Session 1: Microbial genomics and metagenomics

02 | The ocean microbial-environmental interactome

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Determining the relative importance of both biotic and abiotic processes represents a grand challenge in ecology. Integration of organismal abundances and environmental measures from ocean samples allowed reconstructing the first global photic-zone microbial –environmental interactome. Environmental factors are incomplete predictors of the interactome but enhance a proportion of biotic interactions. Confocal microscopy confirms predicted relationships thus demonstrating the value of network-generated hypotheses to guide the discovery of symbiotic relationships. Putative biotic interactions are non-randomly distributed across phylogenetic groups, and show both local and global patterns. The high prevalence of parasitism indicates that parasites contribute to carbon recycling between the different compartments of the trophic web. The virus-prokaryotes subnetwork suggest that viruses are host-range-limited across large sections of host space, but that specialist and generalist phages prey on specific groups within sub-sections of this space. Together, these analyses highlight the importance of top-down effects in the structuring of ecosystems.

03 | Investigating the impacts of stress on the microbiome, immune status and disease resistance of Atlantic salmon

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There is a strong association between the gut microbiome and the immune system. Environmental stressors are known to disrupt microbial community composition, subsequently affecting health status and susceptibility to disease. Knowledge of the microbiome, and how it may be affected by stress, could therefore be valuable for understanding susceptibility to infection and for disease management. However, for fish, relatively little is known about the natural, healthy state of the microbiome in the gut or on the skin, and how this is associated with immune status and disease susceptibility. We characterised the gut and skin microbiome of four wild populations and three hatchery-reared populations of juvenile Atlantic salmon using Illumina sequencing of the V4 hypervariable region of the bacterial 16S gene, followed by analysis in Mothur. Marked differences in microbial abundance, diversity and community structure were apparent between salmon populations and, in particular, between wild and hatchery-reared fish. Wild salmon consistently displayed higher bacterial diversity in both the gut and the skin than hatchery fish. We hypothesise that this is associated with stressful conditions experienced in aquaculture. We are examining potential associations between the microbiome and measures of health and immune status, together with the potential effect of fish genetic background on microbial community composition. We are also specifically investigating the impact of early-life stress on development of the gut and skin microbiome, immunocompetence and susceptibility to infection with Saprolegnia parasitica.

04 | Dynamic changes in the strawberry rhizobiome in response to biochar

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The interaction of soil-borne microorganisms with the plant root is crucial for plant growth and health. This complex microbial community is called the rhizobiome and can constitute up to 109 microbial organisms per gram of soil. Within our research, we investigated the effects of biochar peat amendment on the rhizobiome of strawberry. Biochar is the solid coproduct formed during pyrolysis of biomass for biofuel production. Strawberry plants were grown for three months in peat amended with 0 or 3% dry weight biochar produced from holm oak in the frame of the FP7-Fertiplus project. After 12 weeks of growth, strawberry leaves were infected with Botrytis cinerea. The composition of the bacterial and fungal rhizobiome was studied during the growing season using 16S rRNA gene (V3-V4) and ITS2 amplicon sequencing. We showed that addition of biochar to peat induced changes in the strawberry root microbiome. Temporal profiling of the taxonomic shifts showed that the rhizosphere microbiome stabilised after six to nine weeks for bacteria, while the fungal community only changed in composition and diversity within the first week of plant growth. Biochar addition induced shifts in the bacterial composition of the strawberry rhizosphere from week six of plant growth onwards, whereas the fungal community wasn't affected by the addition of biochar to the peat. These shifts in the bacterial composition due to biochar amendment were linked with increased plant growth and disease resistance, which might partly be explained by the higher microbial diversity observed in the rhizobiome of biochar-amended peat.. Notably, biochar amendment raised the relative abundance of agents described for their biocontrol activity such as Bdellovibrio, Haliangium and Rhodanobacter, which might partly explain the increased resistance against B. cinerea infection. Complementary, only small changes in the chemical composition of the peat were observed by the addition of biochar, confirming that the observed plant growth and health promotion was mostly driven by micro-organisms in the rhizosphere.

05 | The anaerobic digestion microbiome: between active and abundant microorganisms

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Anaerobic digestion relies on complex microbial communities for the conversion of organic waste streams into biogas. The manifestation of high-throughput sequencing techniques resulted in a comprehensive increase in our understanding of the microbial community. Most studies with amplicon sequencing use a DNA-based evaluation, which reflects the total microbial community, but not the metabolically active microbial community. In this research, the microbial community in full-scale anaerobic digestion plants was evaluated through amplicon sequencing of the 16S rRNA and 16S rRNA gene to characterize the difference between the active (RNA) and total (DNA) microbial community. A separate analysis of the bacterial and archaeal community showed that 25.5 and 42.3% of total OTUs, respectively, showed a significant difference in their DNA and RNA profiles. Alpha diversity analysis revealed a significant difference between the DNA and RNA profile of the archaeal community, while this was not the case for the bacterial community. Beta diversity analysis revealed a highly significant differentiation pattern between DNA and RNA for archaea in terms of presence/absence of specific OTUs, based on the unweighted Unifrac measure, while this was less pronounced for bacteria. Canonical correspondence analysis revealed similar operational parameters significantly affecting both the bacterial and archaeal community, yet, the differentiating effect

between DNA and RNA was much stronger for archaea than bacteria. Co-occurrence networks and functional prediction profiling revealed a clear overall differentiating pattern between DNA and RNA. This research demonstrated a strong degree of specialization and organization in the active archaeal community, compared with the total community profile. A higher degree of similarity was confirmed between DNA and RNA for the bacterial community, while the archaea showed a strong divergence between DNA and RNA community profile. Co-occurrence and functional prediction profiles showed a different estimation of functionality between DNA and RNA for bacteria and archaea, but require validation of the actual functionality. In conclusion, a clear difference in active (RNA) and total (DNA) community profiles was observed, implying the need for RNA-based methods to predict community stability in anaerobic digestion.

06 | Automatic identification of causal mechanisms underlying experimentally evolved populations

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In order to understand how ecology and genomics/transcriptomics are related to each other in populations which have recently (been) evolved, for example in an evolution experiment, one approach is to relate the genomics/transcriptomics data in order to search for the causal factors underlying the evolved phenotypes. This is not a trivial task as separating the biological relevant signal from the noise in these data sets, which are typically very large, is cumbersome. Even when data from multiple parallel evolved populations is available, it is often not clear which mutations/transcriptional changes are causal to the evolved phenotype as the exact same genes may not be involved in every repeat but rather similar molecular pathways play a role. To cope with these problems we devised an algorithm, PheNetic, which searches for these molecular pathways by using the interactome of the organism under research. Doing so, we uncovered a plausible additional mechanism to explain the cross-feeding phenotype in the Ara-2 population of Richard Lenski's long term evolution experiment at 6500 generations. We were also able to automatically infer resistance mechanisms for the antibiotic Amikacin in an evolution experiment with four replicated E. coli populations. So far we have only been able to analyze populations of which its constituents originated from a homogeneous ancestral population. Future endeavors will aim at modeling the environments and interspecies interactions to cope with more heterogeneous populations.

07 | Ingestion of microplastics by the freshwater invertebrate Chironomus sancticaroli: effects on PBDE bioaccumulation and the gut microbiome

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Microplastic particles in the environment can associate with persistent organic pollutants (POPs) due to the hydrophobic nature of plastics and organic chemicals. If ingested, the gut environment of an organism may favour desorption of adsorbed chemicals due to gut conditions such as pH and ionic strength as well as the presence of surfactants. Therefore the ingestion of microplastic particles has implications for uptake and bioaccumulation of these chemicals and the potential to disturb the structure and function of the gut microbiome. Given the widespread present of both POPs and microplastic in freshwaters such combined exposures have high ecological relevance. This paper will describe a study in which the chironomid Chironomus sancticaroli was exposed to microplastics and different PBDEs (polybrominated diphenyl ethers) concentrations. Exposure were conducted for Chironomus larvae exposed to a PBDE mix containing BDE-47, 99, 100, 153 and PBB-153 at six

environmentally relevant concentrations (94, 188, 375, 750, 1500, 3000 ng g-1) in sand alone and in sand with 1% of fluorescently labelled < 50 µm nylon particles included. Over the course of the study individuals were sampled during both an uptake (exposed) and elimination (unexposed) phase to allow the time course of effects to be established. Uptake rates and gut transit times of microplastics were analysed by fluorescence microscopy and PBDE bioaccumulation in different treatment measured in chironomid tissues using LC-MS. Gut microbiomes were characterised for both bacterial and fungal composition using bacterial 16s rDNA and fungal ITS sequencing using Illumina technology. Microscopy clearly showed that microplastics were ingested by chironomids over the 48 hour period. Further microplastics were still found to be still present in the gut following the 48 hour elimination period suggesting slow rates of egestion. After the 96 hour exposure there was no mortality observed, either for microplastics or PBDEs. However, characterisation of the microbiome of the chironomids in the presence of microplastics clearly indicate an effect of exposure of the microplastic on the gut microbiome composition. PDBE exposure also had subtle effects on the microbiome that were found to be dependent on whether exposure was in the presence of absence of microplastics. We are currently awaiting results from the chemical analysis to see if these can be explained in relation to PBDE bioaccumulation by the larvae. In conclusion our study demonstrate both direct and indirect effects of exposure to environmentally relevant microplastics concentrations on the chironomid gut microbiome.

08 | Genotype-dependent gut microbiota drives zooplankton resistance to toxic cyanobacteria

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Eutrophication and climate change have caused a widespread occurrence of cyanobacterial harmful algal blooms (cyanoHABs) in lakes, ponds and reservoirs worldwide. Due to the release of powerful toxins in the water, CyanoHABs pose severe threats on livestock and human health, causing diseases from gastrointestinal symptoms to liver cancer. In aquatic ecosystems, cyanoHABs have a strong negative impact on zooplankton grazers, and through the food web, disrupt the whole freshwater community. Deciphering the mechanisms underlying resistance to toxic cyanobacteria in these grazers is thus essential to predict how cyanoHABs can be prevented or controlled. In the freshwater crustacean Daphnia, resistance is influenced by prior exposure to cyanobacteria and genotype, but the underlying mechanisms remain unclear. Through gut microbiota transplants, we here show that the gut microbiota plays a crucial role, and might mediate both genetic adaptation and acclimatization to cyanoHABs. Microbiota from resistant genotypes conferred a higher resistance to recipient Daphnia than microbiota from susceptible genotypes. Resistance to cyanobacteria in recipient Daphnia was not affected by the recipient genotype, but was strongly impacted by the donor genotype. This suggests that the Daphnia genotype acts indirectly on resistance to cyanobacteria, by shaping the gut microbiota. In addition, resistance was higher when donors were previously fed cyanobacteria, suggesting that gut microbiota responded to become more efficient in dealing with cyanobacteria after prior exposure. Next generation sequencing of 16S rDNA shows that resistance is associated with changes in microbiota structure: cyanobacterial exposure favored bacterial taxa involved in digestion of cyanobacterias cells and detoxification of cyanobacterial toxins. Our results provide evidence that resistance to toxic cyanobacteria in Daphnia is driven by the gut microbiota, which might thus be an important mediator of the genetic mosaic of coevolution between toxic cyanobacteria and their grazers, and a key determinant of how freshwater ecosystems respond to climate warming.