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THE INCREDIBLE DIVERSITY OF MICROBIAL ECOLOGY RESEARCH

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Abstract book

INVITED SPEAKERS

Unraveling Complex Interactions in Microbial Communities

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Microorganisms engage in complex interactions with other organisms and their environment. Recent studies have shown that these interactions can be multifaceted, forming intertwined networks. Unraveling these networks requires a multidisciplinary effort, involving expertise in computational methods and applying advanced experimental tools. Lichens, symbiotic associations of photosynthetic algae or cyanobacteria and heterotrophic fungi, are pervasive throughout the environment. How interactions of microbes contribute to resilience and affects fitness of lichens is not fully understood. We integrated genome-scale modeling with metatranscriptomics, metabolomics, and phenotyping to reveal condition-dependent secretion and cross-feeding of metabolites in synthetic lichens. We discovered that interaction among lichen members is highly dynamic and is driven by the availability of organic and inorganic nutrients. Nutrient concentrations impacted lichen stability and shifted members from collaboration to competition. The fitness in these synthetic lichens was highly dependent on the genotype of its members and streamlined genomes, detrimental to growth of microbes in monoculture, improved overall growth of the lichen. Our mechanistic framework provides new insights into lichen physiology and the response of these ubiquitous microbial associations.

Why diversity matters: Symbiont heterogeneity provides multiple benefits to deep-sea mussels from hydrothermal vents

Nicole Dubilier

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Symbioses between chemosynthetic bacteria and marine invertebrates were first discovered at hydrothermal vents in the deep sea but are now known to occur in a wide range of habitats including coral reef sediments, seagrass beds, cold seeps and sunken whale carcasses. In these nutritional associations, the bacterial symbionts use chemical energy sources such as hydrogen sulfide to fix CO₂ into organic compounds and feed their hosts. Chemosynthetic symbioses have evolved multiple times in convergent evolution from numerous bacterial lineages, and occur in at least nine protist and animal groups such as ciliates, flatworms, mussels, clams, snails, annelids, and nematodes.

Similar to Darwin's finches, whose beaks have evolved different shapes and forms as an adaptation to different food sources, the symbionts of hosts from chemosynthetic environments have acquired a wide and flexible repertoire of assimilation pathways in adaptation to the energy and carbon sources available in their environment. Intriguingly, this flexibility appears to have been gained through horizontal gene transfer. In my talk, I will describe how our meta'omic' analyses of symbionts from deep-sea, hydrothermal vent mussels have revealed that horizontal gene transfer and symbiont diversity play a key role in the ecology and evolution of these host-microbe associations.

One carbon metabolism of sulfate reducing bacteria

Alfons Stams

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One carbon substrates, such as formate, carbon monoxide and methanol are excellent energy and carbon substrates for microbial growth of aerobic and anaerobic bacteria. Sulfate-reducing bacteria that grow with one carbon substrates may require an additional carbon substrate, such as acetate. This is e.g. the case for several Gram-negative *Desulfovibrio* strains. We have studied the methanol and carbon monoxide metabolism of Gram-positive sulfate-reducers belonging to *Desulfotomaculum* genus. *Desulfotomaculum* is broad genus with members that are distinct in physiology and phylogeny. We aimed to get insight into the ecophysiology of *Desulfotomaculum* species, especially those that are important in biotechnological processes. Our general approach is to perform genome-based physiological experiments to get insight into the metabolic properties. A key technique is differential proteomics. By analysis of the proteome of cells grown under different conditions, insight is obtained about the pathways involved in the conversion of a certain substrate and in the regulation of the metabolism.

The thermophilic *D. kuznetsovii* possesses two pathways for growth on methanol, one pathway that involves methyltransferases as is common for other anaerobes, such as acetogens and methanogens, and one pathway involving alcohol and aldehyde dehydrogenases (Sousa et al, 2018, Nat Comm 9,239). Genome analysis of other methanol-degrading *Desulfotomaculum* species indicated that some possess only the latter pathway for growth with methanol. Carbon monoxide can be used for growth of e.g. *D. nigrificans* and *D. australicum*, in the presence as well as in the absence of sulfate. In the absence of sulfate, these bacteria convert carbon monoxide to H_2 and CO_2 , and show a similar metabolism as described for *Carboxydothermus hydrogenoformans*. The ability to produce hydrogen from one carbon substrates in the absence of sulfate allows syntrophic growth of sulfate reducers with hydrogenotrophic methanogens.

Ocean microbial community composition and gene expression at global scale

Shinichi, Sunagawa

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Marine microbes cope with a variety of environmental gradients that impact their global distribution and transcriptomic activity. Previous studies have shown that the structure of epipelagic microbial communities in low and mid latitudes are largely governed by seawater temperature. Given that the rate and impact of temperature change is particularly high in polar regions, a better description of microbial community structure in these regions and an understanding of microbial responses to temperature change in general are needed. Here we analyse an extended dataset of 367 epipelagic and mesopelagic metagenomes and metatranscriptomes from 126 sites collected during the Tara Oceans Expedition (2009-2013). The collection of samples now covers a latitudinal range of 143° across main oceanic regions including the Arctic Ocean. On both hemispheres, we observe a strong separation of the polar oceans at around 60°, which is congruent in taxonomic and gene functional composition, as well as community gene expression. An integrated analysis of metagenomic and metatranscriptomic data, using a newly established ocean microbial reference gene catalog of 47 M genes, reveals that the relative contribution of community composition changes and acclimatization differs across the global ocean as a function of temperature and/or latitude. The results are expected to help us differentiating responses of marine microbes to the impact of global climate change.

Evolution of Streptomyces - how to cope with life in a complex environment

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Streptomyces species are prolific producers of specialised metabolites, such as antibiotics, antifungals and metal chelators which have adaptive functions in nature yet have also found utility in human medicine. Whilst the biosynthesis of these specialised metabolites is directed by dedicated biosynthetic gene clusters, little attention has been focussed on how these organisms have evolved robustness into their genomes to facilitate metabolic plasticity to provide chemical precursors for biosynthesis. Here we show that specific expansions of gene families in central carbon metabolism have evolved and become fixed in Streptomyces bacteria to enable metabolic plasticity and robustness that maintain cell functionality whilst costly specialised metabolites are produced. These expanded gene families, in addition to being a metabolic adaptation, make excellent targets for metabolic engineering of industrial specialised metabolite producing bacteria.

Shapes, composition and preservation of photosynthetic microbial communities on the early Earth

Tanja Bosak

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Photosynthetic microbial communities that colonized shallow water environments before and during the rise of atmospheric oxygen left a record preserved in minerals, isotopes, microscopic textures and macroscopic shapes of sedimentary rocks. My laboratory conducts experimental work to interpret this record and understand the parallel evolution of environmental chemistry and microbial communities through time. These experiments explore microbial aggregation and fossilization processes in biological and chemical systems relevant for the past environments. This work helps constrain factors that preserved fine microbial textures and structures before the rise of atmospheric oxygen in dolomite, and contributed to the preservation of some putative and diagnostic cyanobacterial fossils by silicification. Information from experiments, modern cyanobacterial genomes and the very long fossil record of cyanobacteria is combined and used to calibrate molecular clock models and explore the origin and evolution of oxygenic photosynthesis in deep time.

Ocean Sampling Day, an Example for Science 2.0?

Frank Oliver Glöckner and Antonio Fernandez-Guerra for the OSD Consortium

Max Planck Institute for Marine Microbiology & Jacobs University Bremem, Bremem, Germany

The Ocean Sampling Day (OSD) was initiated by the EU "Ocean of Tomorrow" project Micro B3 (Marine Microbial Biodiversity, Bioinformatics, Biotechnology) to obtain a global snapshot of marine microbial biodiversity and function. OSDs are simultaneous, collaborative, global mega-sequencing campaigns to analyze marine microbial community composition and functional traits on a single day. On June 21st 2014, 2015 and 2016 scientists from around the world collected more than 350 ribosomal DNA (rDNA) amplicon datasets and metagenomes plus a rich set of environmental metadata. Standardized procedures, including a centralized hub for laboratory work and data processing, assured a high level of consistency and data interoperability. Since 2015 OSD was accompanied by the Citizen Science campaign MyOSD to enhance the marine microbial community snapshot resolution of the OSD and to increase environmental awareness of the general public.

OSD was an experiment, not only by its research tasks, but also by its innovative character in activating and mobilising marine researchers and citizens alike to form a virtual research community that combines many brains, questions and approaches. OSD has shown that the full potential of recent technological advances can only unfold by moving forward towards an immediate and free exchange of data, technology and expertise to engage many brains right from the start.

The talk will provide an overview about OSD including its first scientific results. It will stress the importance of community driven science and proper research data management for integrative research following the FAIR (Findable, Accessible, Interoperable, Reusable) data principles.

Reference: Kopf A et al. (2015) The ocean sampling day consortium. GigaScience 4:27

Mining zebrafish microbiota reveal broad-spectrum individual and community-level resistance to infection by fish pathogens

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The different functions of the microbiota and its contribution to health are currently under intense scrutiny. However, the protective role of commensal communities against infections is still poorly understood. We developed an experimental model to study the protection provided against fish pathogens by the natural bacterial microbiota of young zebrafish reared in a conventional, germ-free or gnotobiotic conditions. By combining genomics, immunology and microbial ecology, we revealed some of the determinants of infection and colonization resistance. This direct, medium throughput in vivo experimental approach could contribute to inspire novel strategies to use microbiota-based protection towards various pathogens in situations relevant to aquaculture and beyond.

Persistent airway infections – genomic and phenomic dynamics

Søren Molin

The Novo Nordisk Foundation Center for Biosustainability Technical University of Denmark

From a perspective of eco-physiology and evolutionary biology we have studied persistent bacterial airway infections in patients suffering from the genetic disorder cystic fibrosis (CF). Focusing on one of the major pathogens in these infections – Pseudomonas aeruginosa – the adaptive processes developing in the patient airways after migration from the environment to the lungs have been mapped. The presentation will summarize our current understanding of this 'niche invasion' scenario based on a number of investigation scenarios: Whole genome sequencing, in situ meta-transcriptomics, exometabolomics and multi-factor phenomics. Based on fitness determinations performed under in vivo simulating conditions examples of 'large impacts from small changes' will be presented. Translation of these findings and conclusions into medical applications for novel diagnostic and therapeutic strategies is an important focus point.

ORAL PRESENTATIONS

O01 Microbial Ecology of anaerobic phototrophic sulfur bacteria in the meromictic Lake Cadagno

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¹ University of Applied sciences of Southern Switzerland (SUPSI), Department of Environment, Constructions and Design (DACD), Laboratory of applied Microbiology (LMA), Bellinzona, Switzerland. ²: University of Geneva, Microbiology Unit, Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland.

Lake Cadagno (Switzerland) is an alpine meromictic lake that harbors diverse microbial communities. This lake is characterized by high influx of sulfate in the monimolimnion which generates a steep chemocline around 12 m depth. Within this zone is present a dense population of phototrophic purple sulfur bacteria (PSB) of the genera *Chromatium, Lamprocystis, Thiodictyon, Thiocystis,* and of green sulfur bacteria (GSB) of the genera *Chlorobium*. Microbial community dynamics in the chemocline are likely to be relevant – although understudied – since *C. okenii* in the 2017 represents not more than 10 % of the total microbial community in the chemocline of Lake Cadagno. Other anoxygenic phototrophic sulfur bacteria co-occur at the depth in which bioconvection has been observed, especially GSB from the genus *Chlorobium* (contributing about 50 % to the microbial community) and small-celled PSB such "*T. syntrophicum*", *Lamprocystis purpurea* and *Thiocystis spp*. each contributing about 5 %.

Despite its small volume compared to the volume of the lake, up to half of the total CO₂ assimilation in Lake Cadagno occurs in the chemocline. Earlier studies suggest that assimilation in the light was dominated by motile large celled PSB *Chromatium okenii*, whereas in the dark it was dominated by small celled PSB *"Thiodictyon. syntrophicum"*. The latter were, however, also strong CO₂ assimilators in the light.

A recent study, using data from meromictic Lake Cadagno, suggests that the motile PSB *C. okenii* is able to produce an active bio-mixing process, also called bioconvection. This study has shown that the concerted swimming behavior of dense microbial populations may be sufficient to mix stratified fluids in real ecosystems, e.g. meromictic lakes. Moreover, complete genomes of small-celled non-motile PSBs ("*T. syntrophicum*" Cad16^T and *Lamprocystis* sp. CadA31) compared to that of a *C. okenii*, revealed that genes involved in taxis and motility are highly over-represented in the genome of the later, suggesting the ecological importance of these genes for *C. okenii*. However, the details on the environmental drivers of microbial derived bioconvection and the ecological and biological consequences in meromictic lakes remain unknown.

In next years, we will provide understanding of the environmental conditions driving bioconvection and of the ecophysiological impact of bioconvection on microbial communities and on the ecosystem in their natural environment. For this goal, we chose the chemocline of Lake Cadagno for which a wealth of multidisciplinary background data and research experience exists. Our primary hypothesis is that both biotic and abiotic drivers are relevant for *C. okenii* to induce bioconvection and to measurably expand its competitive advantage over other microbial taxa with similar environmental requirements.

O02 Small-scale variability study of Freshwater Bacterioplankton

Natalia Krempaska, Jakob Pernthaler

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On a temporal scale, variability is imposed upon aquatic microbial communities by factors such as weather, or recurrent seasonal changes. Heterogeneity on a spatial scale can be caused by physical structuring (turbulence, stratification) differences in microbial abundance, activity, community composition, or by the interaction between bacteria and organic particles (lake snow, phytoplankton cells). I investigate the seasonal development of small scale spatiotemporal variability of bacterioplankton and of low-molecular weight organic matter in Lake Zurich.

The studied parameters include bacterial abundances, phenotypes, diversity and the concentrations of 22 selected amino acids (AA). Samples are obtained by a custom made device that simultaneously collects 10 samples of 10 ml from the same depth (5m) at distances of 2 cm. The sampling process is repeated 10 times with a time gap of about 5 min between sets, thereby also including the temporal aspect. Bacterial abundances are determined by flow cytometry and their diversity is estimated by 16S rDNA sequencing. AA concentrations are measured and quantified by HPLC-MS.

First results show that, both, bacterial abundances and AA concentrations were highly variable over a 2 cm scale. At the same time, AA data revealed more distinct peaks of elevated concentrations, implying the presence of point sources of these bacterial substrates. Bacterial abundances sometimes seemed to follow the elevated amino acid concentrations and sometimes not, suggesting a stochastic relationship between bacteria and their resources. Currently the extremes in terms of variability levels from several sampling campaigns are being analyzed for differences in community composition.

O03 Which parameters affect the nitrogen cycling microbial community composition in lake sediments?

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Increasing anthropogenic nitrogen input leads to eutrophication and changes in many aquatic systems. Lakes reduce the nitrogen load through several processes, such as denitrification, anammox, and nitrogen burial, thus acting as nitrogen sinks. Other processes such as dissimilatory reduction of nitrate to ammonium (DNRA) and nitrogen uptake conserve nitrogen in the system. The nitrogen removal in lakes thus depends on the activity of microorganisms and several environmental parameters, including dissolved oxygen (O_2) concentrations and organic matter input. Even though the research on nitrogen removal in lakes has received considerable attention, the contributions of different pathways and the main parameters influencing the nitrogen removal processes and the nitrogen cycling microbial community in freshwater lakes are not known.

This study aims to better understand the factors influencing nitrogen cycling dynamics including the key microbial players involved in freshwater ecosystems. Using amplicon sequencing, metagenomics, and metatranscriptomics as well as measurements of porewater chemistry and process rates, a detailed seasonal and spatial resolution of nitrogen removal rates, the microbial community, its nitrogen gene expression activity and environmental drivers in the sediments of two lakes with different trophic states will be measured.

Lake Baldegg is a representative eutrophic lake with high TOC, low O_2 concentration (243 µmol/L), O_2 penetration depth (3 mm) and high nutrient concentration ($NO_3^- 92 \mu mol/L$) in the sediment. In contrast Lake Sarnen is an oligotrophic lake with low TOC content, higher O_2 concentration (308 µmol/L), deeper O_2 penetration depth (6 mm) and low nutrient concentration ($NO_3^- 34 \mu mol/L$) in the sediment. The preliminary data showed a 7 fold higher denitrification rate in Lake Baldegg than Lake Sarnen. Similarly, the DNA and RNA concentration is higher in the sediments of Lake Baldegg.

In the next step the 16S rDNA, Metagenome and Metatranscriptome data will be analyzed to provide details on the genetic potential for nitrogen turnover and the transcriptional activity of the functional groups involved. Ultimately, the data will contribute to better understanding of the main microbial communities and pathways that drive the nitrogen cycle in Swiss lakes and show how environmental conditions influence biological nitrogen removal.

O04 The Microbial N-Transforming Community and N Biogeochemistry in the Redox Transition zone of Lake Lugano

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Denitrification is an important fixed nitrogen (N) removal process in lakes. Organotrophic bacteria are traditionally assumed to drive lacustrine N elimination, yet the role of chemolithotrophic, sulphur (S)-oxidizing denitrifiers may be greatly underappreciated, as well as the role of the nitrate reductase Nap compared to the commonly dominating Nar. We studied the redox transition zone (RTZ) of the meromictic north basin of Lake Lugano, where both modes of denitrification occur. Our objective was to assess the microbial community of N-transforming microorganisms and their activity by i) analysis of N and S compounds, ii) nitrate stable isotope ratio measurements, iii) incubation experiments for denitrification rates, and iv) sequencing and qPCR of 16S rRNA and functional genes.

Water column profiles of dissolved N compounds and microorganisms in the RTZ revealed three main water layers. 1) Above the oxycline, bacterial and archaeal nitrifiers were dominating microorganisms, producing typical nitrate isotope signatures. 2) Within the oxycline, which is the main denitrification zone, the microbial community shifted towards a community with S-oxidizing denitrifier *Sulfuritalea sp.* and methane-oxidizing nitrite-reducer *Candidatus* Methylomirabilis. 3) Below the oxycline, where reduced species such as ammonium and sulphide accumulate, other microorganisms like *Candidatus* Anamoximicrobium and a *Thiobacillus*-related species became more abundant. In incubation experiments, contrasting canonical views with regards to the relative ecological importance of the two main nitratereducing enzymes, S-oxidizing denitrifiers seemed to use preferably Nar, while organotrophic denitrifiers used predominantly Nap. In addition, S-oxidizers reduced a large fraction of nitrate to ammonium, while organotrophs performed complete denitrification to N₂ with low rates. Furthermore, S-oxidizing nitrate reducers were actively growing throughout the year while organotrophic denitrification was limited by the availability of organic substrates in autumn. Whereas concentration profiles of N and S compounds remained relatively stable over the year suggesting homeostatic conditions, nitrate isotope signatures (i.e., isotope effects) as well as the microbial community were subject to major seasonal changes.

O05 Effect of agricultural landscape on the dissemination of β -lactams and sulfonamides resistance genes in the lake of Brêt, Switzerland

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Antibiotic resistance genes, β -lactams, sulfonamides, lake of Brêt, agricultural landscape, agricultural runoff

The use of antimicrobial agents in clinical and agricultural settings revolutionized the treatment of infectious diseases and increased agricultural productivity. However, its use result also in the dissemination of antibiotic resistance genes (ARGs) thus creating a risk hazard for both human and animals' health. Since a decade, there is a growing concern about the link between clinical and environmental resistance genes because the aquatic system is considered as a reservoir of resistance genes. Bi-directional exchange of antibiotic resistance genes and antibiotic resistant bacteria has been suggested thus revealing the need to understand the fate of ARGs in the environment in view to manage their dissemination. This study investigate, the effect of agricultural landfill on the dissemination of β lactams and sulfonamides resistance genes in a small peri-alpine lake used as reservoir for a water treatment plant by investigating the abundance and distribution of ARGs and toxic metal. ARGs (blaTEM, blaSHV, blaCTX-M, blaNDM, sul1 and sul2) total bacterial load were quantified using quantitative PCR (qPCR) in total DNA extracted from the sediment recovered from the lake Brêt in Switzerland. The results highlight the widespread dissemination of ARGs link to the massive use of the 1st generation of antibiotics and their accumulation in the lake over the time. Furthermore, ARGs linked to more recent antibiotic were also ponctually found. These finding demonstrate the role of lake as a catchment area for anthropogenic contaminant and the need to strengthen antibiotic regulation to struggle the dissemination of antibiotic resistance

O06 Environmental toxicity promotes positive interactions in a synthetic bacterial community

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Identifying how different species interact within microbial communities is key to understanding how these communities function and affect our health and our environment. However, disentangling the ecological interactions within natural microbial communities is difficult because they typically contain many species. In our research, we are using a semi-natural ecosystem composed of four bacterial species capable of digesting Metal-Working Fluids (MWF), a coolant and lubricant employed in the manufacturing industry. MWF contains compounds allowing for bacterial growth, as well as biocides that make the environment toxic for bacteria. This MWF ecosystem represents an ideal first experimental system for disentangling ecological interactions. Firstly, we evaluated the growth of each species alone and in co-culture with the others to measure their pair-wise interactions. Surprisingly, we found only positive interactions between the four species, suggesting cooperative behavior within the bacterial community. To explain this observation, we hypothesized that environmental toxicity was killing the species when alone, but that they could cooperate to survive when grown together. To explore this possibility, we built a model to predict pair-wise interactions along nutrient and toxin gradients. Our model predicts that toxicity should induce the appearance of positive interactions, while the addition of nutrients should result in more competitive behavior. We then conducted additional experiments where we manipulated nutrient and toxin concentrations, confirming the theoretical predictions. Our study demonstrates that ecological interactions in bacterial communities are highly context-dependent, and that cooperative interactions between bacteria are likely to depend on sharing a harsh, toxic environment.

O07 Effect of a mixed consortium of three bacillus strains on plant growth promotion of oat and its consequence on native microbial communities

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In conventional agriculture, inappropriate management practices and use of agrochemicals has led to major impacts on both soil and water. Thus, the search for sustainable alternatives in agriculture has become a priority. In the mycorrhizosphere, microbes are considered as crucial actors of soil functioning, as they are involved in many beneficial activities supporting the soil ecosystem. Some endospore forming bacteria are well-known to enhance plant growth through biocontrol and biofertilization activities. They also have the ability to form dormant structures called endospores which allow them to survive under harsh environmental conditions, representing them as a potential solution for bio-inoculation. In this study we selected three mesophilic Bacillus strains with plant growth promoting abilities to investigate their effectiveness to promote oat plants (Avena sativa) growth, either as an individual inoculum or as a consortium. Bio-inoculations in pot, with either a sterile or a non-sterile soil, and field experiments were carried out by coating oat seeds either with vegetative cells or endospores. Pot experiments showed that the consortium increased significantly total dry biomass of plants, suggesting that a consortium of beneficial microbes promotes plant growth better than individual strains. While the effect was not as prominent in the non-sterile soil, it showed that a bacterial consortium seems to be more robust than single strains. Based on this, a field experiment was performed to assess the impact of the consortium on plant growth promotion, as well as the effect on the composition of native soil microbial communities. Bio-inoculation had a positive effect on plant growth and fitness. It showed that the consortium with vegetative cells was more efficient and induced a significant increase in total plant dry biomass. Analysis of the microbial communities of the bulk and rhizospheric soils, as well as the roots, demonstrated that the inoculum successfully colonized the rhizosphere, without modifying the overall structure of microbial communities in soils. Moreover, the direct application of the consortium on seeds favored rhizospheric colonization and resulted in a minimal impact on native bacterial communities in response to the application. In the future, this could provide an ecofriendly alternative to limit the use of agrochemicals.

O08 Manipulation from within – How does Functional Niche Occupancy impact a next Generation Probiotic?

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Next generation probiotics with defined metabolic function have been suggested to address microbial dysbiosis observed in several gastrointestinal disorders. Short chain fatty acid formation (SCFA) is considered a target function of next-generation probiotics. The common gut microbe *Eubacterium hallii* produces butyrate from hexoses, or from lactate and acetate. *E. hallii* furthermore forms propionate from 1,2-propanediol (1,2-PD). The key enzyme in 1,2-PD metabolism is a cobalamin dependent glycerol/diol dehydratase (GDH), which uses glycerol as a second substrate yielding the multicomponent system reuterin. Reuterin exhibits antimicrobial activity and transforms food-derived, carcinogens heterocyclic amines into compounds with reduced mutagenicity. Due to its functional versatility, *E. hallii* is considered a candidate next generation probiotic. However, little studies have been conducted on the role of functional niche occupancy if a gut microbe is added to complex microbiota.

We were interested how the presence of competitors with GDH activity affects the impact of spiked *E. hallii* (10^7 cells/mL) and tested 8 different colonic microbiota in the absence and presence of GDH substrates (glycerol or 1,2-PD). Using a combined molecular and bioanalytical approach, we determined the abundance of *gdh* using quantitative PCR and primers designed based on metagenome prediction, SCFA (HPLC-RI) and reuterin formation (nano LC-MS/MS).

Spiking of *E. hallii* had little impact on SCFA profile. Addition of glycerol led to a decrease in total SCFA if microbiota harboured detectable levels of *gdh* (>log 4 genes/mL) possibly due to the formation of reuterin. Similarly, propionate levels were increased after addition of 1,2-PD if the microbiome harboured >log 4 genes/mL *gdh*. Combined glycerol and *E. hallii* addition decreased acetate levels and increased butyrate production but did not impact reuterin formation only in microbiota with low *gdh* abundance. In agreement, propionate formation of the same microbiota was enhanced after *E. hallii* and 1,2 PD addition.

Our results indicate that targeted manipulation of complex microbiota with a GDH positive gut microbe showed impact on SCFA formation only if competitors with the same functionality were low abundant. Our results suggest the suitability of applying next generation probiotics in the treatment of intestinal dysbiosis if a decrease of microbes with the target function has been observed.

O09 Effect of Fluctuating Environmental Conditions on Spatial Self-organization and Community Stability

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Nearly every microbial community present in nature or utilized for biotechnological applications is exposed to temporal fluctuations in their local environment. The mechanisms that provide stability to a microbial community during environmental fluctuations, however, are unclear. Environmental fluctuations can change how microorganisms interact, which in turn can change how they arrange themselves in space. Spatial self-organization, as well, can be an important determinant of the stability of the whole microbial community. This raises two critical questions. 1) How do temporal fluctuations in environmental conditions affect spatial self-organization? 2) Do these fluctuations affect the long-term stability the survival of the microbial community?

We addressed these questions using a two-strain synthetic microbial cross-feeding community. Our model system consists of two cross-feeding isogenic mutants of P. stutzeri A1501 that differ in their ability to reduce nitrate and nitrite. The two strains interact competitively for space and oxygen under aerobic conditions, but interact mutualistically via nitrite cross-feeding under anaerobic conditions. We experimentally fluctuated the environment between conditions that promote mutualism or competition between the microbial strains. We hypothesized that such fluctuations should destabilize the community.

Analyzing the development of pattern formation over time, we found that fluctuations between mutualism and competition conditions do indeed reduce the stability of the microbial community as a whole, helping to shed light on the role of spatial metabolic interactions in the microbial assemblage. Thus, temporal fluctuations affect the complexity of spatial self-organization, while spatial self-organization also affects stability to temporal fluctuations, highlighting its role as an emergent property of the microbial community.

O10 Interactions within interactions: phage assemblages and their host microbiota in the honey bee gut

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Phages are effective bacterial predators that can potentially have strong ecological effects on microbial populations such as host extinction, host diversification or community homogenization. The true ecological impact of phages on natural microbial communities remains unknown, mainly due to the complexity of bacterial communities and the difficulty of working with phage assemblages. The honey bee gut microbiota is an emerging model system to study host-microbiome interactions due to its relative simplicity (eight core lineages with high strain diversity make up to 98% of the community), and its amenability for experimental manipulation. Here, we aimed to characterize for the first time the characterization of phage assemblages in the honey bee gut microbiota and to determine their interactions with their bacterial hosts.

Our approach included analyzing the phage metagenomes from two different hives, applying a network analysis to define clusters of phage populations and inferring their host using self-organizing maps of k-mer signatures. In parallel, we obtained nine single phage isolates directly from honey bee guts, sequenced their complete genomes and constructed a host-specificity matrix against a culture collection of 100 strains.

A total of 14 complete circular genomes with size range 16.6-51.7 Kbp were recovered from phage isolates infecting strains of *Bifidobacterium asteroides*. These correspond to seven phage populations: three Podoviridae:Picovirinae, and four Siphoviridae. All represent new phages, since best hits to proteins in public databases were always below 20%. The metagenomes produced 1107 viral contigs larger than 1 kbp. The two shared ~10% of their sequences with an average nucleotide identity higher than 95%, meaning they belong to the same viral populations. The largest metagenomic cluster corresponds to phages infecting *Lactobacillus*. The second and third largest clusters correspond to phages infecting Actinobacteria, and two of our phage isolates belong to these groups. This pattern reflects host abundance, since *Lactobacillus* and *Bifidobacterium* are the dominant members in the rectum. Most of the strains tested were not infected, suggesting that evolution of resistance is an important factor for the microbiota. The host-specificity matrix show a nested pattern, with few phages infecting all bacteria and few bacteria being susceptible to all phages. We present a robust framework to assess the effects of phage assemblages on microbial communities *in vivo*.

O11 Mechanisms underlying the inhibition of Phytophthora infestans by cyanogenic Pseudomonas

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Phytophthora infestans is the causing agent of late blight disease, which is associated with a loss of approximately 3 billion dollars per year including the control efforts and production losses. The current control methods like the use of synthetic fungicides or copper-based products are not sustainable as well as harmful to nature. In multiple studies, bacteria of the genus Pseudomonas were shown to act as biocontrol agents and efficiently inhibit P. infestans by producing antimicrobial compounds. Two such antimicrobial compounds are known to inhibit the growth of P. infestans: hydrogen cyanide (volatile) and phenazines (non-volatile). The aims of our study are i) to quantify the relative contribution of known determinants of anti-oomycete activity in the overall activity of potato associated Pseudomonas strains, ii) to identify novel volatile and non-volatile determinants of this anti-oomycete activity. To this end, we are focussing on two fully sequenced Pseudomonas strains and generating mutants of known antimicrobial compounds to confront the bacterial mutants to P. infestans at different stages of its life cycle in vitro and on the potato plants. Since both strains of interest produce HCN, a potent inhibitor of oomycete development, our current experiments are focused on using HCN mutants to determine: 1) the contribution of HCN to invitro and in planta inhibition of P. infestans growth by Pseudomonas strains and 2) the presence of other novel volatile and diffusible compounds with anti-oomycete activity. Understanding the different modes of action of the anti-oomycete compounds and the molecular mechanisms underlying the inhibition could help us designing better control strategies against the devastating late blight disease.

O12 Mycosphere Effects on Phage Transport and Retention

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Phages are viruses that infect bacteria. They have been described as good tracers to assess the transport of water, colloids and viruses. While many studies have examined the effect of phage characteristics and environmental conditions on phage transport, little is known on the interactions of phages with fungi as the prevalent microbial biomass and hotspot of microbial activity. Forming extensive and dense networks mycelia efficiently extend in terrestrial habitats and provide significant surfaces for interactions with phages. In an attempt to study phage-hyphae interactions we quantified retention of phages by mycelia in a microfluidic transport platform that allowed for fluid exchange around hyphae in a defined and controlled manner. Two lytic phages commonly used as tracers for pathogen contamination (coli-phage T4) or for colloidal transport (marine phage PSA-HS2) and two mycelia exhibiting a different surface properties (Coprionopsis cinerea; Pythium ultimum) were employed to evaluate the phage-hypha interactions. Phage transport and retention was quantified in microfluidic devices while phage-hyphae interaction energies were approximated by the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) approach of colloidal interaction. Our results show that phage retention by hyphae depended on both the phage and hyphal properties with surface hydrophobicity of the hyphae being the major driver for phage retention. Our findings suggest that the mycosphere may be a hotspot for the retention of phages and hence influence the subsurface transport of phages and their impact in soil microbial ecology

O13 Oxalotrophy as a biocontrol strategy

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Phytopathogenic fungi have a wide host range and cause economically important damage in crops worldwide. Some of these pathogens use organic acids, and in particular oxalic acid, as a virulence factor. Degradation of this organic acid could be a new approach of biological control of fungal pathogens. Oxalotrophic bacteria are found in various environments and use oxalate as carbon and energy source. Thus, they could provide a protection to the host against the pathogen through nutritional interference.

Confrontation experiments in Petri dishes showed that the fungal growth inhibition change depending on the fungal partner, bacterial partner and trophic factors. Cupriavidus necator and Cupriavidus oxalaticus (two oxalotrophic bacteria) inhibited the growth of Botrytis cinerea and Zymoseptoria tritici but not of Rhizoctonia solani. In the other hand, Pseudomonas putida (a non-oxalotrophic bacterium) inhibited the growth of Z. tritici and R. solani on R2A medium.

Further experiments are planned to investigate the inhibition of germination of spores and sclerotia, as well as the use of oxalotrophic endospore-forming bacteria (EFB) as biocontrol agent, as all studies have be done so far with mycelium and non-EFB. Furthermore, experiments will be conducted in Lactuca sativa in a sterile environment, in order to assess the interactions between plant, bacteria and fungus. As P. putida showed promising results as biocontrol agent, its biocontrol strategy will be investigated. Finally, the swarming behaviour of the bacteria observed in presence of Z. tritici will be further investigated to decipher the trigger behind it.

O14 Overwintering honey bees exhibit a disctinct gut microbiota composition Olivier Emery¹, Lucie Kesnerova¹, Berra Erkosar², Philipp Engel¹

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The honey bee gut microbiota has been shown to consist mostly of ~7 phylotypes, i.e. clusters of strains with >97% sequence identity in the 16S rRNA gene. These phylotypes are consistently present in adult worker bees irrespective of geographical location, life stage or season. To date, most of the knowledge on the adult honey bee microbiota composition has been derived from single time points from different hives, or from a pair of time points between two seasons, raising the question about the stability of the microbiota along seasons. Furthermore, in order to pass the winter, long-lived "winter bees" are produced. They have a different metabolism relative to summer bees (i.e. foragers) and feed exclusively on stored reserves while retaining feces during winter, all of which could impact the gut microbiota composition.

We monitored the gut microbiota composition and pathogen levels of individual bees from a healthy hive monthly for two full years using qPCR. While the microbiota composition of foragers was rather stable along seasons, we detected a marked shift in winter bees with an increase of 10x to 100x for Firm-5 and *Bartonella apis* which resulted in an increase in total bacterial loads for both years sampled. We found that the infection rate of *N. ceranae* was lower in winter bees than in foragers while trypanosomatid infection rates were similar. We also investigated the gut microbiota composition in nurse bees relative to winter bees and to foragers and found that all 3 types of bees have specific community compositions with the one from winter bees being most distinctive. We also assessed the contribution of diet (pollen vs no pollen) to the microbiota composition in bees colonized with an artificial microbiota community, and observed an increase in total bacterial loads in the presence of pollen, as well as an increase in Firm-5 levels but not for *B. apis* which remained at similar levels in both groups.

The repeated change in the gut microbiota composition observed in winter bees suggests that these possess a distinct microbiota composition. However, further experiments are needed to determine how consistent this change is among hives and to determine its causes and consequences for the host, and for the microbiota.

O15 Sulfur cycling in the terrestrial deep subsurface

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Olkiluoto, an island in south-west Finland, has been selected as the site for a deep geological repository for the final storage of spent nuclear fuel. An understanding of the geomicrobial processes occurring within the groundwater at this site is therefore important to ensure long-term, safe storage of the fuel. Of particular concern is the generation of sulfide, as it can induce corrosion of the waste-bearing copper canisters. Sulfide is detected in relatively few drillholes at Olkiluoto despite sulfate being present in the groundwater up to ~300 m depth. To determine what electron donor(s) drive sulfidogenesis we investigated three different groundwaters where sulfate is available but the concentration of sulfide differs from 0–1.5 mM. Geochemical and isotopic data indicated that sulfate reduction was ongoing in two of the three groundwaters. However, combining these analyses with metaproteogenomics revealed that sulfate reduction was active in all three groundwaters. In the groundwater with geochemically undetectable sulfate reduction, a community of sulfate-reducing and sulfide-oxidising bacteria mediate a cryptic sulfur cycle. Hydrogen and small organic compounds provide electron donors for sulfate reduction. The produced sulfide is then cycled to sulfate by the activity of sulfide-oxidising bacteria. The ability of sulfide-oxidising bacteria to limit the accumulation of sulfide was further demonstrated in groundwater incubations amended with sulfide and nitrate. In all instances sulfide was fully removed. The results shed light on the drivers of sulfidogenesis at Olkiluoto and highlight a potentially beneficial sink for sulfide. They also contribute towards our understanding of microorganisms in deep terrestrial subsurface ecosystems and their role in geochemical cycling.

O16 Effect of soil, climatic and biotic factors on bacterial communities across the diverse landscape of Switzerland

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Nation-wide monitoring programs, such as the biodiversity monitoring (BDM) in Switzerland, provide an excellent opportunity to assess structures of bacterial communities in soils and to relate them to other communities, e.g. plants. Furthermore, such frameworks enable studying effects of soil, climatic and biotic factors on bacterial communities and differences among land-use types and biogeographic regions. Within the BDM, 255 sites (mostly with four replicates) were sampled along a regular grid, covering various gradients, e.g., elevation (297-2,741 masl), mean annual temperature (-3-12.2°C), precipitation (758-2,094 mm) and soil pH (2.7-7.8) as well as different land-use types, e.g. meadows, alpine grasslands, forests and arable land. Microbial communities were assessed using metabarcoding of ribosomal genetic markers (V3-V4 of the 16S rRNA gene). Additionally, several soil physico-chemical, climatic and geographic factors as well as plant communities were recorded. In total, 49,280 bacterial operational taxonomic units (OTUs, 97% identity) were detected. Sites harbored distinct bacterial communities, as shown by an overall reclassification success for bacterial communities to their sites of origin of 85%. Also, land-use types harbored significantly different communities and bacterial communities differed among the Southern Alps, the Central Alps and Northern Switzerland (p<0.05). Analysis of environmental factors revealed soil pH as the most important factor explaining variance in bacterial communities (R^2 =0.3, p=0.001), followed by the plant-indicator-value for nutrients and elevation. Soil pH remained the dominating factor also when bacterial communities were analyzed separately for each land-use type. Bacterial and plant communities were significantly correlated and the magnitude of correlation depended on the land-use type (r=0.18-0.55, p<0.05). In this study, an inventory of bacterial communities across environmental gradients was compiled, and data indicate specific associations between soil bacterial communities and environmental factors across land-use types.

O17 Temperature Sensitivity of the high alpine soil microbiome

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Climate change leads to rapid warming of arctic and alpine environments with strong impacts on soil microbial ecology. Microbial activity and diversity are directly linked to ecosystem functioning and geochemical cycles. In this study, we assessed the effect of temperature on high altitude mountain soils linking microbial diversity and function.

We subjected eight high-altitude mountain soils with a different microclimatic history associated with altitude and aspect to different incubation temperatures (4 – 35 °C) for one month. Sequencing of the bacterial 16S rRNA gene at the DNA and RNA level as well as 3H-leucine incorporation as a measure for bacterial growth were used to investigate shifts of abundance, activity and optimal growth temperature, respectively.

All soils intrinsically exhibited an optimal bacterial growth temperature of 25 – 30 °C. Only incubation above 25 °C led to a shift of the optimal growth temperature towards to the treatment temperature. Similarly alpha-diversity indices did not change below 25 °C but showed a dramatic decrease at 35 °C. In all soils, both at the DNA and the RNA level, bacterial community structures shifted gradually with increasing temperature with a very pronounced change at 25 and at 35 °C. Interestingly, north exposed soils reacted consistently more strongly to the temperature treatment than south exposed soils indicating a relationship between temperature sensitivity and microclimatic legacy of the soils. Bacterial community structures showed very similar patterns in response to warming at the DNA and at the RNA level. Changes at the DNA level might indicate enrichment of taxa adapted to the respective treatment temperature in the community as they may gain a selective advantage leading to enhanced turnover relative to less well adapted taxa. On the contrary, shifts in abundance at the RNA level only suggest increase and decrease of activity without concurrent change in abundance. However, major changes in community structures seemed to occur at both DNA and RNA level in our experiment and thus involve turnover.

Collectively, our results indicate that high alpine soil bacteria react sensitively to temperature changes as increasingly imposed by climate change, leading to selective community turnover and partly shifts of activity while the optimal growth temperature is not affected at low and moderate temperature

O18 Effects of freeze-thaw cycles frequencies on arctic and high-alpine soil microbial communities.

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Climate change will lead to an increased frequency of freeze-thaw cycles (FTCs) in cold soils because of less snow cover and larger oscillations in temperatures, resulting in significant changes on the structure and metabolic patterns of soil microbial communities. Here, we evaluated to what extent increased FTCs frequency will alter the structure and functionality of microbial communities in Arctic and alpine soils differing in their FTCs legacies.

We collected top soils at the north and south flanks of a mountain ridge, one in the Arctic and one in the Alps, and subjected them to two different FTCs frequency regimes: (1) shorter cycles (daily) and (2) longer cycles (weekly) of $+5^{\circ}C/-5^{\circ}C$ temperature variations. We analyzed microbial processes such as basal respiration and extracellular enzymatic activities together with the changes of the structure of bacterial and fungal communities by using amplicon sequencing.

FTCs caused a significant decrease in soil basal respiration and led to substantial shifts in microbial community structures in all soils, with stronger effects on bacteria than fungi. No clear changes were observed in C- and N-acquiring enzymes reflecting an uncoupling between microbially-mediated ecological functions and community structures. Contrary to our expectations, alterations in basal respiration and community structures were larger for the soils subjected to longer FTCs than to shorter FTCs whereas the mountain aspect did not show any impact on soil microbial communities.

Our results suggest that increased frequency of FTCs influences the functioning and structures of arctic and alpine soil microbial communities. The *in situ* adaptation of soil microbial communities to continued frozen conditions might have a stronger influence on their resistance to FTCs than the soil FTCs legacy *per se*.

O19 Integrating metabolic versatility into spatially-explicit models of soil bacterial life

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Despite often-inhospitable conditions, soil hosts an unparalleled diversity of bacterial life at all scales. This is partially attributed to the presence of numerous habitat types in small soil volumes, and the versatile metabolic capabilities that enable soil bacteria to adapt different growth strategies and use a wide range of resources. The dynamic and spatially variable nutrient landscape triggers diverse and highly localized growth strategies. Such diverse and adaptive traits are difficult to capture in traditional modelling approaches and require more nuanced representation of bacterial metabolism. We developed a mechanistic mathematical framework that combines metabolic networks with individual based representation of bacterial cells living within complex soil micro-scapes. Pore geometry and associated aqueous phase configuration determine diffusional constrains that influence the nutrient landscape and the resulting metabolic adaptations. In combination with experiments of microbial growth in glass-etched micrometric pore networks, the mathematical model was used to elucidate the spatial self-organisation of a two-species community in response to carbon and oxygen counter gradients. Compared to homogeneous environments represented by shaken flasks, the spatially structured environment enables coexistence of the two otherwise competing bacterial species due to metabolic preferences and opportunistic trophic interactions. The model offers unprecedented opportunities to simulate bacterial life in habitats where diffusional constraints and spatial heterogeneity are significant.

O20 Predatory Behavior of Vibrio Cholerae Strains Highlight possible Adaptations to Different Lifestyles

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Several pathogenic microorganisms have important environmental niches. In fact, some of these organisms can move from an environmental to a pathogenic lifestyle by the horizontal acquisition of key virulence factors. Complementary or minor virulence factors can evolve in case they confer a fitness advantage against environmental pressures, such as competition and predation. Vibrio cholerae is an interesting model organism to study this dual lifestyle as a prominent human pathogen but also a normal member of aquatic habitats. While a limited set of strains are responsible for cholera pandemics, a broad variety of isolates do not cause cholera and are primarily found in the environment. In order to understand pathogen emergence, the knowledge of basic differences between these types of strains is of prime importance. This work aimed at investigating whether pandemic and environmental strains of V. cholerae differ in their predatory behavior towards bacterial competitors and amoebal predators. Specifically, we assessed the involvement of the type VI secretion system (T6SS) and of accessory toxins in the killing of other bacteria and as a defense mechanism against the amoeba Dictyostelium discoideum. Contrary to pandemic strains that tightly regulate their T6SS production, environmental V. cholerae strains constitutively shoot their T6SS, allowing them to efficiently eliminate other bacteria. Conversely, only a limited set of these strains also uses the machinery against D. discoideum, most likely due to the absence of anti-eukaryotic T6SS effectors. We further investigated another molecular weapon of V. cholerae, the pore-forming toxin hemolysin (HlyA), which was shown in in vitro studies to specifically damage eukaryotic cells. Yet again, HlyA is tightly regulated in pandemic V. cholerae while environmental isolates constitutively secrete hemolysin. Our results show, however, that HIyA does not contribute to the defense against D. discoideum. Finally, we tested whether the capacity for formation of extensive biofilms protected environmental but not pandemic strains from the amoebal grazer, which turned out not to be the case under the tested conditions. Collectively, our results highlight important differences in the predatory behavior of pandemic and environmental V. cholerae isolates. We hypothesize that the tight "weaponry" regulation exerted by pandemic V. cholerae can be advantageous in a disease context and under transmission conditions. Conversely, the constitutive production of molecular weapons and defense mechanisms by environmental isolates is probably useful to cope with constant environmental pressures.

O21 Priority Effects Influence Microbiota structure in the Arabidopsis Phyllosphere

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Plants are heavily colonized by microorganisms, many of which are essential to plant growth and health. However, plant-microbe interactions are not well understood. Bacterial colonization patterns in the phyllosphere have been shown to be temporally and spatially reproducible, suggesting that underlying mechanisms exist that shape microbial community structure on various hosts and across geographic boundaries. We conducted drop-out and re-introduction synthetic community experiments using a gnotobiotic system with Arabidopsis thaliana as the host and a synthetic mixture of 62 genomesequenced bacterial strains isolated from native A. thaliana to test how arrival order, known as priority effects, influence community structure and to understand to what extent late-arriving strains can invade an already established microbiota. As a readout, we tracked the relative abundance of all strains in individual plants through amplicon sequencing. Our results demonstrate that synthetic communities establish consistently and that missing strains can, to various degrees, invade an already established microbiota, which is itself resilient and remains unaffected by newcomers. Additionally, we show that priority effects do influence final community structure and that there are winners and losers of late arrival, likely due to niche pre-emption and niche modification. Overall, our results suggest that priority effects play a role in community assembly and structure, although highly competitive strains are largely unaffected by arrival time and can efficiently invade an established community

O22 Dormancy and spores' formers: Investigate non-standard models

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Natural microbial communities have to cope with unstable environmental conditions, which are suboptimal for metabolic activity. There are several strategies to favour survival, among them the production of resistant bacterial spores. A well-studied model of this process is the formation of endospores by Firmicutes. Firmicutes are monoderm and form endospores through endosporulation. Kurthia is a firmicute genus that was previously considered asporogenic and monoderm. Nevertheless, we discovered that Kurthia cells present not only an unusual LPS-free outer membrane (OM), but also form spores by modifying the cell envelope structure. In an evolutionary context, the presence of this atypical LPS free-OM requires an explanation and the most probable one is a *de novo* emergence. Because emergence of diderm cells remains a fundamental question in biology, we designed experiments in order to characterize the cell envelope. We have observed that Kurthia cells changed their morphology after penicillin treatment. In the future, we plan to investigate how Kurthia resists to antibiotics through change in the structure of the OM, using intensive cryo-TEM and classic microscopy analysis. In addition, we are investigating the morphology and resistance of spores, as well as the sporulation process. In order to characterize these elements, the reliable production of spores is essential. We have identified a suitable medium to trigger sporulation. The sporulation process needs to be characterized by direct observation and transcriptomics.

O23 Nutrient fluctuations characteristic of microscale heterogeneity reduce bacterial growth rates

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The characteristic timescale of nutrient fluctuations in many realistic bacterial environments ranges from tens of seconds to several minutes. Bacterial motility, gene expression and rates of translation all respond to nutrient availability within this range. How do nutrient fluctuations affect growth rate? A novel microfluidic device enables us to approach these previously inaccessible questions. We find that second-and minute-scale nutrient fluctuations consistently reduce bacterial growth rates. These growth reductions are timescale-dependent, due to an interplay between the nutrient timescale and timescale of growth responses. Intriguingly, we find that unlike cells grown in steady environments, cells that have experienced repeated nutrient shifts put a pause on active adaptations to changing nutrient. This steadiness in growth rate points towards an alternative physiology: one that provides cells with an advantage when growing in highly dynamic environments.

O24 Following Horizontal Gene Transfer in real time.

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Microbial communities shape our lives at many different levels, from healthcare to waste processing. Consequently, explaining how these communities evolve and adapt is crucial to better understand, benefit and manipulate them towards greater utility and reduced harm. One of the most important mechanisms of microbial adaptation is Horizontal Gene Transfer (HGT). Although the mechanisms enhancing and limiting HGT have been widely studied in vitro in isolated organisms, little is known about their impact in community co-evolution. Accordingly, we are studying the frequency, rate and directionality of HGT in a constructed community consisting of four bacterial species.

We evolved our bacterial community over two different time scales (8 weeks, and 6 months), and sequenced the evolved populations using a whole-community metagenomic approach, with both Illumina and Nanopore. We then measured the mutation rate as well as the HGT rate (defined as the rate of genomic structural variation in the species) compared to the ancestor.

We find that HGT is more common than previously expected, with an important association with the phylogenetic relationship between the donor and the recipient. However, the overall HGT rate is significantly smaller than the point mutation rate, and very few HGT events fully fix in the population at the studied time-scales. Our results also show that the majority of HGT observed was due to mobile genetic elements, especially insertion sequences (IS) that were most abundant between replicons of the same species.

Overall, our results quantify the frequency of HGT and its adaptive effect on a short time scale, with a higher occurrence rate, but a lower fixation rate than we previously anticipated. These results validate previous theoretical results suggesting that, unless a strong benefit is achieved, traces of HGT events are lost over time.

Disclaimer: This abstract represents yet unpublished data.

O25 Small-scale Heterogeneity in Drinking Water Biofilms

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The majority of bacteria reside in biofilms and multiple factors give rise to heterogeneity within these. While this had already been described on large scale (e.g., through a full-scale distribution system), heterogeneity on smaller scales is poorly quantified. In this study, we assessed the heterogeneity of building plumbing biofilms on small-scale. To achieve this, indigenous biofilms were grown inside flexible shower hoses over the course of 12 months under controlled conditions; hoses (1.20 m) were horizontally aligned and exposed to twice-daily shower events (15 min) with non-chlorinated warm water (~ 42°C). For analysis, hoses were dissected into 100 cross sections, which were then halved to yield 200 pieces of approximately 1.2 cm2. Biofilms were characterized by their thickness (µm-scale, optical coherence tomography), total cell concentration (TCC) (cm-scale, flow cytometry), and microbial community composition (cm-scale, 16S rDNA amplicon sequencing). We found considerable heterogeneity on small-scale throughout the length of a hose. Biofilm thickness along the entire hose ranged between $35.8 - 890.7 \mu m$, with significantly higher values for the bottom (323.0 \pm 91.7 μ m) compared to the top part (244.1 \pm 54.4 μ m). On small-scale, the thickness varied up to 75% within as little as 500 µm hose length. TCC varied throughout the hose between 1.2 x 108 – 3.9 x 108 cells/cm2, with successive 1.2 cm biofilm sections showing differences between 0.04 -96.4 %. Interestingly, averaged TCC did not differ between bottom (2.6 \pm 0.7 x 108 cells/cm2) and top (2.6 \pm 0.4 x 108 cells/cm2) as observed for thickness. In addition, changes in the microbial community composition of the biofilm were detected throughout the hose, with a higher α -diversity for the bottom compared to the top. From these biogeographical variations, we conclude that considerable heterogeneity in biofilms does occur already on small-scale, even when biofilms were formed under controlled and consistent conditions within the same hose. These findings are particularly valuable with respect to (1) an improved understanding of biofilm ecology, (2) developing sensible biofilm sampling strategies, and (3) understanding the driving factors for biofilm formation and how small-scale development impacts the microbiology of building plumbing systems on a large-scale.

O26 Influence of Wastewater Composition on the Microbial Communities of Aerobic Granular sludge

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Aerobic granular sludge (AGS) is an emerging technology offering an alternative wastewater treatment with a reduced footprint compared to conventional activated sludge systems. Basic understanding of AGS processes has mainly been obtained in laboratory-scale studies with simple synthetic wastewaters containing volatile fatty acids (VFA) as main carbon sources. Yet, the bacterial communities of AGS treating municipal wastewater have been much less investigated. Two approaches were applied here to assess the impact of fermentable and polymeric compounds on the microbial communities of the AGS and thus make a step toward the comprehension of AGS systems treating municipal wastewater. The first approach was to run an AGS reactor with a simple synthetic wastewater containing VFA as carbon sources and to make a progressive transition to a complex synthetic wastewater, by adding fermentable and polymeric compounds. The bacterial communities of weekly samples were monitored by 16S rRNA gene amplicon sequencing. The second approach was to start in parallel four AGS reactors fed with distinct wastewaters with activated sludge as inoculum. Two reactors were fed with the two synthetic wastewaters used in the first approach, the simple and complex synthetic wastewaters. The other two reactors were fed with either primary clarifier effluent or raw municipal wastewater. In both experiments, the microbial community from the AGS treating simple synthetic wastewater was clearly different from the one treating complex synthetic wastewater. Similarities within the microbial communities fed with the same carbon source were identified. Interestingly the synthetic complex wastewater led to a bacterial community which was closer to the one obtained in the reactors treating real wastewaters. The results of this study show that the type of carbon source is a major factor shaping the bacterial community structure of AGS and that by working with simple synthetic wastewater, one may bacteria which play an important role in the treatment of municipal wastewater. The use of a complex synthetic wastewater may provide a more appropriate model system for the study of the bacterial communities in AGS.

O27 Effects of light / dark diel cycles on the versatile metabolism of purple non-sulfur phototrophic bacteria and their translation for water resource recovery.

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Purple non-sulfur bacteria (PNSB) form a guild of hyperversatile phototrophs that are harnessed for their efficient uptake of nutrients (carbon, nitrogen, phosphorus) and formation of bioproducts. The model genus *Rhodopseudomonas* is recognized for its ability to produce H₂ and poly- β -hydroxyalkanoates (PHAs) under nitrogen limitation and continuous illumination. Impacts of light / dark diel cycles on the metabolism of PNSB have not been revealed yet. Here, natural light / dark cycles were investigated for their effect on the electron- and carbon-based physiology of *Rhodopseudomonas* with focus on the cycling of its primary metabolites H₂ and CO₂ as well as on its PHA storage capacity.

Rhodopseudomonas sp. was isolated as predominant population of an in-house enrichment of PNSB removing all nutrients (98% C, 81% N and P) from an acetate-based synthetic wastewater. Its physiology was examined in an axenic, anaerobic continuous culture subjected to stoichiometrically-balanced growth conditions and three different infrared light conditions: continuous illumination, 16 h light / 8 h dark and 8 h light / 16 h dark. H₂ and CO₂ evolutions were measured in the off-gas by on-line mass spectrometry along with biomass formation by dry weight and PHAs production by fluorimetry.

Under continuous illumination, *Rhodopseudomonas* released spikes of H₂ every 6 h at 0.011 ± 0.002 mmol h⁻¹. As soon as 16 h light / 8 h dark diel cycles were applied H₂ was produced at irregular intervals. PHAs were produced at the beginning of every light phase at a frequency of every 8 h at $1.95 \pm 0.22 \cdot 10^5$ fluorescence counts, concomitantly with peaks of CO₂ production at 0.224 ± 0.033 mmol h⁻¹. Under 16 h dark / 8 h light conditions H₂ was constantly produced under light at increasing rates from 0.02 to 0.06 mmol h⁻¹ from first to seventh cycle, respectively, while PHAs were produced without a specific pattern.

Conclusions

Rhodopseudomonas sp. harbours a very interesting physiology to reallocate excess of electrons into both H₂ and PHA with regular spikes in function of illumination conditions in its environment, suggesting a circadian regulation of its metabolism. Insights gained on this model organism highlight that nature-inspired control of light patterns can foster the recovery of valuable resource from used aqueous and green streams by driving the metabolic versatility of PNSB in mixed-culture environmental engineering systems.

O28 The influence of flow and geometric constraints on bacterial transport and attachment

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The vast majority of microorganisms are exposed to fluid flow, whether in natural environments, the human body, or artificial systems. Flow plays an important role in a broad variety of microbial processes, including nutrient uptake and fertilization, as well as in many industrial applications, ranging from wastewater treatment to the production of biofuels. However, despite the pervasive occurrence and implications of a fluid dynamic environment, its influence on the transport and attachment of bacteria to surfaces remains poorly investigated and understood, especially in complex geometries that best describe real systems. The aim of this work is studying the influence of flow on bacterial transport and surface attachment in complex geometries.

To examine surface attachment in topologically complex geometries, we investigated the effect of laminar flow on motile bacterial suspension around a single pillar in a microfluidics channel. We developed a microfluidics platform where we could study both the effect of pillar diameter and of the local flow velocity on the transport and surface attachment of the opportunistic pathogen *Pseudomonas aeruginosa*. In order to broaden the generality of this study, we considered also corrugated surfaces.

First, we present a phenomenon by which the combination of bacterial motility and shear results in a higher cell concentration near the walls of a channel and consequently in a strong enhancement of bacterial attachment to surfaces compared to quiescent conditions¹. Thanks to the same mechanism, the topological features of the flow in complex geometries promote the attachment of bacteria to specific regions of the surface and shape their distribution.

Thanks to a systematic experimental and numerical study, we show that the combined effect of flow past pillars of different dimensions and bacterial motility can increase the capture efficiency at imposed flow velocity compared to bacterial swimming speed. These results underscore the importance of fluid flow in triggering bacterial attachment and biofilm formation under common environmental conditions, with significant consequences in a broad range of ecological, industrial, and medical problems.

¹Rusconi, R., Guasto, J.S. & Stocker, R.; 2014; Nature Communications; 10: 212–217.

O29 Microcystin Removal by mature Biofilms during Membrane Water Filtration

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Cyanobacterial blooms in aquatic systems are a global concern due the production and release of toxic secondary metabolites, e.g. microcystins (MCs). The health of humans and animals is threatened by the consumption of water contaminated with MCs. The gravity-driven membrane (GDM) filtration system is an efficient means to produce drinking water in a decentralized manner. The biofilm that develops on the filtration membrane is known to cause flux reduction. However, it has the positive effect of reducing organic carbon and MCs in the permeate water.

Complete MCs removal during GDM filtration has been shown to occur only after a period of adaptation around 15 days. We demonstrate that a prior supplementation of biofilms with simple or complex carbon sources, e.g. starch or non-toxic cyanobacterial biomass, reproducibly leads to rapid MC removal (3-6 days). This priming effect could be related to a significantly higher amount of facultative and strict anaerobic bacterial genotypes in these biofilms. GDM experiments performed with water from different sources, moreover, demonstrated that community assembly processes were also an important determinant of MC degradation in these biofilms, likely due to the contrasting establishment of facultative MC degraders.

We conclude that MC removal in GDM biofilms is a facultative trait that can be primed without previous exposure to the toxin. This priming is related to habitat properties rather than direct metabolic stimulation, i.e. oxygen limited conditions. The MC degradation efficiency of GDM biofilm depends both, on the bacterial composition in the source water and on processes that drive community assembly.

END OF ORAL SESSIONS

POSTERS

P01

Synthetic Community Experiments show a role of Community Diversity and Key Players in Plant Protection in Arabidopsis Thaliana

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The phyllosphere of plants harbours an abundant and diverse bacterial microbiota. Most of these microorganisms are not pathogenic and play an important role in plant health, as they can promote plant growth through production of hormones and are involved in plant protection, by producing antibiotic compounds, competing for nutrients or by activating the plant's immune system. Though playing an important role for plant health, microbial interactions and host-microbe interactions are not well understood. To get insights into community members relevant for plant protection, synthetic communities (SynCom) were designed from a collection of 200 genome-sequenced native strains isolated from healthy *A. thaliana* plants and a previously performed screen for protection against the foliar pathogen *Pseudomonas syringae* DC3000 *luxCDABE (Pst)*. It could be shown that distinct SynComs protect the plant against *Pst* to different extent and that the complex SynCom, composed of all 210 strains, is more protective than low complex SynComs, composed of only 16 strains. An intermediate complex community composed of all strains that showed plant protection in a previous screen showed a similar protection potential as the SynCom composed of all 210 strains. These SynCom experiments suggest that not only Alpha diversity, but also presence of key strains play an important role in plant protection.

Antibiotic resistance on bacterial seed banks in sediments from environments with contrasting human impact.

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Since the discovery of penicillin, antibiotics have saved millions of human and animal lives. However the disposal of antibiotics in the environment has led to the selection of antibiotic resistant bacteria, and consequently, to the need for developing new antibiotics. This constant arms race between bacteria and antibiotic developers has left an ecological legacy. Bacterial seed banks are natural reservoir of dormant cells, which have been shown to be able to survive hundreds and up to thousands of years. This make bacterial seed banks a perfect proxy to study the past legacy of antibiotic use. Bacterial seed banks have been shown, to reflect the historical antibiotic use. This was shown through the quantification of antibiotic resistance genes (ARG) on sediment cores from Lake Geneva, a highly human-impacted lake.

In the attempt of obtaining a broader picture of this antibiotic resistance legacy in the environment, we selected two environments with low human impact and one with high agricultural impact. These sites where selected with the objective of getting a background antibiotic resistance abundance from "pristine" environments represented by the Jöri Lakes and Antarctica where humans have had a low influence in the environment. On the other hand Lake Cadagno was selected to assess the historical use of antibiotics for cattle raising.

The Jöri lakes are situated in the Vereina region in the eastern part of the Swiss Alps. These lakes originated from the retreat of the Jöri Glacier and due to its high altitude (2489 - 2730 m a.s.l) they have nearly no human impact. In Antarctica, soil samples where collected from the Terra Nova Bay and from the Barton Peninsula. The remote location of Antarctica and its harsh environmental conditions have preserved the environment from human impact. The third environment is the Lake Cadagno, located in the Piora Valley in the southeaster part of the Swiss Alps; this lake presents a high influence of cattle. The antibiotic resistance abundance in the bacterial seed banks from these three environments would be assess by the combination of a bacterial spore separation method, and the quantification of two ARGs, conferring resistance to tetracycline (*tet*(W)) and sulfonamide (*sul*1).

P03 Resistance, resilience and ecological stability of anammox consortia to transient oxygen disturbances

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Autotrophic nitrogen removal by anaerobic ammonium oxidation (anammox) is an important mechanism of fixed nitrogen elimination, both in engineered and natural systems. Deployed for mainstream wastewater treatment, it may even permit operation under energy autarky. However, process instabilities present ongoing challenges for practitioners. Oxygen and temperature are two crucial factors affecting stable operation of the anammox process in mainstream treatment. A better understanding of the key ecological mechanisms underlying microbial community performance and stability is crucial for maintaining process stability and thus for assessing the feasibility of autotrophic N removal at larger scale. Here, we investigated the performance dynamics of anammox biofilms to a series of dissolved oxygen perturbations under different temperature regimes (20°C, 14°C). Triplicate laboratory-scale bioreactors allowed us to conduct these disturbances under comparably steady conditions and in combination with high resolution process monitoring. By combining measurements of performance parameters, and omics-based community composition, biodiversity and gene transcription analysis, we aim to unravel potential key mechanisms likely conducive to the resistance and resilience of complex microbial communities.

In our experiments, transient exposures to different oxygen concentrations led to the reversible inhibition of the anammox process and, depending on the oxygen concentration, significant differences in resistance periods but not recovery times of the anammox activity. Contrary to our expectations, these stability metrics were not significantly affected by lower temperatures. Further analysis of the obtained community data and gene transcripts will give new insights into the ecological stability of complex microbial communities on the functional level, which in turn will contribute to our understanding of microbial process stability in engineered systems.

P04 Microbial carbon utilization in dry and wet Arctic tundra soils under elevated temperature

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High-arctic tundra soil ecosystems are particularly sensible to global changes due to their proximity to freezing, snow cover, light availability and scarcity of vegetation. Hydrological fluctuations in these soils are also important, where drier soils are expected to be less buffered to temperature variations, directly impacting soil biological activity. Yet, little is known on the combined effects of soil moisture and elevation of temperature on the regulation of microbial activities in high-arctic soils and their impact on carbon (C) degradation. In a laboratory experiment, the utilization of two C sources (cellulose and glycine) and the responsive microbial community involved in their degradation was investigated in dry and wet tundra soils using stable isotope probing techniques. To assess for the effect of elevated temperature on microbial C processing, the soils were incubated at two different temperatures, the averaged summer *in situ* temperature of the soils (8°C) and an increased temperature (16°C).

Microbial C utilization, as measured by fluxes of $^{\delta_{13}}$ C-CO₂, was drastically higher in the soils incubated under elevated temperature, especially in the soils supplied with the easily assimilable glycine C source. Contrastingly, soil moisture had only a limited effect on the microbial C respiration. Responsive microbial community to C utilization was mainly constituted of a few dominant taxa. In contrast to microbial C utilization, soil moisture had a major effect on both responsive bacterial and fungal community structures while incubation temperature did not affect them. Moreover, most bacterial responsive taxa were significantly correlated with utilization of cellulose rather than glycine. They mainly included taxa from the Actinobacteria, Chloroflexi and Gemmatimonatedes phyla. Fungal responsive taxa were scarce compared to the bacterial ones, suggesting the large dominance of bacteria in the C processing in these high-Arctic tundra soils. This experiment indicates a high dynamism of microbial activities and associated active microbial taxa in relation to carbon cycling in high-Arctic tundra soils, especially under warmer conditions.

P05 The *taxis* physiology of the purple sulfur bacterium *Chromatium okenii*

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Owing to their miniscule size, aquatic microorganisms (length < 100 μ m) cannot efficiently mix water by swimming. However, when in high numbers, microorganisms can trigger bioconvection, a physical mechanism in which fluid mixing is induced by a density increase at the surface of a water compartment, at this day observed only at laboratory level (in vitro). By combining in-situ measurements in meromictic Lake Cadagno, laboratory experiments and numerical simulations we recently demonstrated that bioconvection, so far observed only under laboratory settings, could induce mixing in a stratified natural water body. C. okenii, a positively phototactic and negatively aerotactic species, was able to create a sustained well-mixed layer, varying from 0.3 m to 1.2 m in thickness, located at around 12 m depth (e.g., in the chemocline of Lake Cadagno). Despite its now-demonstrated occurrence, the environmental drivers of bioconvection and its impacts on the ecological community in the chemocline remain unknown. The long-term aim of the studyis to understand the environmental drivers and ecophysiological effects of bioconvection in the chemocline of a meromictic lake from studies along spatial, temporal and ecological gradients. Here we present the taxis physiology of the motile large celled purple sulfur bacterium (PSB) C. okenii. To reach this goal, gradient of light, sulfide (H₂S), oxygen (O₂), temperature and salinity are used to determine the motile behavior of pure cultures of C. okenii. Parameters influencing the induction of bioconvection by C. okenii will be manipulated and tested using quantitative microscopy and physiological tests under controlled laboratory conditions. Phenotypic traits of *C. okenii* populations related to swimming activity will be inferred from visualization-based tools used to quantify swimming activity in response to various stimuli (e.g. gradients of sulfide and oxygen). Moreover, physiology at different incubation conditions of C. okenii will be analyzed using fitness parameter such as growth rate, ATP concentration, ¹⁴CO₂ fixation, and on a transcriptional level.

P06 Local cell distribution and habitat fragmentation affects the spread of plasmids in SOIL bacterial populations

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Transfer of conjugative plasmids among soil bacteria is an important evolutionary driver for fostering adaptation to environmental stresses such as antibiotic or xenobiotic contamination. However, plasmids impose a metabolic burden on host cells and the reason for their persistence over evolutionary times remains unclear. Here, we hypothesize that soil environments favor plasmid maintenance due to habitat fragmentation and nutrient spatial heterogeneity that promote plasmid transfer rates and reduce competition in microniches. We aim to test that hypothesis and disentangle the contributions of transmission and selection.

The soil bacterium *Pseudomonas putida* was used as donor and recipient of the conjugative plasmid pIPO2tet (conferring resistance to tetracycline). A tagging system with fluorescent proteins allowed us to visually discriminate recipients, donors and transconjugants using microscope image analysis and plating on selective media. Bacteria were grown in controlled systems of varying complexity, from agar surfaces to micromodels, and in presence or absence of tetracycline as a selective agent.

Experiments on homogeneous agar surfaces showed that transfer rate and the final size of the plasmidcarrying population increased with cell density, while competition (as measured by selection coefficient) tended to decrease. The presence of antibiotics at sub-inhibitory concentrations also affected plasmid transfer rate and selection. To address the role of spatial isolation of microhabitats, we have designed micromodels that allow us to observe local variations in the spread of bacterial plasmids, with results still pending.

Local (microscale) conditions such as cell density and spatial confinement can enhance or suppress plasmid transfer among bacterial populations, as well as affect the selective effects of carrying a plasmid. The study offers new insights linking soil microhabitats to ecological and evolutionary adaptations of soil bacteria.

P07 CO₂ and H₂S Assimilation rate of most important Phototrophic Sulfur Bacteria from the Lake Cadagno Chemocline

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The Lake Cadagno (Ticino, Swiss Alps) has always been a subject of interest because of its meromicticity. The lake is that way stratified in two layers and the chemocline, with steep gradients of especially oxygen, sulfide and light, is the contact zone between the upper oxic (mixolimnium) and the lower anoxic (monimolimnium) water. It is also called crenogenic because of the double origins of the water composing the two layers. Underground sources charged with salts (sulfates, calcium, magnesium, carbonates) compose the lower stratum. Torrents of granitics moutains origin fill the upper layer.

The presence of different gradients such as oxygen, sulfate and light influence the distribution of the microorganisms in the water column.

Particularly, the chemocline is a particular niche for anoxygenic phototrophic sulfur bacteria; green-sulfur bacteria (GSB) and purple sulfur bacteria (PSB).

Previous studies have shown that the carbon assimilation rate of the different species wasn't the same. Surprisingly the large-celled PSB Chromatium okenii representing 0.3% of total bacterial cells contributed up to 70% of the carbon fixed in the lake during the day (in presence of light). Moreover, another PSB population, the small-celled Thiodictyon syntrophicum, constituting only 1.5% of all the phototrophic sulfur bacteria fixed up to 26 % of all bulk inorganic carbon in the chemocline during day and night. GSB species Chlorobium clathratiforme constituting 73 % of total bacteria is however the least efficient in terms of carbon assimilation.

These data arouse the curiosity, yet there is a lack of consistency between the results of the different studies. That's why there is a need of specific study to have a larger and more accurate view of the carbon assimilation in the chemocline of Cadagnos' lake.

The aim of this master project is to analyze the different rate of CO_2 and H_2S assimilation of the pure cultures of the main phototrophic sulfur bacteria population of Cadagnos' chemocline. On this purpose, the strains will be studied in different condition using ¹⁴C and the bubling method. The presence or absence of light will be important to see the dark fixation of CO_2 observed by PSB. The study will be completed with transcriptomic analysis in order to have a deeper insight in metabolic mechanisms.

P08 The abundance and diversity of viruses in alpine stream biofilms

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Viruses are increasingly recognized as important drivers of microbial community dynamics and diversity, yet, we don't currently know their ecological roles in stream biofilms. The advent of metagenomic sequencing of viral genomic material now allows us to address fundamental questions related to the diversity, composition and turnover of phages in environmental settings. Here, a collection of laboratory protocols were compared to describe the purification of viruses from stream biofilm samples with the specific aim of generating viromes. We present an optimized protocol for viral metagenomics of stream biofilms including the concentration of viral particles, removal of contaminant cells and free DNA and processing of viral single- and double-stranded DNA. Overall, we present a first systematic estimate of viral abundance and diversity along a gradient from high alpine to lowland stream biofilms and relate those to key environmental parameter and biofilm properties. Our results indicate that phages play an important role in structuring biofilm communities which dominate microbial life in streams.

P09 Imaging the aerobic granular sludge microbial community using light-sheet fluorescence microscopy

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One of the aims of wastewater treatment is the removal of phosphorus before the water is discharged into the environment. Since phosphorus concentrations in wastewater exceed the requirement of bacterial growth, biological phosphorus removal is based on the ability of a group of microorganisms, named "Polyphosphate Accumulating Organisms" (PAO), to store large quantities of intracellular phosphate in form of polyphosphate.

In this study, we are focusing on the PAO actively involved in bioreactors operated with Aerobic Granular Sludge (AGS) technology. This process based on dense microbial biofilms is a cost-effective and land-saving alternative to the conventional biological wastewater treatment with activated sludge. This promising technology has received a significant commercial interest, but questions remain unanswered regarding the system performances in the context of full-scale applications.

Like many natural microbial communities, the microbial structure of the AGS is complex. Its spatial organization is shaped by the diffusion of nutrients from the external environment and by the diffusion of microbial by-products formed in the biofilm. Understanding how the AGS microbial community works requires the elucidation of its spatial architecture and development.

Light-sheet fluorescence microscopy is a powerful tool for examining microbial communities by overcoming the limitations of the classical three-dimensional confocal fluorescence microscopy. This technique shapes the excitation laser into a thin sheet providing an optical sectioning in order to illuminate the sample on a single plane. Then the emission signal is collected by a perpendicular lens. The large datasets generated are analyzed with a pipeline on the FIJI platform^[1] to extract quantitative information.

Preliminary results indicate that AGS are composed of heterogeneous biofilm aggregates. Interestingly, our observations are contrasting the AGS model structure previously described^[2].

We would like to highlight the necessity of multiple observations when highly variable structures, like AGS, are analyzed in order to capture data which are representative of the studied system.

[1] Schindelin et al. in Nat. Methods 28;9(7):676-82 (2012)

[2] Winkler et al. in Water Res. 15;46(16):4973-80 (2012)

P10 Systemic: System Efficiency of Microbial Consortia

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After decades of debates, ecologists agree that both, deterministic and stochastic processes play a role in shaping the structure of microbial communities. However, the relative importance of both processes and their implication for community functioning are still unknown. In microbial communities, the link between community structure and functioning remains elusive due to the high metabolic plasticity of bacteria. This project aims to investigate whether similar abiotic conditions can lead to communities with different structure and functioning due to stochastic community assembly.

Bacteria from the epilimnion of lake Zurich were cultivated at 3 different seasons in 20 replicated microcosms of 200 mL under identical abiotic conditions. Each microcosm contained bacteria from the same inoculum and 2 carbon sources: glucose (10μ M) and its dimer cellobiose (100μ M). Community functioning was assessed via substrate consumption and growth parameters. Community structure at the end of the experiments was elucidated by 16s rDNA amplicon sequencing.

The emerging communities showed a diverse genotypic structure and also a high variability in functional parameters. No particular relationship was found between the structure of the community and its functioning. About 1/3 of the identified genotypes were exclusive to a single microcosm. Overall evenness was low, with 3 of an average of 27 OTUs per microcosm accounting for >50% of total sequence reads. Stochastic assembly processes, likely associated with biotic interactions, had a fundamental role in shaping the structure of the experimental communities. Moreover, the observed variability in functional aspects and the absence of a link between community structure and function may have severe implications for our ability to predict ecosystem functioning and response to climate change

P11 To spore or not to spore an insight into the environmental sporobiota

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In most habitats fluctuating environmental conditions create periods of compromised survival for active organisms. In response, various strategies have evolved, including the formation of durable resting cells. We used a functional, phylogenetically unbiased approach to investigate the diversity of these resting cells in the environment. This fraction of the bacterial community was defined based on the ability of resting cells to withstand a harsh lysis method prior to DNA extraction. The community analysis yielded three highly enriched phyla: Firmicutes, Actinobacteria and Proteobacteria, all known for the production of resting and durable cells structures such as spores. However, detailed analysis of the detected taxa unearthed many genera hitherto not known to sporulate or to display an equivalent specialized lysis-resistant cell structure. The comparison of this fraction to the total bacterial community reveals that the lysis-resistant community is distinct from the total community. The identification of new taxa capable of generating a lysis-resistant cellular state beyond known spore-formers suggest that production of a durable cell structure is a widespread adaptation of environmental bacteria.

P12 Quantification of phage-to-host ration by next generation sequencing

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In human feces, viruses are found at the concentration of 10⁸-10⁹ particles per gram of feces. Major parts of the intestinal viruses are present as lysogenic phages (prophages). Prophages can be induced by environmental stimuli like antibiotics (Mitomycin C) or inflammation, leading to host lysis and release of new phage particles. Patients suffering from intestinal disease have been observed to have increased numbers of free phage particles in the intestine, raising the question whether those originate from lysogenic phage induction. The narrow host-specificity of most phages and the small number of cultivable intestinal bacteria is major limitation when studying the impact of prophage on the intestinal microbiota. We are therefore establishing a next generation sequencing approach to investigate prophage induction in uncultivable host bacteria. To this end, we use a lysogenic model, *Salmonella enterica* and its p22 phage, and murine intestinal bacteria, in which prophages where computationally predicted.

With next generation sequencing produced reads, we localize prophage in the host genome and quantify prophage induction after antibiotic treatment. Reads from lytic phage mapping to lysogens increases the coverage over the region of the lysogenic phage. By analyzing read coverage, we can localize p22 prophage in *Salmonella* and two inducible prophages in *Akkermansia muciniphila*. Furthermore, relative orientation and distance between paired end reads, will be use do indicate the origin of reads from lytic or lysogenic phages. Identification of read origin allows estimation of a ratio between lytic and lysogenic phage, where a high ratio is indicative of prophage induction. The possibility to localize prophages in metagenomics samples and to analyze their induction rate can bring further insight into phage-bacteria interaction and deeper knowledge into the impact of prophage in human health.

P13 Anaerobic Biodegradation of organohalide pollutants: a crucial step towards the elucidation of proteins involved

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Halogenated organic compounds (so-called organohalides) represent one of the major class of groundwater pollutants. The exploration of how organohalides are used as energy source is important in terms of ecosystem remediation but is also essential for the complete understanding of microbial metabolic interactions in the environment.

Organohalide respiration (OHR) is a bacterial anaerobic process in which chlorinated compound, *e.g.* tetrachloroethene (PCE), is used as terminal electron acceptor. In the present work, *Desulfitobacterium hafniense* TCE1 and *Dehalobacter restrictus*, our model organohalide-respiring bacteria (OHRB) harbouring the *pceABCT* gene cluster, will be considered for the study of PCE respiration. To date, the function of PceA, the key catalytic enzyme in the process, and PceT, the dedicated molecular chaperone for PceA maturation, are well defined. However, the roles of PceB and PceC are not yet elucidated and the biochemistry of OHR electron transfer is still relatively elusive. Based on the genetic composition of the *pce* gene cluster, the hypothesis of a possible PceABC respiratory complex is tempting but the question remains largely unanswered.

The present work represents an evaluation of the stoichiometry of PceA, PceB and PceC proteins via quantitative proteomics applied to the membranes fractions of our model organisms. In a second phase, the use of Blue-Native electrophoresis technology will be considered to investigate whether PceC participates in a membrane-bound protein complex together with PceA and PceB. The complementary results of both techniques will lead to identify the three proteins of interest in the membrane and is expected to shed light on the presence and composition of a PCE respiratory complex.

P14 Spatial niches and temporal dynamics of planktonic freshwater methanotrophs

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Lakes and reservoirs significantly contribute to global methane budgets, but Methanotrophs in these systems are important regulators of methane emissions. In stratified lakes with an anoxic methane-rich hypolimnion, the water column is the main site of methane oxidation. We have conducted studies in several Swiss lakes to determine the niche ecology of methanotrophs in such systems.

Lakes were characterized physicochemically using a combination of in-situ probes and laboratory analysis. Microbial populations in general and methanotrophs in particular were characterized by amplicon sequencing of 16S rRNA and *pmoA* genes and their transcripts.

Overall, the lakes were shown to each harbor distinct communities of methanotrophs, while sharing a small number of ubiquitous taxa. The traditionally proposed site of methanotrophy, i.e. the lower end of the oxycline overlapping with the methane gradient, was often marked by a maximum of the overall methanotroph abundance and activity but a clear abundance peak in this region is only observed for few taxa. Methanotroph activity and transcriptionally active methanotrophs are present over a much larger segment of the water column. Some methanotrophs are mostly found above the transition zone or even peaking in the high oxygen, low methane epilimnion, potentially taking advantage of epilimnetic methane production. Others reach maximum abundance in the anoxic bottom waters, where their presence may be linked either to cryptic photosynthetic oxygen production, alternative electron acceptors (i.e. Ca. Methylomirabilis limnetica in some permanently stratified lakes), or perhaps to switching to alternate catabolism. Autumn lake mixing was shown to lead to a methanotroph bloom that largely prevented methane outgassing and that was accompanied by a succession of methanotroph species that successfully competed under these conditions.

Similar to the "paradox of the plankton", aerobic methanotrophs compete for simple resources (O_2 and CH_4) and the question how they maintain diversity in the lake environment was poorly understood. Our results indicate that methanotrophs have distinct trait combinations that allow them to occupy specific spatial and temporal niches. This trait diversity may be important for the efficiency of the methane filter of lakes and provides a basis for improved modeling of methanotrophy in dynamic systems.

P15 Diversity of the insect pathogenic fungi *Metarhizium* spp. and *Beauveria* spp. in soils of three different habitats

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Insect pathogenic fungi of the genera *Metarhizium* and *Beauveria* are important natural antagonists of arthropods. Due to their function, they have a high potential for use in biological control of pest insects. Presence and abundance of these fungi in the environment depend on many abiotic and biotic factors as well as habitat-type and composition. Furthermore, in an agricultural context, factors like crop management and protection may affect their presence and distribution. Comprehensive knowledge on all these factors is important for the application of these fungi as biological control agents, particularly in conservation biological control approaches, which rely on natural occurrence of the fungal antagonists.

The goal of this study was to investigate abundance and population structure of *Metarhizium* spp. and *Beauveria* spp. in three habitat types, i.e., wheat, permanent grassland and forest and to assess, how they may be affected by habitat types and/or various physical, *chemical* and microbiological parameters.

The study was performed at 30 sites that are part of the national soil monitoring network (NABO), in which soil physical, chemical and microbiological parameters (biomass, soil respiration) are monitored in wheat, permanent grassland and forest sites since 1984. The 30 sites included 10 sites of each of the three habitat types, distributed across Switzerland. Fungi were isolated using a selective medium (SM) from three bulk soil samples, each consisting of a mixture of 25 soil cores collected from a 10x10 m area per site in 2016. A total of 346 *Metarhizium* spp. and 202 *Beauveria* spp. isolates were collected from the 30 sites (0 to 18 isolates per species and site). *Metarhizium* spp. were present in 8, 10 and 4 of the 10 wheat, permanent grassland and forest sites. Whereas *Beauveria* spp. were present in 8, 4 and 9 of the 10 wheat, permanent grassland and forest sites.

Multi-locus genotypes were determined for all the isolates applying 15 microsatellite markers each for *Metarhizium* spp. and *Beauveria* spp. The genetic structure for *Metarhizium* spp. and *Beauveria* spp. will be determined for each site and correlations with physical, chemical and microbiological parameters available assessed to determine factors affecting presence and abundance of *Metarhizium* spp. and *Beauveria* spp. in soil.

Dissimilarities in polyphosphate accumulation between *shewanella loihica* and the model bacterium *shewanella oneidensis*

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Polyphosphate plays an important role in the physiological adaptation of bacteria during growth and development, as well as in their response to nutritional and environmental stresses. Moreover, polyphosphate accumulation is of environmental relevance and is linked to the formation of phosphate minerals. In particular, ferrous iron phosphate minerals represent a key step in the recycling of phosphorous in aquatic and terrestrial ecosystems. In this study, we investigated the polyphosphate accumulation potential of two selected species of iron-reducing *Shewanella*.

We studied polyphosphate accumulation in *Shewanella loihica* by a combination of methods including indirect and direct polyphosphate measurements. During iron reduction in absence of an external phosphate source, we observed a correlation between polyphosphate accumulation, orthophosphate release and production of ferrous phosphate minerals. We also used *Shewanella oneidensis*, the well-characterized iron-reducing bacterium, as model organism in our study. In contrast to *S. loihica*, the ability to accumulate polyphosphate appears to be less pronounced in *S. oneidensis*. These physiological data were confronted with the analysis of the genomes of both species. Genes for phosphate transporters (*pit, pst*), polyphosphate kinase (*ppk*) and exopolyphosphatase (*ppx*) were identified in the genome of *S. loihica* and *S. oneidensis*, but the *ppk* gene is disrupted in *S. oneidensis*. Expression of these genes during aerobic growth and anaerobic iron reduction are currently under scrutiny by RT-qPCR analysis. We will discuss the results obtained in the context of biogenic iron phosphate mineral formation, the formation of such minerals in natural and manmade ecosystems, as well as their potential use for the recovery and recycling of phosphorous.

The assembly of finished genomes from low complex communities and novel insights we gain into strain diversity and an active phage systems

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The completeness and contiguity of genome assemblies greatly improves the quality of subsequent systems-wide functional profiling studies and the ability to gain novel biological insights. While a *de novo* genome assembly of an isolated bacterial strain is straightforward in most cases, more informative data about co-existing bacteria as well as synergistic and antagonistic effects can be obtained from a direct analysis of microbial communities. However, the complexity of metagenomic samples represents a major challenge. While third generation sequencing (TGS) technologies have been suggested to enable finished Metagenome-Assembled-Genomes (MAGs), it has not been shown so far. Natural whey starter cultures (NWCs) are used in the production of cheese and represent low complex microbiomes. Previous studies of Swiss <u>Gruyère</u> and selected Italian hard cheeses, mostly based on amplicon-based metagenomics, concurred that three species generally pre-dominate: *Streptococcus thermophilus, Lactobacillus helveticus* and *Lactobacillus delbrueckii*.

We subjected two NWCs from Swiss Gruyère producers to shotgun metagenome sequencing using both Pacific Biosciences (PacBio) Sequel and Oxford Nanopore Technologies (ONT) MinION platforms.

We achieved the complete assembly of all dominant bacterial genomes from these low complex NWCs. Moreover, we successfully co-assembled two distinct *L. helveticus* strains from the same sample. Besides bacterial genomes, we were also able to assemble bacterial plasmids as well as phages and a corresponding prophage. We could uncover biologically relevant insights by linking all the plasmids and phages to their respective host genomes using DNA methylation data on the plasmids and by matching several prokaryotic CRISPR spacers with the corresponding protospacers on the phages. These results could only be achieved by employing long reads able to span intragenomic as well as intergenomic repeats.

Here, we demonstrate the feasibility of complete *de novo* genome assembly of all dominant strains from microbial communities using third generation sequencing data. This was achieved directly from low complex NWCs. This enables new biological question to be answered and is the fundamental basis for all further downstream 'omic analyses, functional profiling and phenotype to genotype analysis of microbial communities.

P18 RDHK Family, Regulators dedicated to Organohalide Respiration - Sequence Diversity and Functional Prediction

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Organohalide respiration (OHR) is an anaerobic metabolism by which bacteria conserve energy from the use of organohalide molecules as terminal electron acceptors. Because most organohalides of anthropogenic origin are persistent pollutants, the study of bacteria capable of OHR (OHRB) has a strong environmental interest.

OHRB appear in three major phyla (Firmicutes, Proteobacteria and Chloroflexi) and encode at least one reductive dehalogenase (RdhA) in their genomes. RdhA enzymes are the key catalytic subunit in OHR and their corresponding genes are often surrounded by accessory genes organized in *rdh* gene clusters. Among them, *rdhK* is coding for a member of the CRP/FNR superfamily of transcriptional regulators and is dedicated to the regulation of *rdh* genes in Firmicutes. Upon binding of an effector molecule (i.e. organohalide) the regulator recognizes a specific DNA motif in the promotor region of the target genes in order to recruit the RNA polymerase and activate transcription. So far, based on the characterization of only three RdhK proteins, it was assumed that one RdhK regulator senses specific organohalide compounds which are the substrates of the RdhA enzyme encoded in the same *rdh* gene cluster. Therefore, the identification of the binding partners (both effector molecule and target DNA sequence) for each new RdhK regulator can be used as an indirect way to define potential substrates of yet uncharacterized RdhA enzymes.

Firstly, we aimed to study the diversity of RdhK regulators in Firmicutes. In this regard, BLAST search was used to identify CRP/FNR regulators encoded by genes located in *rdhA* direct vicinity in the available genome sequences of *Dehalobacter* spp. and *Desulfitobacterium* spp. This resulted in a list of 96 RdhK sequences that will be used for sequence similarity analysis in order to define RdhK subgroups. Moreover, a tentative identification of signature motifs will be done through the analysis of individual subgroups. Finally, our RdhK sequence database will be confronted to the available knowledge on organohalide specificity of characterized RdhA enzymes. This should help predicting the range of effectors and substrates for pairs of RdhK regulators and RdhA enzymes, respectively. Such correspondence could possibly lead to a common type of effectors for a given subgroup of RdhK regulators which would have to be verified through biochemical work.

P19 Harnessing the Power of Plant-associated Bacteria for Crop Protection

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Plants are colonized by a diverse microflora both at the root and at the shoot level. These plantassociated microbes have evolved traits that enable them to compete with other microbes for these mostly nutrient-rich plant habitats. Different plants haven been shown to select different microbial communities when grown in the same environment. Recently, it was demonstrated that infection of plants with pathogenic organisms could lead to the recruitment of specific microbial populations, which contribute to plant defence against diseases. The overall goal of our research is to understand by which mechanisms plant-associated bacteria contribute to their host growth and defence against pathogens. Using the model plant A. thaliana as well as agronomically relevant plants such as potato and grapevine, we present examples of recent and ongoing research demonstrating how volatile and non volatile compounds produced by plant-associated bacteria belonging to the genera Pseudomonas, Streptomyces and Paenibacillus, contribute to plant growth and health. In particular, we show that in addition to the already described effect of indole, other volatile metabolites trigger changes in root growth pattern in Arabidopsis as well as induction of resistance genes. We further show how non-volatile metabolites from soil-borne Actinomycetes inhibit Botrytis development both in vitro and in planta. We finally discuss different strategies on how to best harness the protective potential of potato-associated Pseudomonas against Phytophthora blight, comparing the efficacy of using either the bacterial metabolites or the strains, alone or in combinations. In view of the challenges of food production in a changing world, we believe that a better understanding of the crops as holobionts and of the services provided by their associated microbes will help us design more sustainable strategies for crop protection.

Preferred mode or presentation: oral Presenting author: Laure Weisskopf

P20 Dissecting the Bistable Switch Governing the Activity of a Mibile Element in Pseudomonas

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Evolution has shaped a myriad of genetic programs allowing bacteria to colonize specific niches and adapt to changing conditions. More surprisingly, among populations of isogenic cells, adaptive strategies have evolved that lead to subpopulations displaying strong phenotypic differences. The case we study here is a bistable program invoked on host cells of the bacterium *Pseudomonas knackmussii* by a mobile genetic element named ICE*clc*. In most cells, ICE*clc* is and remains stably integrated in the bacterial chromosome, but in 3-5% of cells the element becomes active, leading to its excision as a circular molecule that can transfer to other cells by conjugation. Transfer requires the initiation and completion of a differentiation program named transfer competence development, but the mechanisms controlling the bistable switch between non-active and transfer competence have remained unclear. Using targeted mutations, RNA-seq, single-cell fluorescent ICE*clc* activation. We further reconstructed the minimal gene set necessary for developing bistability in a *Pseudomonas* strain devoid of ICE*clc* and showed how to control variable outputs. A stochastic mathematical model was built that explains the network structure necessary to generate bistability. This model may be further used to describe different types of bistability in various biological processes.

Hydration dynamics alters species composition of a representative soil bacterial community inhabiting unsaturated porous Microcosms

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The central role of soil hydration in controlling soil microbial diversity and function has been studied with focus on static effects on spatial arrangement of soil microhabitats. The aim is to explore systematically effects of hydration dynamics on microbial community composition and species abundance in a well-defined soil bacterial community.

We have used a representative soil community of 11 soil bacterial species inoculated in unsaturated glass bead microcosms. We hypothesized that hydration dynamics will affect species abundance and community composition over time. The microcosms were exposed to either dry/wet constant conditions or to repeated 6-days cycles of drying and rewetting mimicking soil conditions following irrigation or rainfall. Community DNA was extracted every two days and species abundance was assessed using microfluidic qPCR.

Results illustrate that the main changes in community composition occurred within the first 2 days of incubation irrespective of hydration and nutrient conditions. The initially even bacterial community became dominated by *Arthrobacter and Pseudomonas* species under all conditions, while other species (*Bacillus subtilis, Micrococcus luteus*) rapidly declined. The community composition was relatively similar across hydration and nutrient conditions. However, certain species exhibited distinct growth dynamics and significant differences in their relative abundances as function of hydration after 12 days. Several species thrived under constant wet conditions (*Rhizobium etli*) while others (*Streptomyces violaceoruber and Escherichia coli*) were more abundant under dynamic regimes, supporting our hypothesis.

This work demonstrates that hydration dynamic in soil-like habitats play an important role in shaping soil bacterial community composition and species abundance irrespective of nutrient and biological factors. The dominance of community selection by the physical environment stands in stark contrast to behaviour in well-mixed laboratory studies (liquid cultures).

P22 Crowdsourcing and functional redundancy in the mobile gene pool of the honey bee gut microbiota

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The honey bee gut microbiota has recently emerged as a model system for the study of host-microbe interactions and community ecology due to its simplicity and experimental manageability. Eight core lineages make up to 98% but also show high intra-species diversity. Horizontal gene transfer (HGT) has recently emerged as the main evolutionary force driving protein family evolution, yet little is known about its impact on community assembly and structure and its frequency in natural communities. We hypothesize that HGT plays a central role maintaining intra-species diversity by dispersing functional redundancy across strains or as a means to distribute the burden of metabolically costly functions as common goods.

With a genome database of 230 strains, we characterized the mobile gene pool of the honey bee gut microbiota. We identified mobile element machinery (vectors) using a structural detector of macromolecular systems, and we inferred recent putatively transferred regions (cargo) independently through a statistical approach. We defined a set of HGT regions as those with vector-cargo associations, and determined recent activity by looking at coverage discontinuities in metagenomes.

Transposons and insertion sequences were the most abundant vectors in the mobile pool, but integrative elements contained the largest regions. A large number of genes were involved in restriction-modification and secretion systems. Functional characterization of cargo revealed a large bias towards carbohydrate and aminoacid metabolism, which suggests the mobile pool is predominantly increasing functional redundancy of functions with small or temporal selective advantages, and on defense and self-identification systems.

Flow plays a key role in determining the bacterial degradation dynamics of prototype marine aggregates.

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The oceanic biological carbon pump is an important part of the global carbon cycle, generating a massive carbon flux of particulate organic carbon (POC) from the ocean surface to its interior. While it is known that microbes are key players in controlling the fate of POC, we lack a mechanistic understanding of the biochemical and physical interactions by which microbes drive POC degradation. Here we test the effect of flow, a natural consequence of sinking, on bacterial degradation dynamics of model marine snow. Using microfluidics to mimic sinking, we visualize a colonized synthetic aggregate in laminar flow while quantifying bacterial growth and degradation. We found that by altering the concentrations of breakdown products within the particle boundary layer, flow changes the bacterial growth and degradation dynamics. While in low flow rates, aggregate degradation was slower, at high flows, lag time of the bacterial population increased dramatically and hypothetically extended the distance traveled on the particle before dispersal. Altogether, these findings demonstrate the significance of sinking speed in altering marine-snow degradation dynamic and controlling the distribution of particle-habituating bacteria in the water column. Characterizing the bacterial behaver as it changes with sinking speed can potentially advance the way we model and understand the biological carbon pump of the ocean

P24 Unveiling biofilm architectural differentiation in sedimentary environments

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Hydrated porous systems with their high surface availability are attractive substrates for biofilm growth. A prime example of such a system is the hyporheic zone of streams hosting diverse microbial communities concealed within the sediment interstices. Within this sedimentary environment, topographic heterogeneity and fluid flow constrain biofilm growth by altering solute supply and hydrodynamic stress. The interplay between hydrodynamics and biofilm architecture and function in porous systems remain poorly understood. To better appreciate these links, we designed porous-like fluidic devices, exposed to streamwater flow containing bacterial cells. Biofilm formation and local hydrodynamics were investigated using time-lapse microscopy and micro-particle image velocimetry. We found two different architectural differentiation was the result of biofilm growth on the grains and of cell retention by the streamers. We also found that biofilms competed for space and substrates, and that the differentiation into streamers and coating biofilm was beneficial to the entire biofilm. Our work advances previous studies on streamer formation in porous systems and highlights the importance to work with diverse microbial communities rather than monospecies systems if the biophysical processes that shape the most successful mode of microbial life is to be understood.

Keywords: biofilm morphology, porous-media, filtration

P25 An automated system for OCT imaging of biofilms

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OCT is an emerging imaging technique in biofilm research. It permits the characterization of biofilms 3D morphology and structural parameters at the mesoscale, effectively implementing new opportunities to understand the structure-function relationship in these microbial communities. However, currently the amount of data that can be acquired and processed by OCT is limited to few cm², restricting statistical inference of large scale spatial patterns in biofilm research. We introduce an automated OCT imaging system that allows covering larger spatial (up to 100 cm²) and extended temporal scales (up to months) of biofilm morphogenesis. To achieve this, we have combined a commercially available OCT system with a robotic platform and developed a suite of software solutions to control the positioning, as well as acquisition and processing of 3D biofilm imaging data.

We demonstrate the possibilities offered by this system with a flume experiment investigating structural differences of phototrophic biofilms grown under a gradient of flow velocity.

P26 Biodiversity in soils: the role of fungal driven dispersal in the spatial structuration of bacterial competitors

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Understanding why soil bacterial communities are so diverse is a fundamental question in soil microbial ecology. One outcome to competition is the spatial segregation of competitors. Therefore, the spatial structuration of bacterial communities is a key concept in the understanding of how competitors might coexist. Spatial structuration of bacterial communities has been particularly addressed for colonies. The structuration occurring in dense bacterial colonies on agar plates has been shown to stem from mechanisms as simple as the physical constraints linked to the shape of the bacterium or the genetic drift occurring at the expanding front of a colony. This latter case was shown to be tightly linked with the outcome of bacterial interactions, because it segregates different genotypes in clonal sectors. Reciprocally, the type of interaction is thought to modulate spatial segregation. Even if a considerable amount of work has been done on these colonies, a key element that has been poorly addressed is dispersal. In soils, bacteria can disperse along fungal hyphae, in a process called "fungal highways". "Fungal highways" have the potential to profoundly modulate the structure of soil bacterial communities by connecting otherwise separated bacterial populations, allowing access to inaccessible resources, or by promoting differently the dispersal of bacteria. Therefore, we are interested in understanding how fungal-driven dispersal might affect the spatial structuration of soil bacterial competitors. This will be addressed under three perspectives: the fungal network as 1) a dispersal event affecting an already-structured community 2) a segregation opportunity for mixed colonies 3) a connection between spatially distinct populations. To explore this, we will use a two-strains system: Pseudomonas putida UWC1 (mCherry-tagged) and its wild-type counterpart KT2440 (GFP-tagged). These two strains have the same niche, but differ in growth rate as UWC1 carries a costly antibiotic resistance. Their position will be monitored by fluorescence microscopy and imaging. The fungal partner will be Trichoderma rossicum, on which both bacterial strains can disperse. Experimental work will be completed by spatially-explicit modelling. Our study aims at understanding how the dispersal on a structured biotic network modulates the coexistence of competitors. Moreover, we also aim at exploring the mechanisms behind the potential structuration and partition on the fungal network.

P27 The fungal microbiota: who is there and why?

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Many organisms live in symbiosis with endobacteria. Such interactions are well-known in animals and plants. However, even though endobacteria are common in fungi, little is known about their relationship with the fungal host. Fungal endobacteria has been mainly studied in arbuscular mycorrhizal fungi as well as in the rice pathogen Rhizopus microsporus. In the case of the latter, virulence is actually due to a toxin produced by its endobacterium Burkholderia rhizoxinica. The same bacterium manipulates sexual reproduction in the host to ensure its transmission across generations. These examples highlight the very significant impact that endobacteria can have on the biology of their fungal host. Therefore, studying other fungal species and their endobacteria is mandatory to understand the biology of fungi and their role in different ecosystems. The aim of this study is to assess the impact of endobacteria on fungi under different environments. Three fungal species are used: two strains of Fusarium culmorum, one strain of Trichoderma harzianum and one strain of Trichoderma rossicum. First, the 10 most abundant endobacteria present in the different fungal host were identified by direct sequencing. In order to assess the impact of endobacteria on the different fungi, an antibiotic treatment will be used to create endobacteria-free strains. The treatment consists of a mixture of kanamycin, ciprofloxacin and imigenem at different concentrations. Once a curated strain is obtained, a comparison of the fungal phenotypes will be performed between the curated and the non-curated strains. The fungal phenotypes will be assessed under different pH, humidity and temperature conditions. In order to ensure that the potential phenotypic changes observed are due to the lack of endobacteria and not to the side-effects of the antibiotic treatment, the endobacteria will be reintroduced inside the curated strains. The results of this project will contribute to our understanding of the role of endobacteria in fungi, especially in the case of the cosmopolitan Fusarium and Trichoderma species.

P28 Inactivation of humal viral pathogens by protists: toward biocontrol of viral contaminants?

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Human viruses are widespread in the water environment and pose a risk to human health. Viral pathogens can persist in aquatic ecosystems and cause human viral infections via water or food. Among the different factors governing the environmental persistence of enteric viruses, the removal of viruses by indigenous microorganisms, in particular virus predation by protists, has received little attention to date. This study aims at determining the contribution of indigenous protists to human virus inactivation in different surface waters. Materials and Methods. Samples were taken from Lake Geneva, the Mediterranean Sea and the Atlantic Ocean, and were filtered to discriminate microorganisms based on their size (total, eukaryotic, bacterial and sterile fractions). Additionally, protists were isolated from marine water samples by serial dilution. Inactivation experiments were performed by incubating the different water fractions with Human Echovirus type 11 (E11), and monitoring its inactivation over time. The effect of temperature on the antiviral action of protists was also investigated. Results. Incubation of viruses in biologically active waters led to efficient inactivation of E11 (2.5-log reduction within 48 hours at 22°C), whereas inactivation in sterile controls was minor (0.8-log reduction). This inactivation could mainly be attributed to the action of protists in the eukaryotic fraction of the samples. The inactivation of viruses was shown to be temperature-dependent, with a complete inhibition of biological inactivation at 4°C. In addition, inactivation depended on both the species of virus and protist. For all waters tested, the removal of E11 was more efficient compared to that of the larger Human Adenovirus. Among three protist isolates tested (Paraphysomonas sp., Uronema marinum and Caecitellus paraparvulus), Caecitellus paraparvulus was the most efficient at removing_E11 (3-log reduction over four days with an initial protists concentration of 103 cells \times ml-1). Conclusions. Protists present in surface waters are important biological contributors to the inactivation of E11. These results pave the way for further research to understand better how protists control human viral pathogens in aquatic ecosystems and how microbial inactivation could be exploited as a water treatment solution to enhance the inactivation of viruses.

Keywords: Protists, Grazing, Human viral pathogens, Virus inactivation, Water treatment

P29 Biofilm formation in porous media: a microfluidic approach

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The subsurface is a complex environment in which the presence of several phases creates numerous interfaces (solid-liquid, liquid-gas and solid-gas). In the fields of bioremediation and ecology, it is of growing importance to understand the interplay between hydrodynamics and biogeochemical processes. In natural subsurface environments, microorganisms are found in large numbers in the pore space, within a mosaic of regions of high and low flow velocity. These diverse conditions permit microorganisms to live in the free-swimming phase and to form surface-attached communities known as biofilms. The growth of biofilms influences pore geometries and thus redirects the flow, which in turn affects biofilm development and mass transport. To obtain a mechanistic understanding of this interplay at microscale, we study a soil-born microorganism, Bacillus subtilis, in porous media analogs created in microfluidic devices.

Our microfluidic experiments were performed in carefully designed porous geometries, in which different flow rates could be imposed. The devices were exposed to a constant flow of nutrient broth after being seeded with bacteria, and over a period of 48 hours, biofilm growth was continuously imaged using automated video microscopy.

The rate of biofilm growth is influenced by both hydraulic and geometric parameters of the porous medium. For the same porous geometry, the initiation of biofilm formation and the definition of preferential flow paths occurs earlier in time with increasing flow rate. For higher flow rates, the opposite is found. After the formation of preferential flow paths, their intermittent opening and closing behavior takes place. Besides the flow rate, this behavior is also controlled by the biofilms rheological properties, which enables it to accommodate flow and pressure differences.

The results shed light on the mechanisms involved in biofilm formation, clogging and its impact on the hydraulic properties of porous media, with implications for industrial and environmental systems.

P30 Quantifying the dynamics of motility and chemotaxis of marine bacteria in situ

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Chemotaxis and motility are used by many heterotrophic bacteria to exploit nutrient hotspots in the heterogeneous physicochemical landscape of the ocean. This is of great ecological importance because rates of biogeochemical cycling by microbes within these microscale hotspots are thought to greatly exceed rates in the surrounding bulk. However, we know little about the proportion of bacteria that are motile, the key molecules inducing chemotaxis, the environmental conditions that promote these traits, and the identity of the bacteria involved. These knowledge gaps limit our ability to scale up the role of bacteria in key biogeochemical processes.

Here we present results from newly developed field-based microfluidic devices that allow in situ chemotaxis (ISCA) and motility (ISMA) assays. We quantified chemoattraction during a phytoplankton bloom towards selected carbohydrates. Our results show that bacteria were attracted to laminarin, dissolved organic matter and healthy phytoplankton extracts at specific time points of the bloom. Combined with a suite of environmental data, these assays promise to be precious tools to study the dynamics of motility and chemotaxis, the conditions that select for these traits, and ultimately to get a better handle on their ecosystem-level consequences.

Differential gene expression of pseudomonas veron II 1YdBTEX2 in different polluted soils: an overview of how microorganisms adapt to polluted environments

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The natural recovery of the soils polluted by aromatic hydrocarbons such as BTEX (benzene, toluene, ethylbenzene and xylene) can be enhanced by introducing specific biodegrader bacteria in a process named bioaugmentation. Owing to their unique metabolic properties and to their adaptation potential these bacteria survive and propagate in the contaminated environment at the expense of the degraded pollutant. Yet, inoculation attempts frequently have not the expected success due to insufficient adaptation and survival of the inoculants during transition into the new systems. In this project, we studied the adaptive response of the BTEX biodegrader Pseudomonas veronii 1YdBTEX2 to different soils using laboratory scale microcosms. We performed an RNA-seq analysis of the immediate genome-wide transcriptional response of P. veronii to toluene in three different non-sterile soils (sand, silt and clay). The genes whose expression changed the most were genes encoding for transposases and integrases, electron transport systems, and pili-flagela synthesis. Other interesting individual genes whit increased expression includes several genes for hypothetical proteins. These genes might play an important role in coping with adaptation to soil environments. Stable growth and survival of P. veronii 1YdBTEX2 in the microcosms for extended periods of time (7-10 days) suggest that the immediate response mechanisms disclosed in this study are efficient for long-term establishment in the polluted soil. Yet the general understanding of factors that leads a better strain survival in non-sterile soils with native communities is very limited, our work may contribute to identify important features to enhance the survival of strains with biodegradation potential or to design synthetic communities de novo.

High-throughput monitoring of microbial community assembly in controlled microenvironments to understand the building blocks of the ocean's microbiome

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Marine bacterial communities play a pivotal role in the ocean's carbon pump, the process responsible for sequestration of carbon into the deep ocean and a major mechanism regulating atmospheric CO2 levels. Carbon degradation by these communities depends not on the properties of a single species, but rather the composition of the bacterial ensemble in which a diverse set of microbes perform different functions. However, what determines the composition of these diverse bacterial communities remains poorly understood. Therefore, I study the initial, critical phase of development of such diverse communities of marine bacteria that degrade particulate carbon. I will test two alternative hypotheses, namely whether the community composition is mostly determined by migration from the bulk or interactions within the community. To do so, I will be applying state-of-the-art time-lapse microscopy to quantify the migration towards and subsequent growth on organic surfaces in controlled micro-environments, in which I can monitor many communities forming in parallel.

I will use a set of seven strains derived from a natural chitin degraded community in which different strains have different properties in terms of motility and metabolism. The inclusion of both controlled migration using motility and growth on organic surface substrates is an important departure from both well-mixed batch cultures and traditional biofilm studies and may provide new connections between the role of bacterial motility and ecology.

Preliminary results show the successful filling and solidification of chitosan hydrogel plugs in wells of 0.3 mm in diameter, while obtaining little to no hydrogel deposition outside the wells. Current effort is involved in simplifying the protocol expanding this protocol for alginate hydrogels. Next step will be testing and observing the influx and growth of a single fluorescent labelled marine bacterium.

This direct, quantitative and temporally resolved view will be an important new vantage point to quantify microbial processes in the ocean and, more broadly, will contribute to a better understanding of the processes driving biological diversity.

P33 Harnessing bacterial fungal interactions for the biorecovery of valuable metallic compounds in industrial waste

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Industrial waste is a growing fraction of the waste produced worldwide. It consists of both inorganic (e.g. discarded electronic equipment - e-waste), and organic waste (e.g. digested sewage sludge). Both contain valuable compounds such as precious metals and phosphorous, which are non-renewable resources. To sustain the development of modern societies, the demand for these resources will keep on growing, along with the depletion of natural mining sites. This results in environmental, ethical, and economical concerns. This has led to the development of the concept of urban-mining, which proposes to use industrial waste as a mine for traditionally non-renewable resources. At present-day, the recycling of industrial waste is still in its infancy. Current methods in metal recycling consist in relatively polluting approaches (pyro- and hydrometallurgy). As a result, industrial waste recycling is a timely issue that requires innovative and sustainable approaches. Industrial waste typically consists of a heterogeneous matrix of materials and thus a parallel can be drawn to other complex systems such as soils. Microbial interactions in soil are essential to maintain biogeochemical cycles. In this project, we aim at exploring the possibility of using bacterialfungal interactions (BFI) to develop an innovative process for the biorecovery of selected valuable compounds from industrial waste. We propose to harness both, BFI and the fungal highway (FH) mechanism as a logistic tool. The idea is to take advantage of bacterial and fungal biogeochemical capabilities towards metals, together with synergistic mechanisms that take place upon BFI and FH in order to recover metals in minute concentration from heterogeneous matrices such as e-waste and digested sewage sludge. The experimental design consists in enrichment and isolation of metal-resistant bacterial-fungal consortia from metal-rich samples, followed by the assessment of their metal mobilization and immobilization capabilities. In parallel, microcosm trials with actual industrial waste are set-up to design a process that could be scaledup. By harnessing the natural interactions that exist between microbes, along with their ability to act as chemical reactors, an ecological, economical, and ethical strategy for the biorecovery of metals could be developed for the field of urban-mining.

P34 Isolation of bacterial fungal couples and their interaction with AG and CU in order to ensue nanoparticules (NPS)

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Natural ecosystems are drastically affected by the environmental pollution from hazardous heavy metals and waste materials, to the detriment of sustainable development of humans. The source of these pollutants can be anthropogenic from urbanization, industrialization and extra usage of agricultural practices, but also from natural disasters such as hurricanes and volcanic eruptions. Fungi, alone or in synergistic interaction with bacteria, display a large pallet of features that can be harnessed in the bioremediation of polluted water, soil and air. Bioremediation is a natural and costeffective approach that implies the application of natural organisms such as bacteria, fungi, yeast, algae, plants to recover metals for instance. One way to improve bioremediation is to properly isolate and discover specific tolerant microorganisms from soils with high concentrations of metals. Some species of bacteria and fungi have been described as being able to produce nanoparticles (NPs) during bioremediation procedures. As the application of NPs has become an emerging area of nanoscience and nanotechnology, where they can provide solutions to technological and environmental challenges, generating NPs through waste material represent an innovative approach in bioremediation. In this project, different type of microbial consortia, consisting of bacteria and fungi already present in the soil, will be tested for their ability to degrade and bioremediate metal-containing industrial waste. Suitable heterotrophic consortia of bacteria and fungi able to bioremediate silver (Ag) and copper (Cu) in the form of intracellular NPs will be enriched and isolated. To do this, microcosms with four different type of matrices: forest soil, digested sewage sludge, soil from a shooting stand and urban compost will be setup. Each type of matrix will be incubated with a printed circuit board (PCB) to select for metal tolerant organisms. After a given incubation time, organisms that will have actively colonize the PCB will be analyzed using fungal highway columns in order to select for interacting bacteria and fungi. Developing and also understanding microorganism communities response to metals, will allow us to better take advantage of the multidisciplinary technique 'bioremediation'.

P35 Endofungal bacteria – New insights into bacterial-fungal coexistance

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Bacteria and fungi coexist in various microhabitats and establish interactions ranging from mutualism to antagonism. Such interactions can impact higher trophic levels, as well as nutrient cycling. The interaction between fungal-bacterial interacting partners is dynamic and can rapidly change in response to changes in environmental factors. The same is true for interactions occurring at the cellular level. Bacteria and fungi in close physical contact show relationships yet consists in bacterial colonizing inner hyphae (endobacteria).

We have investigated the diversity of both, endobacteria and bacteria firmly attached to hyphae in 130 fungal strains. Amplicon sequencing of the 16S rRNA gene was used to identify bacterial species in DNA extracted from individual fungal cultures.

We have discovered that endobacteria are much more frequent than assumed. Moreover, they seem to appear equally distributed in the phyla Basidiomycota, Ascomycota and Zygomycota, and also occur in the distinct phylogenetic lineage of the eukaryotic fungus-like Oomycota. We have started to investigate the rules underpinning this close association. Under environmental conditions affecting negatively the fitness of the fungal host, we have observed for several fungal models that this tight association turns to a loose coexistence.

Defining the conditions triggering changes in the type of interaction between both partners are key to understand the dynamics of bacterial-fungal interactions. Such discovery is essential for a better definition of the general mechanisms behind these interactions and their role in microbial ecosystem functioning.

P36 DIRECT MEASUREMENT OF THE PHYCOSPHERE VIA RAMAN MICROSPECTROSCOPY

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The phycosphere is the region immediately surrounding a phytoplankton cell that is enriched in organic molecules exuded by the cell into the surrounding water. Its size determines the scope of ecological interactions between phytoplankton cells and associated bacteria in aquatic environments. In this study, we aim to provide direct measurements of the size of the phycosphere around live phytoplankton cells using Raman microspectroscopy.

As a test sample, we used a marine planktonic diatom, Chaetoceros affinis, measured within a glass chamber in a still fluid. Using a commercial Raman microspectroscope operating with a 532-nm laser, we measured the resonance Raman signal of the carotenoid secreted from the phytoplankton cell by line scanning from the cell surface to the surrounding seawater in depth and lateral directions.

We determined that the phycosphere of C. affinis measures up to 40 μ m and 100 μ m in lateral and depth directions from the cell surface, respectively. As the density of the carotenoid is lower than that of the surrounding artificial seawater, the secreted carotenoid tends to travel upward and forms a plume above the cell.

The size of the phycosphere is strongly influenced by the size of the phytoplankton cell. We envisage that further Raman measurements of variously-sized phytoplankton cells (from Ceratium of 20–200 μ m to Prochlorococcus of 0.5–0.7 μ m) will provide realistic insights into how far organic compounds secreted from phytoplankton cells travel into the surrounding water. This would provide a foundation for the study of interactions between these primary producers and bacteria, mediated by bacterial chemotaxis.

P37 Pheno-chip: A single cell photo-phenomic platform

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There is increasing demand for the identification of alga with specific phenotypes and their use in e.g. energy and food production, ideally under representative environmental conditions. Here we present 'pheno-chip', a high-throughput phenotypic ('phenomic') screening device for single algal cells. The phenochip is based on a versatile microfluidics platform with the ability immobilize cells, reproducibly deliver dynamic environmental conditions while observing photophysiological responses of single phototrophs. In a first proof-of-concept, single cells from two strains of the marine dinoflagellates Symbiodinium were tested for their ability to withstand periodic up- and downshifts of progressively increasing temperatures (+1°C to +4°C above ambient) and to perform photosynthesis under various pH. Strain specific differences emerged in both treatments; during temperature cycling, mean maximum quantum yields ((ΦΙΙ)max) decreased more for CCMP2467 than for CCMP421 (83% vs. 25% loss at +4°C, respectively) and revealed a higher sensitivity to, and more rapid recovery from, temperature stress in CCMP2467. Single cell analysis demonstrated that initial values of (Φ II)max are reliable predictors of later thermal stress tolerance and uncovered the existence of a thermal sensitivity threshold in strain CCMP2467, above which only subpopulations of cells resisted elevated (+3°/4°C) temperatures ((Φ II)max ~0.385). For pH, effective quantum yields (OII) were proportionally higher for CCMP2467 at lower pH (<6) and higher for CCMP421 at pH >6, with distinct pH optima and large phenotypic variations for both strains. Finally, cells were successfully removed from the pheno-chip using laser assisted catapulting, a procedure causing a minimal reduction of cellular (OII)max and principally enabling the future selection and investigation of select phenotypes. In conclusion, pheno-chip provides a non-invasive platform for the characterization of photosynthetic phenotypes under desired physico-chemical microenvironments and will significantly advance directed evolution research and bioenergy production.

P38 Dissecting promoter bistability of ICE*clc*

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There is increasing demand for the identification of alga with specific phenotypes and their use in e.g. energy and food production, ideally under representative environmental conditions. Here we present 'pheno-chip', a high-throughput phenotypic ('phenomic') screening device for single algal cells. The phenochip is based on a versatile microfluidics platform with the ability immobilize cells, reproducibly deliver dynamic environmental conditions while observing photophysiological responses of single phototrophs. In a first proof-of-concept, single cells from two strains of the marine dinoflagellates Symbiodinium were tested for their ability to withstand periodic up- and downshifts of progressively increasing temperatures (+1°C to +4°C above ambient) and to perform photosynthesis under various pH. Strain specific differences emerged in both treatments; during temperature cycling, mean maximum quantum yields ((ΦΙΙ)max) decreased more for CCMP2467 than for CCMP421 (83% vs. 25% loss at +4°C, respectively) and revealed a higher sensitivity to, and more rapid recovery from, temperature stress in CCMP2467. Single cell analysis demonstrated that initial values of (OII)max are reliable predictors of later thermal stress tolerance and uncovered the existence of a thermal sensitivity threshold in strain CCMP2467, above which only subpopulations of cells resisted elevated $(+3^{\circ}/4^{\circ}C)$ temperatures ((Φ II)max ~0.385). For pH, effective quantum yields (Φ II) were proportionally higher for CCMP2467 at lower pH (<6) and higher for CCMP421 at pH >6, with distinct pH optima and large phenotypic variations for both strains. Finally, cells were successfully removed from the pheno-chip using laser assisted catapulting, a procedure causing a minimal reduction of cellular (OII)max and principally enabling the future selection and investigation of select phenotypes. In conclusion, pheno-chip provides a non-invasive platform for the characterization of photosynthetic phenotypes under desired physico-chemical microenvironments and will significantly advance directed evolution research and bioenergy production.

P39 Manganesellron-supported sulfate-dependent anaerobic oxidation of methane by candidatus methanoperesdens in lacustrine sediments

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Anaerobic oxidation of methane (AOM) coupled to sulfate reduction is an important sink of the greenhouse gas methane (CH₄) in marine environments, yet evidence for this process is very rare in lacustrine environments. We investigated methane oxidation in the anoxic sediments of Lake Cadagno. Direct radio-label based CH₄ oxidation rate measurements provided clear evidence for AOM within the sediments, with maximum activity of ~15 nmol/cm³/d at a sediment depth where sulfate concentrations became depleted. ¹³CH₄ tracer incubation experiments confirmed that AOM is coupled to sulfate reduction, and stable isotope probing of lipid biomarkers suggests that sulfate-reducing bacteria are involved in AOM. Common lineages of anaerobic methanotrophs (i.e., ANME-1, -2 and 3) were not detected. However, 16S rRNA gene sequencing revealed the presence of Candidatus Methanoperedens (i.e., ANME-2d), where maximum AOM rates were observed, suggesting that this phylotype is primarily responsible for the observed methane oxidation in the anoxic sediments, and supporting evidence on the methanotrophic capacities of this clade. Although novel ANME strains belonging to ethanoperedenaceae were previously reported to conduct nitrate- or iron/manganese-dependent AOM, our results suggest that the direct involvement of these alternative electron acceptors in Lake Cadagno is negligible. Instead, we demonstrate for the first time that the methanotrophic archaea perform canonical sulfate-dependent AOM, which under sulfate-starved conditions is supported by metal (Mn, Fe) oxides through the reoxidation of reduced sulfur species to sulfate. The relatively high abundance and widespread distribution of Candidatus Methanoperedens in lake sediments highlights their potentially important role in mitigating methane emissions from terrestrial freshwater environments to the atmosphere, analogous to ANME-1 and -2 in marine settings.

Current and future spatial distribution of the tick Ixodes ricinus-host of the Rhadbochlamydiae bacterial pathogens-in Switzerland

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The bacteria Rhabdochlamydiae are candidate pathogens causing possible complications such as pneumonia. To estimate the risk of contracting Rhabdochlamydiae in Switzerland, we modelled the spatial distribution of one of their known host, the tick species Ixodes ricinus. This is also useful to precise the potential transmission risk of other Ixodes-associated pathogens such as Borrelia spp., Anaplasma phagocytophilum and tick-borne encephalitis virus.

We used 164 location sites of ticks collected by a Swiss Army field campaign and 2034 tick positions reported by users of a smartphone application (A&K Strategy, https://zecke-tique-tick.ch). For each location, we retrieved from Swiss federal datasets the environmental factors reflecting the topography, climate and land cover. We then used two presence-only modelling techniques (MaxLike and MaxEnt) to estimate the current and future spatial distribution of I. ricinus. Future distribution are estimated up to year 2090 and according to various scenarios for climate and land-use changes. We also studied the influence of the environmental factors on the infection rate by Chlamydiae bacteria based on the observed prevalence of bacteria within the ticks collected by the Swiss Army field campaign (8440 pools of ticks in 164 location sites) using correlations, regressions and ANOVA analyses.

The most influencing factors explaining the current suitability for I. ricinus' presence are PCA-components correlated with monthly temperatures, vegetation indexes (NDVI), percentage of grassland areas and precipitation (Figure 1). From 2016 to 2070, the suitability is predicted to increase in many areas above 800 meters in altitude and to decrease in areas converted from natural to artificial land-use or where the climate will be significantly dryer. The infection rate by Chlamydiae bacteria seem to be homogenous over the various areas in Switzerland and no significant correlation could be observed between the environmental factors and the infection rate.

The distribution maps offer an important support for the delimitation of health risk areas, where Rhabdochlamydiae's presence should be monitored in priority and where specific prevention or control campaigns may be useful to prevent transmission of all Ixodes-associated pathogens.