Meeting the challenge of applying single-cell analyses to terrestrial systems

Understanding the active microbial participants in key processes within the terrestrial nutrient cycles and the factors that govern these activities is an on-going challenge. In my group we meet this challenge by taking a multidisciplinary approach that combines process level measurements, sequencing-based analyses and single-cell activity measurements. This combined approach is applied to different terrestrial systems, including arid ecosystems, temperate soils and plant-microbe interactions. In many projects, single-cell analysis techniques such as high-resolution secondary ion mass spectrometry (NanoSIMS) and Raman microscopy are being employed to elucidate the in situ function of microorganisms. However, the application of these techniques to terrestrial samples is not straightforward, since in soil microbial cells are dispersed in a large background of particles and important structural information needs to be considered. As such, the development and optimization of single-cell analysis methods for the application on terrestrial samples is a constant focus in my group. One example where structural information is of great importance is the association of microorganisms with plant roots, and performing single-cell measurements while maintaining this spatial-scale information is a major goal. To that end, we are exploring the interactions of paddy soil microorganisms and rice roots, with particular emphasis on N₂-fixing (diazotrophic) microorganisms. In addition to the factors influencing the rice plant microbiota assembly, we aim to elucidate the N₂-fixation activity of plant-associated diazotrophs. For this purpose, we are currently developing the combination of cell-identification via gold-FISH followed by NanoSIMS investigations of root surfaces. Such analysis would allow one to identify and investigate active diazotrophs associated with plants and could also help to answer the question whether these microorganisms represent a source of nitrogen for the plant.

New microscale genomic and functional approaches for microbes

The Blainey lab develops ways to access new information about biological systems and alleviate bottlenecks in data collection. I will present examples in three activity areas: Integrated sample preparation for highly deployable genomics. Readout by next-generation sequencing (NGS) no longer limits throughput for many project types, but deployment for many applications demands low input quantities, streamlined workflows, and/or high throughput. We developed a family of lab-on-a-chip microfluidic systems to realize major advances in real-world throughput, cost, and sensitivity by integrating entire sample preparation workflows from crude biological samples for whole genome shotgun sequencing and a demonstration project with thousands of clinical isolates. High quality single cell genome sequencing. Microfluidics and whole-genome amplification are enabling single-cell genomics. At the same time, these technologies limit single-cell genomic studies by imposing cost and complexity barriers. Here I will present a method that requires no microfluidics or specialized equipment for direct single-cell genome amplification. Droplet based microbial culture with combination capacity. Droplet microfluidics methods are dramatically increasing the throughput of single-cell genomics assays. However, droplet methods have not yet impacted quantitative ecology/physiology studies due to the challenges of manipulating droplets, acquiring longitudinal data from droplets, and platform chemical compatibility. I will describe a new system for processing and tracking tens of thousands of droplets in parallel that prevents crosstalk of small hydrophobic solutes and allows time-series measurements.