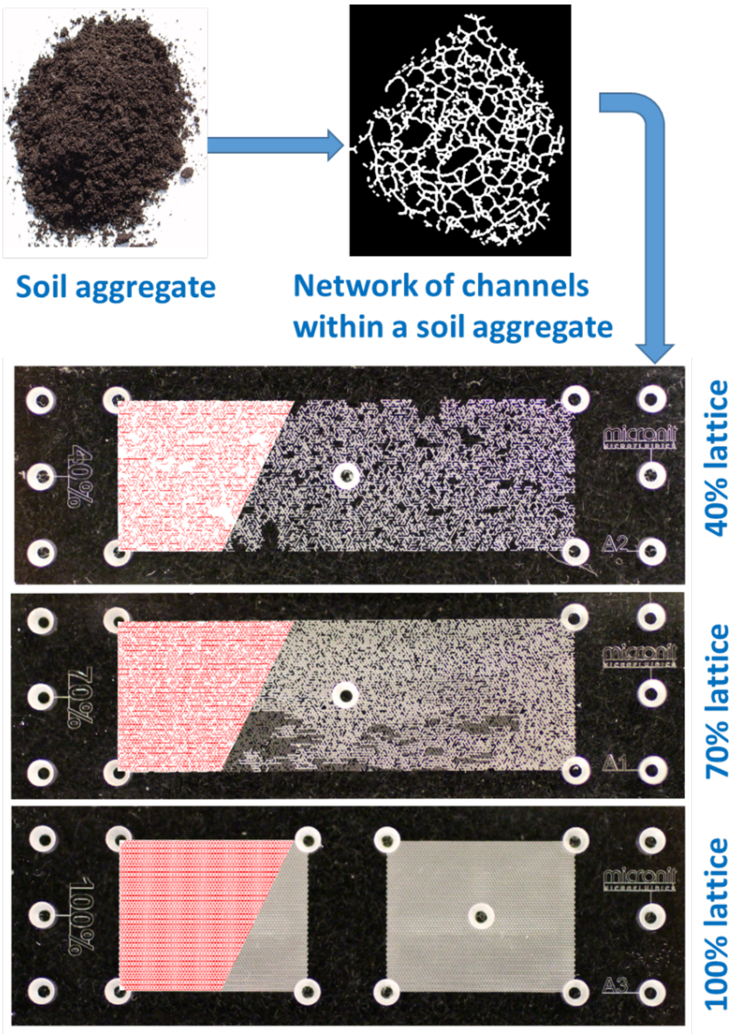
More details about MicroScapesX activities and partners can be found on the projects' webpage: http://www.microscapesx.ethz.ch/microscopesx-3.html

MicroScapesX Research Activities within STEP

We are using both experimental and theoretical models to better understand the spatial self-assembly of bacterial consortia as a consequence of cell-cell interactions and cell-environment interactions. For this purpose (1) an experimental model for cultivation and observation of multiple bacterial species in microfluidics devices has been developed in parallel with (2) a numerical time dependent model in which bacteria are represented individually based. These two approaches complement each other leading to a more profound understanding of the microbial processes occurring in soil.

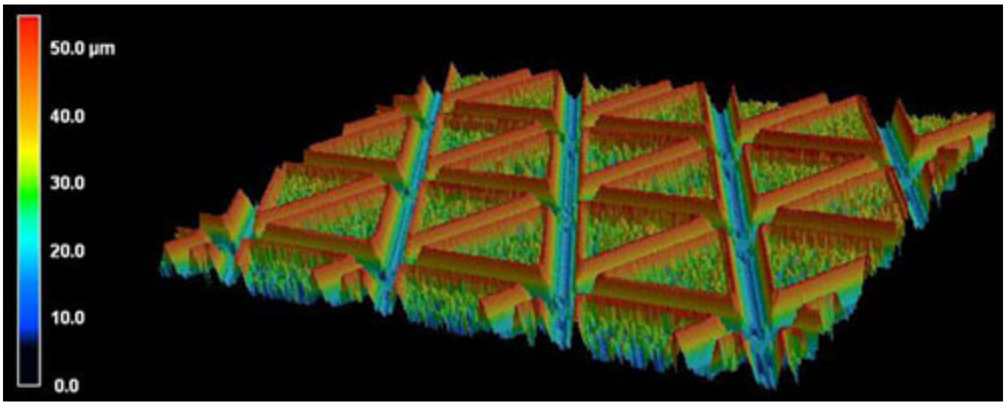
Experimental model

The complex soil structure has been abstracted to a network of channels with different degrees of connectivity (named 100%, 70%, and respectively 40% connectivity) to simulate soil heterogeneity and fragmentation at different values of matric potential. The *100% connectivity* corresponds to a homogenous situation when the soil is fully saturated and all the channels are ideally connected, while the *40%* and *70% connectivity* lattices correspond to heterogenous cases when fragmentation of the aquatic habitat occurs.



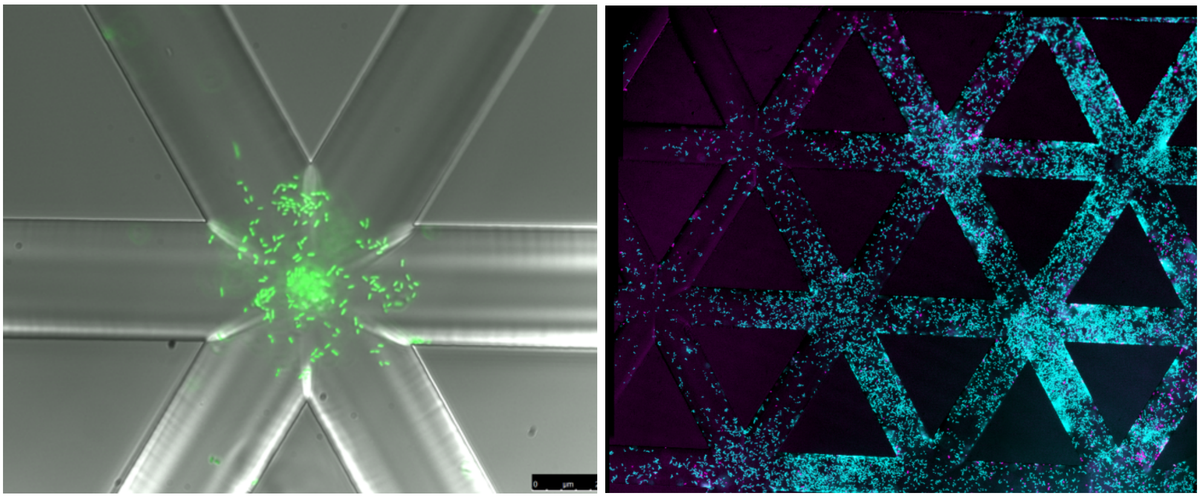
Glass etched lattices of microchannels

We etched these lattices in glass creating networks of microchannels with the channel dimensions of 200 µm x 40 µm x 15 µm (L x W x D).



3-d scan using laser microscopy of the 100% connectivity lattice

By cultivating bacterial consortia in such controlled micro-environments, both in saturated and unsaturated conditions, the cell level interactions can be studied and the basic principles that promote and control spatial organization and function of multispecies microbial communities can be researched.

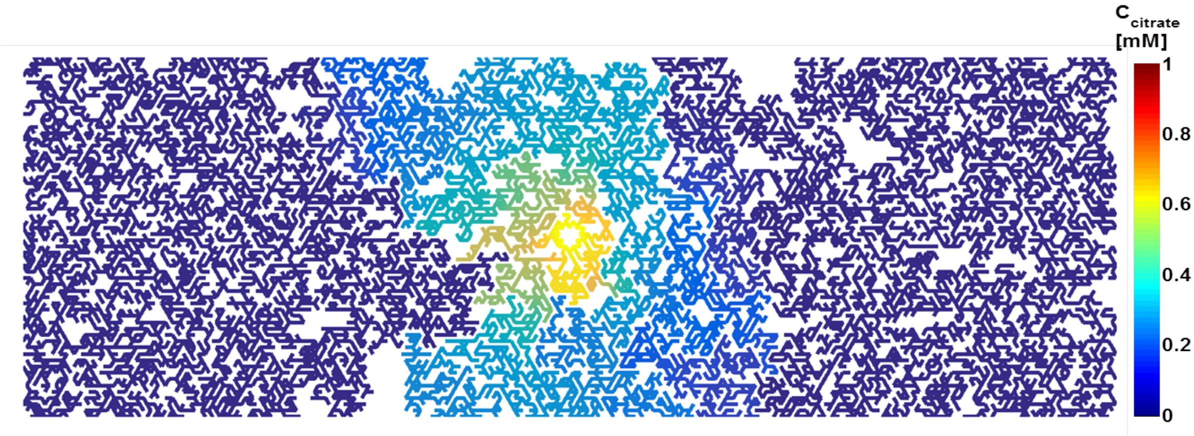


(Left) GFP tagged *P. putida* (green) cells forming a colony at the intersection of microchannels. (Right) Dual culture pf GFP tagged *P. putida* (cyan) and mCherry tagged *P. stutzeri* (magenta)

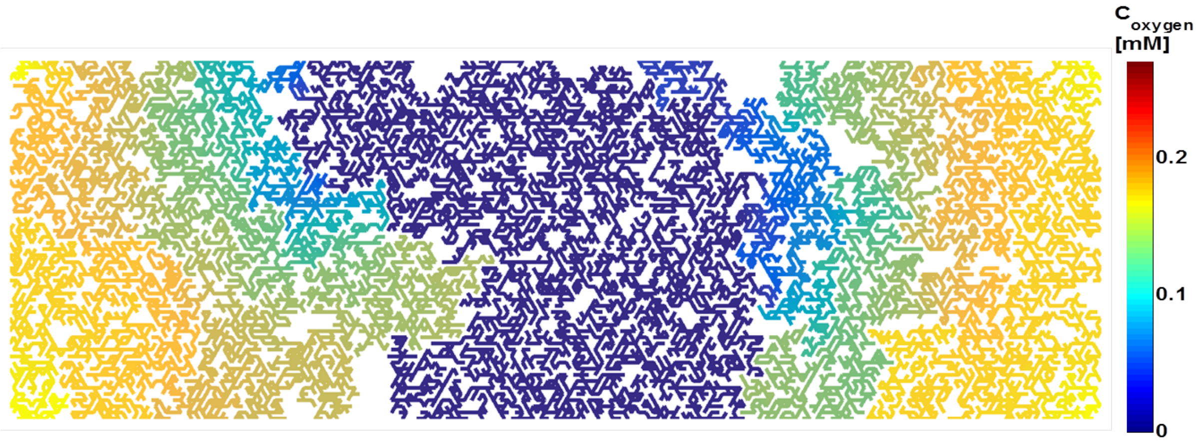
Numerical model

A one-dimensional (1-d) time dependent numerical model has been developed to (i) gain a better theoretical understanding of the processes leading to bacterial self-organization in unsaturated media, (ii) to assist with the interpretation of the experimental results and (iii) to guide future experiments. In exchange, the experimental model will be used to validate the mathematical one.

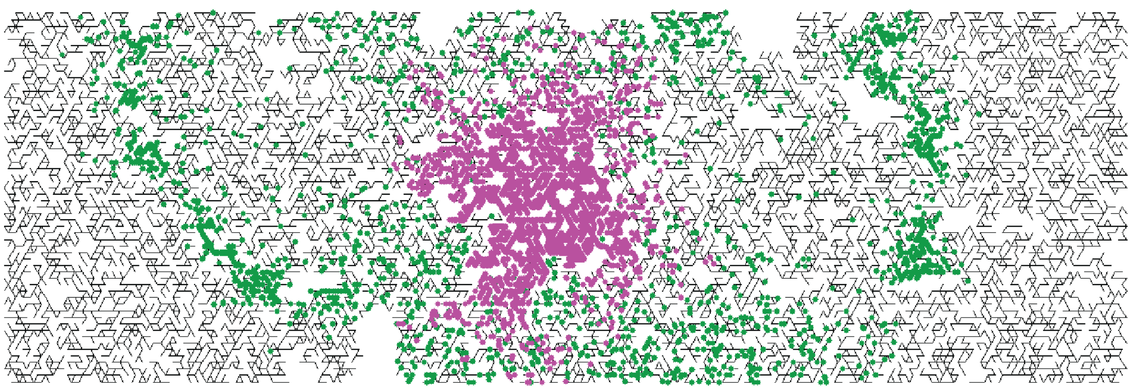
The numerical model integrates physical processes (mass transfer) with bacterial processes (motility) and metabolism (microbial growth based on consumption and production of solutes). The geometry of the computational domain is identical with the one etched on the microfluidics chips from the experimental model. Bacteria are represented as individual agents using the well-established individual based modelling approach pioneered by Kreft et al. in 1998 (Kreft J.U., Booth G., Wimpenny J., 1998, BacSim, a simulator for individual-based modelling of bacterial colony growth, Microbiology, 144, 3275-3287).



Calculated citrate concentration profile in a 70% connectivity lattice where the solute is being supplied from the middle of the lattice and the corners act as an infinite sink



Calculated oxygen concentration profile in a 70% connectivity lattice where the solute is being supplied from the corners of the lattice and the middle port acts as an infinite sink

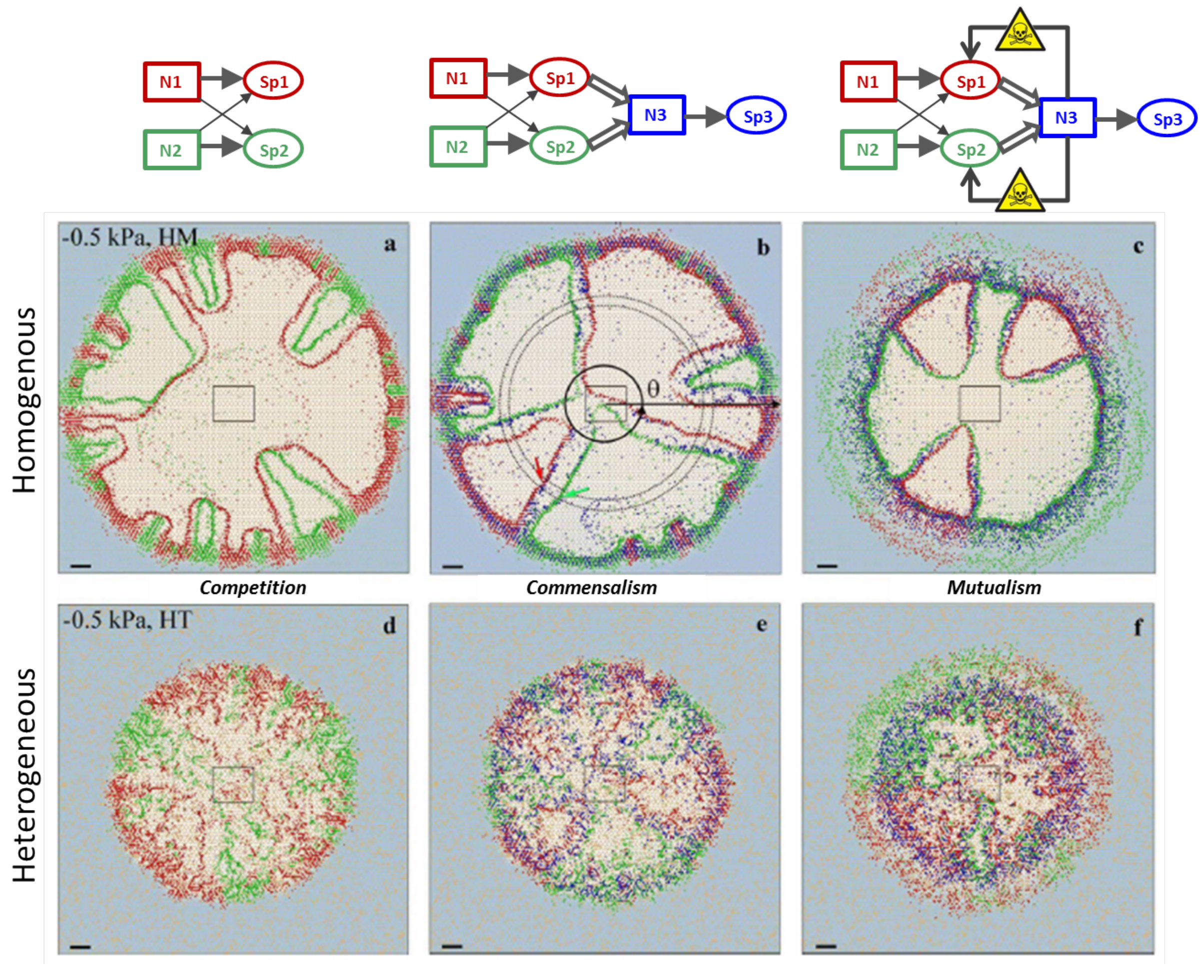


Result of a numerical simulation representing spatial organization of two generic bacterial species: an aerobic one (green) and a facultatively anaerobic species (purple) growing in a

70% connctivity lattice where the Carbon-Source is supplied from the middle and the oxygen from the corners of the lattice

Previous Numerical Studies

Previous studies carried in our group focused on microbial self-organization mediated by trophic interactions in both homogenous and heterogeneous unsaturated media.



(Wang and Or, 2014, Scientific Reports)

Simulated microbial consortia patterns on homogeneous (HM) and heterogeneous (HT) hydrated rough surfaces at -0.5 kPa at 50 h after inoculation for different consortium types marked by schematic trophic relations (panel 1, solid thick arrows represent large specific growth rates and thinner ones of smaller growth rates, empty arrows represent byproduction, and conditional arrows represent byproduct inhibition). Red, green and blue spots represent individual cells of sp1, sp2 and sp3, respectively. Light blue background marks normalized concentration of *N1* (white area means *N1* was depleted). Squares mark original inoculation positions. The scale bar is 1 mm.

MicroScapesX Members

Prof. Dani Or (http://www.step.ethz.ch/people/scientific-staff/prof-dani-or.html) – Principal Investigator

I am interested in modeling, measurement and interpretation of mass and energy flow and transformation in porous media; in situ characterization of soil hydraulic and mechanical properties. Developing new measurement methods. Current research interests: (1) evaporation from porous media (2) hydromechanical triggering of landslides (3) acoustic emissions for interfacial motions (4) diffusion and microbial activity in unsaturated soils

Dr. Robin Tecon (http://www.step.ethz.ch/people/scientific-staff/dr-robin-tecon.html) – PostDoctoral Scientist

I am an environmental microbiologist with a specific expertise in fluorescence microscopy and bioreporter technology. I am particularly interested in observing individual bacteria that colonize artificial or natural surfaces. I will contribute to the experimental dimension of SoilLife through the visualization and quantification of bacteria growing in a variety of set-ups (e.g. on porous surfaces, in microfluidics chambers). I am a PhD graduate from the University of Lausanne, Switzerland, and I have worked as a postdoc researcher in the Netherlands Institute of Ecology and at the University of California, Davis.

Dr. Olga Ilie (http://www.step.ethz.ch/people/scientific-staff/dr-olga-ilie.html) – PostDoctoral Scientist

My current research activities are focused on understanding the mechanisms that promote the incredible bacterial diversity found in soil, with an emphasis on: - bacterial interactions in different types of consortia (e.g., commensalism, mutualism, competition); - predicting the spatial organization of multispecies microbial assemblies and recognize the ecological functions associated to these "landscapes"; - study the role of EPS on short term (hours, days) and long term (months, years) bacterial survival strategies. I work at the intersection of several disciplines (mathematics, microbiology and soil physics) by building mathematical models that integrate mass transfer, microbial conversions, bacterial motility, nutrient availability and soil composition and hydrology.

MSc. Benedict Borer (http://www.step.ethz.ch/people/phd-students/benedict-borer.html) – PhD Student

My main interest is to study microbial interactions at the cell and community level using experimental and computational resources. I am currently using a microfluidic experimental setup to study bacterial interactions (competition, commensalism and mutualism) and developing a mathematical model (individual based model) of the system for predictive and analytical purposes.

MSc. Ali Nejad Ebrahimi (http://www.step.ethz.ch/people/phd-students/ali-ebrahimi.html – PhD Student

My main scientific interests are in the field of transport phenomena and biological processes in porous media, in general and the study of microbial life affected by pore scale heterogeneities and hydraulic conditions, in particular. My research contributions in ERC advanced project include the combination of computational and experimental aspects:

- Developing 3D functional pore network structure which contains effective properties of porous media

- Investigation of traveling time and age probability distribution of first bacteria which crosses the network in a single species population
- Investigation of competitive growth of microbial species and studying the effects of pore structure heterogeneities in the framework of 3D pore network
- Construction of 3D-printed pore network in order to study microbial life in the real structure
- Investigation of competitive growth of aerobic and anaerobic microbes in porous media

MSc. Minsu Kim (http://www.step.ethz.ch/people/phd-students/minsu-kim.html) – PhD Student

I am interested in modelling complex systems from the perspective of physics andapplications of physics to environmental science. In this research, I focus on microbial life in soil. Currently, I am developing a model to describe bacterial life on rough hydrate surface and to simplify highly dynamic and heterogeneous aqueous networks so that it enables us to upscale the system. The model is applied to two-dimensional lattices, which represents spatially-correlated-patchy surfaces mimicking soil grain surfaces preserving hydration physics and unsaturated transport properties. This model will be extended to study how trophic interactions influence microbial community and its spatial self-organisation of larger scales of systems in long-term prospects. The model system corresponds to in-situ bioremediation activities and potentially simple biogeochemical processes in soil. This research will shed light on the importance of interaction between physical and biological properties. This interaction is essential for developing quantitative understanding of complex ecological micro systems such as soil.

MSc. Hannah Kleyer (http://www.step.ethz.ch/people/phd-students/hannah-kleyer.html) – PhD Student

I am interested in soil bacteria since they play a central role in many ecosystem services such as formation of rhizosphere communities, nutrient transformation and global biogeochemical cycles. Molecular techniques based on rRNA genes analysis have uncovered the tremendous bacterial diversity in soil, but the mechanisms that control the assembly, functioning and maintenance of complex microbial communities remain largely unknown. My study aims to link dynamics of soil hydration conditions and related biophysical factors with variations in microbial composition and ecological functioning. To assist with definitive community level observations I designed a synthetic microbial community comprised of 10 well-characterized bacterial species spanning a wide range of soil phyla to be inoculated onto model porous surfaces mimicking soil habitats. The experimental system consists of sand layer placed on porous ceramic surface connected to nutrient reservoir. Fluctuations in hydration conditions are induced by changing the reference nutrient reservoir level to simulate wetting-drying cycles. The activity and composition of the bacterial community is studied using 16S fingerprinting and quantitative PCR.

MSc. Samuel Bickel (http://www.step.ethz.ch/people/phd-students/samuel-bickel.html) – PhD Student

Relevant publications

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- Wang G. and Or D., 2014, Trophic interactions induce spatial self-organization of microbial consortia on rough surfaces, Nature Scientific Reports, 4, 6757, DOI: 10.1038/srep06757
- Wang G., and D. Or, 2013, Hydration dynamics promote bacterial coexistence on rough surfaces, The ISME Journal, 7, 395-404, doi:10.1038/ismej.2012.115
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- Dechesne, A., G. Wang, G. Gülez, D. Or and B. F. Smets, 2010, Hydration controlled bacterial motility and dispersal on surfaces, The Proceedings of the National Academy of Sciences USA, 107, (32), 14369-14372, doi:10.1073/pnas.1008392107
- Wang, G., and D. Or, 2010, Aqueous films limit bacterial cell motility and colony expansion on partially saturated rough surfaces, Environmental Microbiology, 12, (5), 1363-1373, DOI: 10.1111/j.1462-2920.2010.02180.x
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- Long, T., and D. Or, 2007, Microbial growth on partially saturated rough surfaces: Simulations in idealized roughness networks, Water Resour. Res., 43, W02409