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Bacteria drive our planet's health

ABSTRACTS

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Content

ISME - Microbial Ecology

AL001

Unseen Invasions: How Microbial Encounters Reshape Communities

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Microbial species are currently spreading across various ecosystems at an unprecedented rate, leading to their inclusion in the Invasive Alien Species Assessment list provided by the IPBES (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services). Microbial invasion is a key process that influences the composition, dynamics, and stability of microbial communities. However, the specific mechanisms driving species interactions during microbial invasions, especially in cases of community-driven invasions (such as community coalescence), remain largely unexplored. In my presentation, I will explore the ecology of both single-species and multispecies-driven invasions within the context of community assembly. Using experimental evidence, I will initially explore how scenarios of single-species invasion can help us understand the process of community coalescence. Next, I will examine the mechanisms behind community coalescence, with a focus on understanding the outcomes for both the source (invading) and resident microbial communities. Finally, I will discuss how ecological principles governing species interactions can be leveraged to facilitate the establishment of invasive species. These findings offer valuable insights for comprehending and predicting the implications of human-mediated microbial invasions, such as the application of individual microbial strains or synthetic communities in agriculture and human health.

ISME - Microbial Ecology

AL002

Marine symbioses for ecosystem conservation and the blue bioeconomy

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The diverse and phylogenetically unique associations between marine hosts and their microbial symbionts present untapped potential for advancing ecosystem conservation and fostering a blue bioeconomy. Here, I shed light on the natural product biosynthesis and carbon metabolism capabilities of foundational marine symbioses, encompassing the microbiomes of marine sponges, corals, and algae. By integrating field surveys and experimental approaches with multi-omics and synthetic biology techniques, the phylogenetic breadth and coding potential of uncultivated and cultivated bacterial symbionts associated with keystone marine eukaryotes can be uncovered. Moreover, metabolic features of relevance to conservation and sustainable biotechnology can be revealed and validated. Notably, novel lineages of abundant, uncultivated symbionts from marine sponges and corals exhibit the ability to degrade chitin, the most prevalent polysaccharide in the ocean, highlighting the integral role of symbiotic bacteria in global carbon and nitrogen cycling. Moreover, chitin catabolism is a distinguishing feature of several culturable bacteria enriched from the microbial rare biosphere of sessile marine invertebrates, with species in the genus *Aquimarina* identified as exceptional chitin degraders that produce novel bioactive natural products. This positions them as valuable models for investigating the interplay between polysaccharide catabolism and secondary metabolite biosynthesis, its consequences to host health and disease, and potential applications across the human health, environment, and food production sectors. I further highlight the pivotal roles of coral and algal microbiomes in promoting (i) host resilience against the compounded stresses of climate change and pathogenesis and (ii) host development in aquaculture, respectively, discussing microbiome intervention strategies for improved ecosystem conservation and food production.

ISME - Microbial Ecology

AL003

Endophytes as Drivers of Bioactive Compound Production in Plants

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Endophytic microorganisms, residing within plant tissues, represent a vast and largely untapped reservoir of novel bioactive compounds. Far from being passive inhabitants, endophytes engage in dynamic symbiotic relationships with their plant hosts, exchanging metabolic resources and responding to environmental cues. These symbiotic partners engage in sophisticated molecular dialogues with their hosts, driving the production of structurally diverse secondary metabolites with remarkable pharmaceutical potential. Through co-evolutionary processes and ecological pressures, endophytes have become prolific producers of bioactive compounds that often surpass those of their plant hosts. This work explores the therapeutic arsenal offered by endophyte-derived metabolites while highlighting their chemical diversity, including alkaloids, terpenoids, and phenolic compounds, among others. It showcases their wide range of bioactivities, such as antimicrobial, anti-inflammatory, antioxidant, and potent anticancer effects. Notable examples include the anticancer agent taxol from *Aspergillus fumigatus*, the neuroprotective compound huperzine A from *Colletotrichum gloeosporioides*, and the anti-inflammatory sesquiterpene purpurolide from *Penicillium purpurogenum*. By examining the mechanisms through which these compounds exert their therapeutic effects, we demonstrate that endophytic microorganisms are not merely passive residents but key players in producing valuable medicinal agents, offering promising avenues for drug discovery and the development of new therapeutic strategies.

Key words: endophytes, metabolites, compounds, pharmacy, biotechnology

ISME - Microbial Ecology

AL004

Molecular cues shaping ecological adaptations during pathogen-probiotic interactions

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Microbial interactions shape the function and structure of microbial communities and play a crucial role in biotechnological and biocontrol applications. Our previous studies demonstrated that the beneficial bacterium *Bacillus subtilis* PS-216 exhibits strong pathogen-inhibitory effects both *in vitro* and *in vivo*. Unpublished results further show that broilers receiving PS-216 spores for 42 days exhibit increased body weight, improved immune function, and enhanced microbiota structure-function relationships, supporting the probiotic potential of PS-216. However, the molecular mechanisms and cues shaping pathogen-probiotic interactions remain poorly understood.

To address this gap, we investigated the interactions between *B. subtilis* PS-216 and *Salmonella enterica* serovar Typhimurium, a major diarrheal pathogen and one of the top ten antibiotic-resistant bacteria of concern. Under nutrient-rich conditions, *B. subtilis* PS-216 effectively inhibits *Salmonella* growth and biofilm formation by secreting the antibiotic bacillaene. However, *Salmonella* is frequently found in nutrient-limited environments (e.g., soil, plants, aquatic ecosystems), where its interactions with beneficial bacteria remain unclear. Our results show that under such conditions (diluted TSB), *B. subtilis* does not eliminate *Salmonella*; instead, it alters its sporulation behavior. Specifically, in co-culture with *Salmonella*, *B. subtilis* avoids sporulation via activation of the SigB-dependent general stress response. This response is critical, as a *sigB* null mutant remains unresponsive to *Salmonella*-induced cues. Moreover, this response is novel, as previous research shows that competition induces sporulation.

Furthermore, reduced sporulation in *B. subtilis* requires direct cell-cell contact and is influenced by *Salmonella*'s type VI secretion system (T6SS). In response, *B. subtilis* appears to trade spore quantity for higher spore quality through SigB-dependent regulation. Transcriptomic analyses of pathogen-probiotic co-cultures versus monocultures in nutrient-limited medium confirm *sigB* transcriptional activation and reveal interaction-dependent differential gene expression in both species.

These findings enhance our understanding of the molecular cues shaping probiotic-pathogen interactions and provide insights into the ecological effects of *B. subtilis* within the broiler gut microbiota.

ISME - Microbial Ecology

FT001

Distribution of bacteria fauna in *Anopheles gambiae s.l* larval habitats in the central region of Ghana

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Question: The bacterial fauna in mosquito breeding habitats play a pivotal role in larval development, habitat selection, and vector competence. The question is, can the presence or absence of a particular bacteria in mosquito breeding habitats predict the breeding habitat of *Anopheles gambiae,* a major malaria vector in Africa?

Method: Larval survey was conducted in the Central Region of Ghana in twelve (12) communities. The communities were selected based on the ecological zones of the region (Coastal and Forest Zones) and the degree of urbanisation (Urban and Peri-urban). In each selected community, three Anopheles Positive Breeding Habitats (APBH) and three Anopheles Negative Breeding Habitats (ANBH) were identified. Larvae and water were sampled from each habitat. Three replicate water samples were taken from each habitat at 10 m intervals. Samples were collected in 2019 and repeated in 2020. Water for microbial samples was collected into 500 ml sterile samples bottles in three replicates and placed on ice, they were transported to the laboratory for analysis.

Results: A total of 12 bacteria species were identified from 112 breeding habitats. All 12 species were found in both APBH and ANBH sites. Also, similar bacteria fauna occurred in both APBH and ANBH surveyed for both 2019 and 2020. *Bacillus* and *Streptococcus* were the most prevalent bacteria in the mosquito breeding habitats in both coastal and forest zones. Furthermore, both *Bacillus* and *Streptococcus* species were more dominant in *Anopheles-positive* sites than in *Anopheles-negative* sites. However, *E.coli* was the least prevalent bacteria in the study area as well the least dominant bacteria in both *Anopheles* positive and negative sites.

Conclusion: from the results in this study, the bacteria communities in *Anopheles gambiae* and other mosquitoes in the Central region of Ghana have been established. However, both Anopheles breeding habitats and other mosquito breeding habitats shared similar bacteria fauna, and none of the 12 bacteria species was able to predict *Anopheles gambiae* breeding habitats.

ISME - Microbial Ecology

FT002

Plant pathogens and their sinsiter social lives – a pathobiome appraoch to disease aetiology

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Microbes share a vibrant social life with each other and their hosts.The same organisms can attack, defend, trade, compete, and cooperate, depending on who is around them and what space they share. We are now starting to recognize the limitations of categorizing microbes as "pathogens" or "beneficials". Instead, the pathogenic potential of a microbe is represented in the interaction with its surrounding biotic environment. This research supports an unorthodox transition from 'pathogens' to a sociomicrobiological phenomenon called the 'pathobiome'.

We used a modified baiting assay together with high-resolution confocal microscopy to isolate 30 bacterial communities that colocalize with the pathgoenic fungus, *Fusarium,* in different soil types. We characterized these bacteria with amplicon targetted sequencing and biochemical assays. We tested their impact on disease development when co-inoculated together with the fungal pathogen in greenhouse experiments. Finally, we also investigated the impact on the pathogen metabolome with untargetted mass spectrometry.

We found diverse bacteria to co-localize with *Fusarium* in the rhizospheric soil, where the composition of the recruited bacteria was specific to the species of *Fusarium*, irrespective of the bacteria reservoir in the different soil types. Some of these communities, when co-inoculated with *F. oxysporum,* expedited development of Fusarium wilt in tomato seedlings. These 'pathobiome' bacteria were not able to infect tomato plants independant of the pathogen. Furthermore, in a simulated rhizosphere environment, the pathobiome was found to significantly alter the metabolomic profile of the pathogen, in a community-specific manner.

In summary, we found *Fusarium* to recruit a species-specific pathobiome from differing soil environments. These bacteria are phylogenetically diverse, but show a conserved fucntional influence on the metabolome of the pathogen. These results support the investigation of a social model of disease development, where the pathobiome refers to the microorganisms and their social interactions that modulate disease development.

ISME - Microbial Ecology

FT003

Discovery of microbial bioremediation capabilities for sustainable water management

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The demand for freshwater is rising as the global population constantly grows, exerting ever-increasing pressure on natural ecosystems through anthropogenic pollution. This pollution poses significant threats to aquatic ecosystems across all trophic levels, including microbial communities. As pollutants accumulate, microbial communities adapt, resulting in an alteration of both microbial species composition and community-level functionality. This study investigates the impact of human activities, specifically anthropogenic pollution, on environmental microbiomes across ecological and evolutionary time scales, focusing on pollution-driven changes and adaptations in constantly contaminated environments. We take a microbial population genomics approach applied to metagenomic time-series datasets to explore changes in microbial dynamics and gene evolution with respect to their changing environment. We found increase in genomic variation of genes following external perturbations in the microbiome of waste water treatment plant sampleled monthly for 9 years. We aim to identify potential biomarkers and bioremediators for better pollution management. We found increase in genomic variation of genes following external perturbations in the microbiome of waste water treatment plant sampleled monthly for 9 years.

ISME - Microbial Ecology

FT004

The potential of global and local datasets to assess occurrence, abundance, and diversity of the entomopathogenic fungi *Metarhizium* and *Beauveria*

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The use of synthetic agrochemicals has raised significant concerns about off-target impacts at multiple ecosystem levels affecting human and overall ecosystem health. However, insect pests relevant to agricultural production are expected to increase in abundance, while overall insect diversity is expected to further decline, with yet unpredictable environmental impacts. To meet the One Health goals, our approach to crop protection under abiotic and biotic stress conditions must consider environmental sustainability. Therefore, insect pest management should become more targeted while off-target effects should be minimized. Entomopathogenic fungi (EPF) are natural antagonists of insects and a promising approach to complement and replace synthetic insecticides. Some EPF species comprise a saprophytic life stage in soil, making them suitable for managing soil-dwelling insect pests while also exposing them to the complex soil microbial communities and their metabolites. Consequently, biotic and abiotic soil conditions should be considered for the isolation of climatically adapted EPF strains, maintenance of biologically active EPF inocula in soil, and successful field application. Using amplicon and metagenome datasets, we modeled the global cropland suitability for two EPF genera, *Metarhizium* and *Beauveria*, as a function of soil physicochemical and climate properties. Our data analyses suggest that *Metarhizium* predominantly occurs in temperate and tropical regions with high primary soil productivity, while *Beauveria* occurs more frequently in drier, more mountainous regions. These data were further compared to a projection of global insect population changes. The ecological inferences obtained using global datasets were compared to inferences obtained from a local dataset of agricultural soils with detailed measurements of the physicochemical and microbial community properties. Our analyses revealed similar correlation patterns with site and soil properties for the occurrence and abundance of *Metarhizium* and *Beauveria* at global and local scales. Agricultural lands that are threatened by potentially increasing insect pest populations are suitable for the application of EPFs. Our results can guide future isolation strategies for locally available EPF strains. They emphasize the importance of considering the ecological niche of EPF in their saprophytic life stage while further demonstrating the potential of biological insect control.

ISME - Microbial Ecology

FT005

Unravelling the plasticity of the tomato seed microbiome

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The seed microbiome was shown to have implications for plant health. As a result, seeds are a target for the delivery of innovative microbiome solutions for supporting sustainable agricultural systems. However, there is limited knowledge regarding the factors that influence the composition and assembly of the seed microbiome, particularly in nutritionally and economically important crops such as tomato (*Solanum lycopersicon*). Here, a microbiome analysis of so far largest microbiome dataset involving 100 tomato cultivars was conducted to examine plant genetics and environmental factors influencing the seed microbiome composition. By using predictive models, we explored whether plant traits such as insect resistance, yield, seed weight, number of ovaries, berry colour, berry taste, e.tc might affect the seed microbiome. We hypothesized that the heterogenous genetic background of tomatoes reflects on its seed microbiome, and that it is dependent on the region of production as well as certain host traits. We detected high effective bacterial diversity in the range 20 to 150 amplicon sequence variants (ASVs). Tomato genetics more than geographic region of tomato production were found to shape the tomato seed bacterial community (R2=56% vs. 11%). All genotypes were mainly composed of bacterial classes *Bacilli* (mean relative abundance=52%), *Gammaproteobacteria* (37%), and *Alphaproteobacteria* (5%). Interestingly, significant variations in the tomato seed microbiome could be linked to specific plant and berry traits, like insect resistance (R2=7%), tomato yield (R2=3%), weight seed (R2=3%), number of ovaries (R2=2%), berry colour (R2=2%), and berry taste (R2=2%). A core microbiome of 21 ASVs was revealed in atleast 50% of the samples, mainly dominated by *Lactobacillus*, *Leuconostoc , Ralstonia*, and *Pseudomonas*. Our study highlights the crucial role of plant genetics in shaping the seed bacterial community, uncovers the plasticity of the seed microbiome, and provides insights into the potential of using seed as a target for seed microbiome engineering.

ISME - Microbial Ecology

FT006

Host-species traits shaping feather and preen gland bacteriomes and their functional properties

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Diverse bacterial communities found in the preen gland and on feathers of several bird species are hypothesized to synthesize volatile organic compounds and antimicrobial compounds, potentially contributing to host olfactory communication and antimicrobial defence. To date, little is known about host phylogenetic and environmental factors that influence feather and preen gland bacteriome composition and their functional properties, including the production of volatile organic compounds in preen oil. Given the functional roles of the bacteriomes, we expected that preen gland and feather bacteriomes are shaped by their host species traits, like habitat types and different social systems. We aimed to determine the composition of feather and preen gland bacteriomes, and preen oil VOC profiles, in relation to host phylogeny and differences in sociality and habitat humidity. To this end, we sampled feathers and preen oil from 530 individuals of 40 temperate bird species varying in degrees of sociality and habitat humidity conditions. We found that preen oil bacteriome, preen oil VOC and feather bacteriome diversity and composition significantly vary among bird species, where preen oil bacteriomes show a phylogenetic signal. Host sociality and habitat humidity affected feather bacteriomes, but not preen oil bacteriomes and VOC diversity. We detected various preen oil VOCs, with high proportions (40% and 24%) of putative microbial VOCs and VOCs with antimicrobial potential, respectively. Additionally, enrichment analysis revealed dominant sensory and olfactory functions of preen oil VOCs.

ISME - Microbial Ecology

FT007

Fungal dysbiosis driven by *Monilinia fructicola* – implications for microbial interactions, ecosystem stability, and plant health

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Microbial community dynamics, including fungal and bacterial interactions, are crucial for maintaining plant health, ecosystem stability, and resilience against diseases. *Monilinia fructicola*, a quarantine pathogen of *Prunus persica*, is hypothesized to act as a dysbiosis driver by altering not only fungal diversity but also broader microbial ecological interactions and functional roles. This study investigated how *M. fructicola* impacts fungal community composition, trophic dynamics, and microbial network structures in peach orchards.

Using ITS metabarcoding and high-throughput sequencing, we analyzed fungal communities in flowers and branches of infected and non-infected *P. persica* trees. The pathogen-induced significant shifts in community structure, most evident in flowers, where increased richness (Chao1), diversity (Shannon), and dominance (Simpson) metrics highlighted a restructuring of the fungal microbiota. In wood samples, higher species dominance suggested ecological filtering driven by *M. fructicola*. Network analysis revealed that the pathogen disrupts not only fungal but potentially also fungal-bacterial interactions, causing fragmentation and reduced complexity within microbial networks.

Infected trees exhibited diminished node connectivity and increased modularity, undermining ecological cohesion and potentially weakening orchard resilience. The shifts in fungal trophic modes, including enhanced tissue decomposition, indicate that *M. fructicola* may facilitate secondary infections through microbial imbalance. Taxonomic shifts showed a decline in beneficial fungal genera and the rise of pathogen-associated taxa, emphasizing the pathogen"s role as a community disruptor.

The isolation of *Epicoccum nigrum* as a potential biocontrol agent introduces a promising nature-based solution to mitigate the negative impacts of *M. fructicola*. These findings not only position *M. fructicola* as a driver of fungal dysbiosis but also highlight its broader implications for microbial network stability and plant ecosystem health. By deepening our understanding of these microbial shifts, this study supports the development of microbiome-informed disease management strategies, promoting sustainable orchard practices that leverage beneficial microbial interactions, including those involving bacterial communities that underpin our planet's health.

Keywords: Mycobiota, Dysbiosis, *Monilina fructicola, Prunus persica*; Fungal Community Structure, Disease Ecology

ISME - Microbial Ecology

FT008

For better or for worse – how bacteria positively or negatively affect *Fomitiporia mediterranea, a fungal pathogen involved in grapevine trunk disease*

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Due to climate change and socio-economic constraints, viticulture is facing several threats. Among these, Esca, a grapevine trunk disease, causes losses estimated at €1 billion per year in France due to 12% of unproductive vineyards. Esca is a complex disease involving several fungal pathogens such as *Phaeomoniella chlamydospora, Phaeoacremonium minimum* and *Fomitiporia mediterranea*. However, these fungi do not systematically induce Esca symptoms, even though *F. mediterranea* is highly abundant in typical wood necrosis observed in diseased plants. We therefore hypothesise that disease development is due to multiple fungal-fungal interactions, but also to fungal-bacterial interactions. Bacteria are known to inhibit fungal growth, but the reverse is also true. We screened 160 bacterial strains isolated from grapevines and selected strains that were able to reduce fungal growth, but also those that could promote it. We then selected 3 strains with a positive effect (2 *Pseudomonas* and 1 *Paenibacillus*) and 3 others (3 *Paenibacillus*) with a negative effect on *F. mediterranea* growth. Each of the 6 bacteria was co-cultured with the fungus, and the 6 bacteria and the fungus cultured alone were used as controls (4 replicates per condition, 52 samples in total). To decipher the positive and negative interactions between bacteria and fungus, we used the meta-omics approach consisting of the analysis of the volatile metabolome (GC-MS), the diffusible metabolome (LC-MS) and the transcriptome (RNAseq). Our initial results revealed very different metabolomes depending on the bacterial genus, with many molecules known to have antifungal activities, such as pyrazine and fusaricidine.

ISME - Microbial Ecology

FT050

Effect of Nickel and Sulfamethoxazole on Zebra Mussels: Interplay Between Physiology and Gut Microbiota

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Heavy metals and pharmaceuticals can be found in the aquatic environment worldwide and can be harmful to organisms, impacting the host"s physiology and gut microbiota (GM) communities. It is essential to understand the complex network connecting biological systems and entities (GM) for improving the biomonitoring of the aquatic environment. In this study, we investigate the effect of two contaminants- sulfamethoxazole (SMX), a frequently prescribed antibiotic in concentration 520ng/L (PNEC) and Nickel (Ni), a heavy metal naturally occurring in the water that may be associated with oxidative stress and biomarker disruption in higher concentrations, in concentration 20µg/L (PNEC) —on zebra mussels (Dreissena polymorpha).

The aim is to find a link between GM and biomarker responses to the pollutants. We hypothesise that GM response might be perturbed in SMX exposure, while Ni may primarily alter the biomarkers" activity. We included the mixture of both pollutants to observe additive and interactive effects. We also distinguished two types of feeding, Chlorella and mix feeding of Chlorella and Cyanobacteria (to create an algal bloom situation), to observe the alteration of GM and biomarker activity.

Zebra mussels are exposed for 7 and 21 days under controlled laboratory conditions. The measurements were performed at the start (T0), day 7 and day 21. Gut microbiota composition was assessed through high-throughput sequencing of 16s rRNA gene amplicons, while biomarker analyses included an analysis of physiological stress indicators, antioxidant enzymes, and lipid metabolisms. With comparison over time and across conditions, we aim to determine i. if the pollutants affect the GM and biomarkers responses, ii. if GM shifts occur before shifts in biomarker activities and vice versa, iii. how factors (duration, feeding) affect GM and biomarkers and iii. asses the core GM.

This study leads to a better understanding of how microbial and physiological endpoints respond to environmental contaminants and if GM can serve as an early warning of aquatic pollution. Our results may help to improve biomonitoring by connecting microbial indicators alongside biomarker measurements.

ISME - Microbial Ecology

FT009

Beyond the clinic – ecological adaptations of *C. difficile* in non-host environments

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Clostridioides difficile inhabits both hosts and environmental reservoirs, with all clades—normal (C1–C5) and cryptic—present in these niches. However, normal clades are more frequently associated with pathogenicity, while cryptic clades, despite overlapping ecological roles, rarely cause disease. These groups are genetically distinct, sharing less than 90% average nucleotide identity (ANI), suggesting divergent adaptations. In the gut environment, *C. difficile* spore germination is driven by bile acids and amino acids, yet the ecological potential of all strains, particularly cryptic clades, in non-host environments remains underexplored. To address this, we assessed *C. difficile* growth in sterilized compost using strains from clade 1 (normal) and cryptic clade I. Over 20 days, both exhibited robust proliferation, confirmed by culture-dependent (CFU) and -independent (16S rRNA gene copy number) methods, demonstrating that non-host environments sustain diverse *C. difficile* populations.

To elucidate the genetic basis of this adaptability, we conducted comparative genomic analysis of ~30,000 *C. difficile* genomes, identifying clade-specific gene content linked to environmental survival. These differences, especially in cryptic clades, likely underpin metabolic flexibility outside the host. To test this, we are initiating BIOLOG plate assays to characterize the carbon and nitrogen sources utilized by strains across clades, with preliminary results expected to reveal their nutritional versatility. Our findings highlight environmental reservoirs as critical for C. difficile persistence and transmission, with cryptic clades exemplifying the species" ecological diversity. This challenges its clinical-centric view and underscores the interplay between ecological niches and human health risks.

Anthropocene and biogeochemical cycles

AL005

The soil microbiome – Mitigator or origin of climate change?

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The soil microbiome plays a pivotal role in biogeochemical cycles, particularly in carbon and nitrogen cycling, which are central to the regulation of Earth's climate. As global climate change accelerates, understanding whether the soil microbiome functions primarily as a mitigator or as a contributor to this threat is increasingly critical.

Recent advances in metagenomics, isotope tracing, and environmental modeling have revealed that soil microbial communities significantly influence carbon fluxes between terrestrial ecosystems and the atmosphere. Microbial processes such as SOM decomposition, nitrification, denitrification, and methanogenesis govern the turnover of key greenhouse gases, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). In stable ecosystems, especially unmanaged forests and undisturbed grasslands, microbes facilitate carbon sequestration by stabilizing organic matter in soils and promoting plant-microbe symbioses that enhance biomass productivity and drought resilience. These functions position the soil microbiome as a *potential climate change mitigator*.

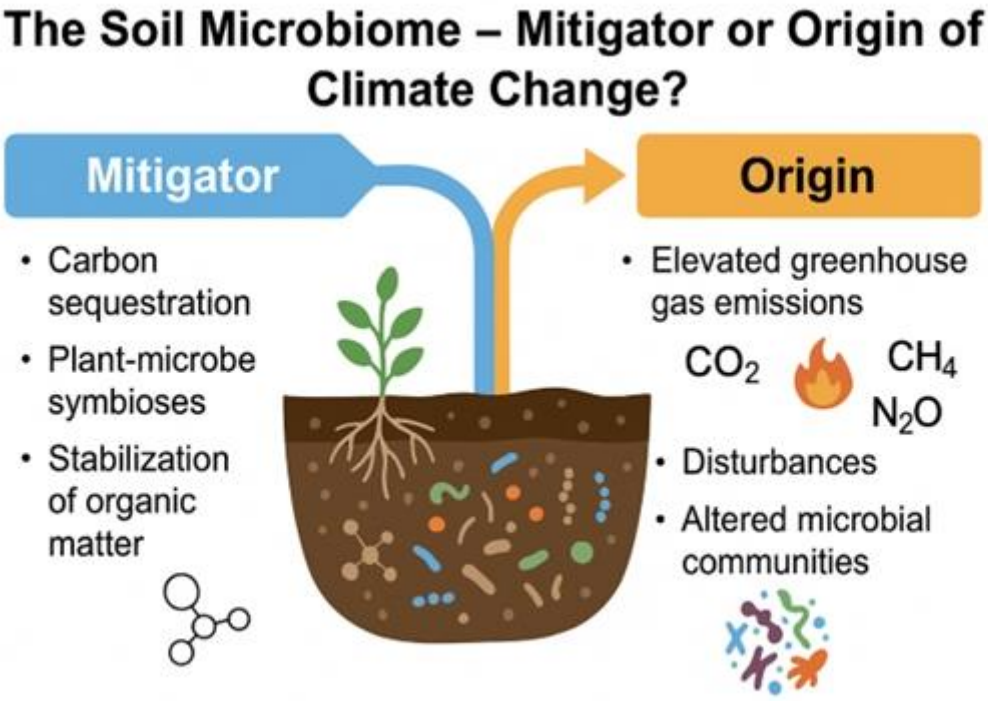
However, microbial contributions to climate change are highly context dependent. Disturbances such as intensive agriculture, deforestation, and permafrost thawing alter microbial community structure and function, often leading to elevated greenhouse gas emissions turning the soil microbiome into a *potential accelerator of climate change*.

Thus, the answer to the question - whether the soil microbiome is a *mitigator or origin of climate change?*— is not binary. The soil microbiome is both, and its trajectory is ultimately shaped by human stewardship as well as environmental conditions including climate change itself.

The key question is: How can microbial processes be managed or engineered to enhance their climate-mitigating capabilities? Promising strategies include promoting beneficial microbial taxa through conservation agriculture, rewilding degraded lands, applying biochar, and developing microbial inoculants designed to stabilize soil carbon. However, the high complexity and variability of microbial communities on the one hand and differences in site specific properties like soil type, management or climate on the other hand exacerbate prediction and control. Thus the "One fits all solution" might be never achieved. Furthermore, current Earth system models inadequately represent microbial processes, underscoring the need for more integrative research at the intersection of microbiology, soil science, and modeling.

The presentation will give an overview about current state of the art describing the dual role of the soil microbiome that can either buffer or exacerbate climate change. The presentation will also address the question of the stoichiometry of nutrients and if subsequent adaptations in management might be a possibility to induce targeted changes in the soil microbiome composition and activity pattern, which could also influence greenhouse gas emission pattern.

Fig. 1


The Soil Microbiome – Mitigator or Origin of Climate Change?
Mitigator
• Carbon sequestration
• Plant-microbe symbioses
• Stabilization of organic matter
Origin
• Elevated greenhouse gas emissions
CO_2
CH_4
N_2O
• Disturbances
• Altered microbial communities

Anthropocene and biogeochemical cycles

AL006

Tiny Giants: The Overlooked Role of Cryptogamic Communities in Shaping Planetary Biogeochemistry

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Cryptogamic communities (CC) consist of assemblages of photoautotrophic non-vascular organisms, such as cyanobacteria, algae, lichens, and bryophytes, which are accompanied by a wealth of heterotrophic bacteria, microfungi, and archaea. They colonize soil, rocks, and grow epiphytically on trees. CC have been shown to impact global biogeochemical cycles, as the cycling of carbon, nitrogen, and water.

In drylands, CC form biological soil crusts (biocrusts), which colonize the uppermost millimeters of the soil. These biocrusts, covering ~$18 * 10^6$ km^2 around the globe, provide multiple ecosystem services, as they stabilize the soil surface, fertilize the soil, and influence water cycling and plant growth. Contrastingly to their growth under extreme dryland conditions, biocrusts are severely impacted by both climate and land use change, with an expected decrease by 16-39% of their global coverage until the year 2070. This loss in biocrusts is expected to only marginally affect their input in global carbon cycling, whereas the reduction in N fixation is suggested to be substantial. As biocrusts stabilize dryland soils, their loss will also affect the global cycling of aeolian dust. According to our calculations, biocrusts currently reduce the emission and cycling of dryland dust by 60%, thus preventing the release of ~0.7 Pg of dust per year. Until 2070, biocrust loss will cause an increased dust burden, affecting biogeochemical cycling, functioning of ecosystem, and human health. Due to all these effects on biogeochemical cycling, CC need to be incorporated in Earth System Models.

Anthropocene and biogeochemical cycles

AL007

Diversification and niche adaptation shape long-distance transported dust microbial community

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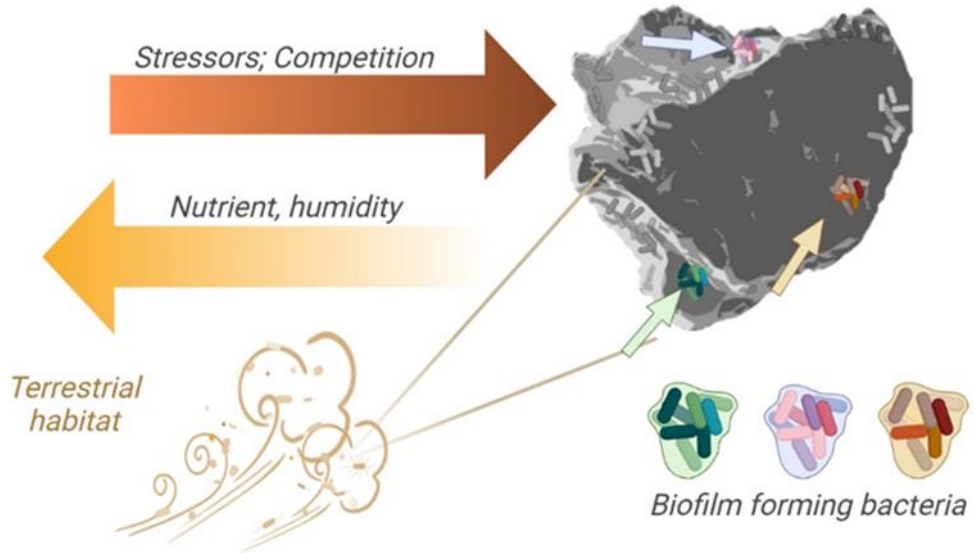
The long-distance transport of microorganisms via dust events is expected to become more prominent and intensified. At the same time, the survival mechanisms of air/dust-borne bacteria and their possible contribution to global processes remain poorly understood. In this study, we characterized *Bacillus* species from transitional season dust storms, previously identified as a significant component of the bioactive community of the dust microbiome. Our results demonstrated substantial growth and biofilm formation diversification linked to niche adaptation and surface-associated biofilm formation within heterogeneous dust particles. Most dust-storms isolates form biofilms while exhibiting different preferences for media composition. Sterile dust induced biofilm formation, growth, matrix gene expression of *B. subtilis,* and robust biofilms in key related dust isolates. It was superior to the inorganic dust components, representing a role for the organic substrate from previous community members. Overall, our results highlight the significance of biofilm formation as an adaptive mechanism in the distinct habitat of dust storms, with niche adaptation potentially playing a role in microenvironments within the dust particle. These results hold significant potential for implications on terrestrial and aquatic ecology and health, suggesting a pivotal process by which bacteria survive and evolve in this understudied habitat.

Fig. 1



Anthropocene and biogeochemical cycles

AL008

The ASEAN Industrial Wastewater Genomics Consortium (ASEAN-IWGC) – Applying genome-resolved metagenomics to understudied, economically important wastewater bioprocess microbial communities in South East Asia

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[2]National University of Singapore, Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore
[3]University of Malaya, Chemical Engineering, Kuala Lumpur, Malaysia
[4]King Mongkut's University of Technology Thonburi, Bangkok, Thailand
[5]Yokohama National University, Yokohama, Japan
[6]Taylor's University, Selangor, Malaysia
[7]Tokyo University of Agriculture, Tokyo, Japan
[8]National Centre for Genetic Engineering and Biotechnology, Bangkok, Thailand
[9]National University of Singapore, Department of Life Sciences, Singapore, Singapore

Question: We report the initial data release from a research consortium focused on applying genome-resolved metagenomics to study microbial communities from wastewater treatment bioreactors in industrial factories in the ASEAN region. We have performed a metagenome survey of samples obtained from bioreactors treating wastewater from citric acid production, glove manufacturing, oleochemical production and palm oil mill effluent, in Malaysia and Thailand.

Methods: From 21 samples across 5 field sites, genomic DNA was sequenced using short read sequencing (Illumina), and in selected cases, additional long read sequencing (ONT), obtaining around 0.5T bp of sequence.

Results: A total of 3,774 metagenome assembled genomes (MAG) were recovered, which represented 2,192 non-redundant genome clusters (defined at >95% average nucleotide identity). Of these, 526 clusters (24%) contained at least one putatively high-quality MAG. In regards taxonomic novelty, 970 unique taxa (GTDB) annotations were identified, of which 235 (24%) were annotated to species level and 503 (52%) were annotated only to genus level. Presential analysis of genome clusters across sites showed strong site-specific patterning ($P=0.001$, $R^2=0.63$; PERMANOVA), probably reflecting the influence of deterministic community assembly due to the presence of extreme physico-chemical conditions. Members of (GTDB) families Bryobacteraceae, Burkholderiaceae, Paludibacteraceae, Rhodobacteraceae,Rhodocyclaceae, PWPN01 (class Phycisphaerae) and UBA8517 (class Microgenomatia) were observed in at least 4 of 5 system types.

Conclusions: These pilot data build a preliminary dataset to explore the ecophysiology of these understudied, but socio-ecological-economically important, microbial communities, which may also be suitable model systems for studying deterministic community assembly in the moderate complexity regime.

Anthropocene and biogeochemical cycles

FT010

Global ecological insights from metabolic modeling of resource requirements in archaea and bacteria

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Microbial interactions are vital for niche colonization, nutrient exploitation, and environmental adaptation. However, the global-scale formation of these interaction networks, along with their taxon or habitat specificity and universal patterns, remain unclear.

Utilizing the EMP500 dataset from the Earth Microbiome Project (Shaffer et al., 2022), we generated community-scale metabolic models from genomes representing 1,261 strains using GapSeq and performed flux balance analysis with MICOM. We simulated minimal growth requirements for 414 metagenomic samples, predicting growth rates, metabolite exchanges, and significant associations along environmental gradients.

We investigated microbial interactions through provided, received, and co-consumed metabolites and predicted overall metabolic exchange scores for each habitat. Key drivers for clustering minimal media by metabolite composition included oxygen, ammonia, and D-Fructose. Higher metabolic exchanges were observed in animal and saline-associated samples. Halobacteriota Bog-38 sp003170935 and Prevotella were top generalists, while Collinsella and Haloarculaceae QS-5-70-15 were top specialists. The fungal and animal corpus (non-saline) provided the broadest growth niches, whereas the animal distal gut (saline) provided the narrowest. Cholate emerged as a key metabolite for many metadata categories, including a host's total mass, overall microbial diversity, and increasing environmental temperatures. Oxygen and thymidine were significantly associated with a free-living lifestyle, while D-Arabinose and GABA were associated with a host-associated phenotype.

Focusing on archaeal interaction patterns, species-specific interactions dominated over habitat-specific interactions. Additionally, our analysis revealed Rhodothermales and Conexivisphaerales DTJL01 as main interaction partners of Nitrososphaerales and Haloarculaceae for co-consumed metabolites in non-saline soils, Prochlorococcus_A pastoris for providing metabolites to Thalassarchaeaceae MGIIb-O2 sp902511005 in saline waters, and Escherichia coli as a receiver of metabolites from Bathyarchaeia in non-saline soils and from Methanobrevibacter_A sp900769095 in the animal distal gut.

This study provides, for the first time, a global-scale data resource simulating microbial interactions across diverse habitats using standardized multi-omics.

Shaffer, J.P. et al. Nat Microbiol 7, 2128–2150 (2022)

Anthropocene and biogeochemical cycles

FT011

Transcriptomic analysis of *Rhodococcus* sp. strain SC26 under chromium stress

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Chromium contamination poses a significant threat to both human health and ecological balance (Pushkar *et al*., Journal of Environmental Management, 2021). Among the various remediation strategies, biological approaches show great promise for mitigating chromium contamination, by reducing it into its less toxic form Cr(III) (Roșca *et al*., Processes, 2023). Gaining a deeper understanding of the metabolic response of bacteria to hexavalent chromium [Cr(VI)] is crucial for elucidating resistance mechanisms and enhancing the bioremediation of Cr(VI)-contaminated wastewater (Ramli *et al., Microbiological Research,* 2023).

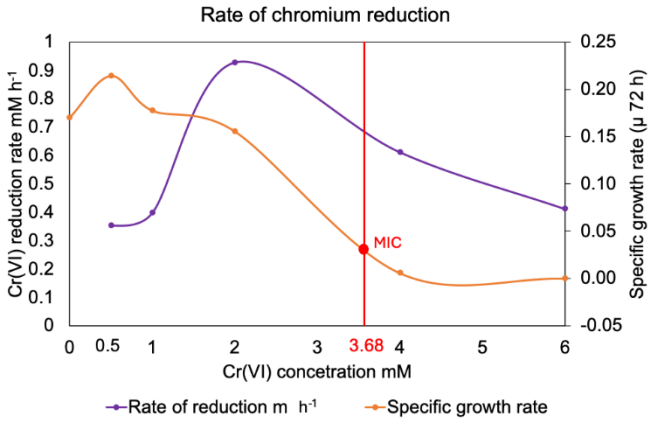
Rhodococcus sp. strain SC26 demonstrated high resistance to Cr(VI) (MIC of 4 mM). During growth in the presence of 1 mM Cr(VI), SC26 cells achieved up to 98% reduction to Cr(III) within 72 hours (Fig. 1). When tested in real wastewater containing 2mM Cr(VI), the strain maintained a reduction efficiency of 82%. NADP-dependent oxidoreductase and glutathione S-transferase were found to be overexpressed by Real Time q-PCR and enzymatic determinations.

Despite the promising potential of strain SC26 in Cr(VI) reduction, detailed Cr(VI) reduction and related ROS scavenging pathways remain poorly documented within the *Rhodococcus* genus. This gap may be attributed to challenges in gene annotation, which can obscure potential metabolic routes. Additionally, the taxonomic complexity of the *Rhodococcus* genus (Garrido-Sanz *et al.,* Microorganisms, 2020) complicates the accurate characterization of these pathways due to frequent reclassification and merging of species.

To address this knowledge gap, this study aims to elucidate Cr(VI) reduction pathway in *Rhodococcus* sp. strain SC26 through whole-genome sequencing and transcriptomic analysis. By comparing differential gene expression profiles, this research seeks to identify key genes and regulatory networks involved in chromium detoxification, providing a theoretical basis for the bioremediation of Cr(VI) in contaminated environments.

Acknowledgments: Research supported by Fondazione CARIPLO project 1069-2024 and by PSR 2021 Linea 6 1H_HUB project. A.M. is awarded by a PhD fellowship by the University of Milan - Food Systems PhD Program and Erasmus+ traineeship fellowship.

Fig. 1


Rate of chromium reduction
Cr(VI) reduction rate mM h-1
Specific growth rate (μ 72 h)
MIC
3.68
Cr(VI) concetration mM
Rate of reduction m h-1
Specific growth rate

Anthropocene and biogeochemical cycles

FT012

Agricultural land use reduces bacterial diversity and increases potential pathogens in the gut of mayfly larvae from adjacent streams

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Host-microbe interactions shape host health and function. Besides vertical transmission, hosts acquire part of their microbiome from the environment. Thus, environmental microbiome shifts can disrupt the host microbiome and cause dysbiosis. We propose that agricultural land use alters bacterial community structure in adjacent stream sediments and biota. Specifically, we hypothesize that higher nutrient runoff and suspended particulate organic matter (sPOM) from intensive agriculture will reduce bacterial diversity in stream sediments (<5 cm) and mayfly larvae (*Ephemera danica*), that feed on sPOM and live within these sediments. We also expect an enrichment of potential pathogens in the larval gut due to increased pathogen input from runoff or proliferation.

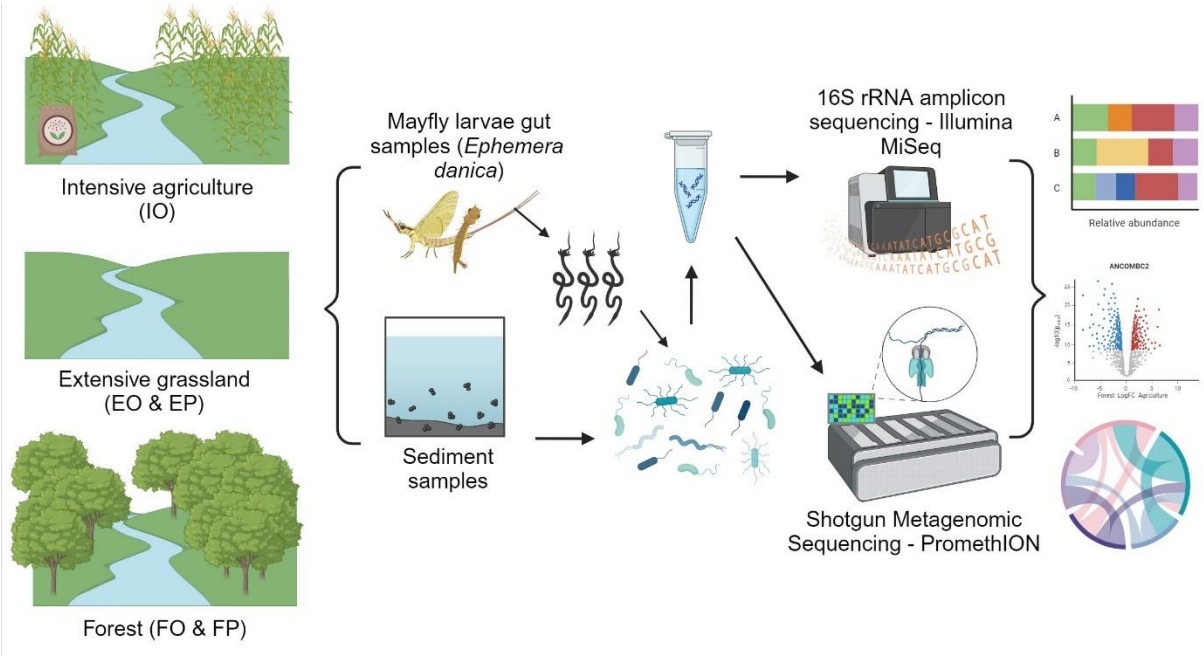
We analysed sediment and larval gut bacterial communities in relation to three land use types (forest, extensive grassland and intensive agriculture) along two streams in the Forstmühler Forest, Upper Palatinate, Germany. Five sediment replicates and larvae samples were collected from each site in August and November 2023. Bacterial community composition was assessed through 16S rRNA amplicon sequencing, obtaining amplicon sequencing variants (ASVs) which were taxonomically classified using DADA2 and a SILVA classifier. Moreover, long-reads shotgun metagenomic sequencing was carried out to further characterize the larval gut taxa associated with each land use type. The resulting reads were assembled with metaFlye and annotated with Prodigal, DIAMOND and BLASTx.

Grassland and intensive agriculture reduced bacterial richness in the larval gut samples, while sediment communities showed no clear trends. Using ANCOMBC2 to compare the ASVs from the forest with those from the grassland and intensive agriculture sites, we mainly identified wastewater-associated taxa in the sediments and potentially pathogenic bacteria in the larvae guts from the intensive site, such as *Flavobacterium*, *Tyzzerella* or *Clostridium*, which were also detected with the metagenomics sequencing carrying pathogenic elements such as *blaOXA-209*, *katG* and genes coding for membrane damaging toxins such as *hlyIII*, *plc* or *tlyc*.

Our findings demonstrate downstream effects of agricultural land use on adjacent freshwater ecosystems, leading to lower bacterial diversity in hosts and an increased abundance of bacteria harbouring pathogenic elements, which may be transferred to higher trophic levels within the stream ecosystem.

Fig.1 Experimental design.

Fig. 1


Intensive agriculture
(IO)
Extensive grassland
(EO & EP)
Forest (FO & FP)
Mayfly larvae gut
samples (*Ephemera
danica*)
Sediment
samples
16S rRNA amplicon
sequencing - Illumina
MiSeq
Shotgun Metagenomic
Sequencing - PromethION
A
B
C
Relative abundance
ANCOMBC2

Anthropocene and biogeochemical cycles

FT013

Assessing the impact of sublethal concentrations of chemical pollutants on the gut microbiome of pollinators – insights from *Osmia bicornis*

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Pollinators provide a key ecosystem service that underpins sustainable agriculture. For this reason, the recent and widely documented declines in pollinator populations have raised global concern. One of the leading causes of this decline has been identified as the exposure of pollinators to chemical pollutants, which can indirectly affect insects" health by disrupting their gut microbiome. Most studies on the effects of chemical pollutants on the gut microbiome of pollinators have focused on a few species (*Apis mellifera*, *Bombus* spp.), mainly examining insecticides and herbicides at typical field application doses. However, the impact of sublethal concentrations of various chemicals on the gut microbiome of other pollinators, including wild bees, remains largely unexplored, as does the structure of their gut microbiome. Therefore, the aim of this study is to assess the impact of various categories of chemical pollutants on the gut microbiome of one of the most common wild pollinators, *Osmia bicornis*. In laboratory trials, adult *O. bicornis* specimens were exposed to sublethal doses of four chemicals - boscalid, copper chloride, glyphosate, and ivermectin - administered both singularly and in combination, to explore potential synergistic effects. These compounds were selected because they have been detected as residues in bee-related matrices (e.g., pollen, nectar), making them relevant for studying their impact on pollinator health. The gut microbiome was studied using quantitative PCR to assess bacterial abundance and high-throughput Illumina sequencing of the 16S rRNA gene to examine the taxonomic composition. The results showed significant reductions in gut bacterial abundance following exposure to copper chloride and boscalid. Moreover, the microbiome composition and diversity were altered by all chemicals under study. Information on the effects of chemical pollutants on pollinator survival will be integrated with the knowledge gained from studying the impact of these substances on the gut microbiome of *Osmia bicornis*, providing new insights into the chronic effects of sublethal doses of chemicals on pollinator populations.

Anthropocene and biogeochemical cycles

FT014

Multiomics approach untangled sunflower ability to recruit beneficial microbiome for hydrocarbon rhizoremediation of contaminated soil

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[2]Università Cattolica del Sacro Cuore, Piacenza, Italy

Question

Industrial activity can represent a cause of land degradation and fertility loss due to pollutant accidental leakage and their subsequent release into the environment. Growing interest is devoted to developing efficient methods for remediating contaminated soils. A nature-based approach is phyto-rhizoremediation, which relies on the "cry-for-help" concept—i.e., the ability of plants to release specific root exudates upon stress and steer the composition of the rhizosphere microbiome.

Methods

(i) Based on phytotoxicity assays, sunflower demonstrated the ability to grow in a historically hydrocarbon (HC) polluted soil and was exploited to recruit and isolate HC-degrading and plant-growth-promoting bacteria through culturomics.
(ii) To monitor the dynamics of rhizosphere bacterial communities during the plant recruitment experiment, 16S rRNA Illumina sequencing and qPCR targeting HC catabolic genes were applied, comparing biostimulation (i.e., plant effect) and bioaugmentation (i.e., plant inoculated with HC degrading strains) interventions.
(iii) To identify root exudates potentially involved in plant response, metabolomic analyses were applied on exudates collected from plants grown under HC contamination.

Results and conclusion

Sunflower demonstrated a remarkable ability to withstand the phytotoxic effects of the target HC polluted soil, making this species ideal for rhizoremediation intervention. Molecular analyses revealed significant shifts in rhizosphere microbiome composition and HC degrading potential, shaped by both plant growth (biostimulation) and inoculation with degrading strains (bioaugmentation). Parallel experiments suggested that sunflower excretes specific molecules in response to HCs, potentially facilitating the recruitment of beneficial microbiomes. A bacteria collection was established from the sunflower rhizosphere, with most strains capable of thriving on various aliphatic and aromatic hydrocarbons. These strains showed the potential for alleviating plant stress by balancing phytohormones and increasing the bioavailability of hydrophobic pollutants. Based on these results, novel synthetic microbial communities were designed to enhance the bioremediation potential of the native soil microbiome.

Overall, our results highlight the importance of ecological interactions in steering the rhizosphere microbiome in polluted soils, selecting specific microbial populations that could enhance bioremediation efficiency.

Anthropocene and biogeochemical cycles

FT015

Harnessing desert rhizosphere microbes for enhancing plant resilience to abiotic stress – a case study on *Avicennia marina* and *Indigofera argentea*

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Desert ecosystems harbor a rich diversity of microbial life uniquely adapted to thrive under extreme environmental conditions, particularly in the rhizosphere and endosphere — the zone around/inside the roots. In this study, we investigate the potential of desert rhizosphere microbes from mangroves and Indigofera to enhance crop plant resilience against abiotic stress. Employing an interdisciplinary approach integrating microbiome, transcriptome, and metabolome analyses, we aim to elucidate the mechanistic underpinnings of plant-microbe symbioses.

The microbiomes associated with *Indigofera argentea* (Fabaceae) and *Avicennia marina* (Acanthaceae) ecosystems exhibit significant potential for bolstering plant resilience in arid and coastal environments, respectively. Through comprehensive investigation, we identify key bacterial strains capable of promoting plant growth and tolerance to salt, heat, and drought stress. Our research elucidates the functional characteristics of pivotal microbial taxa within the rhizosphere and endosphere, uncovering microbial consortia linked to advantageous traits such as nutrient acquisition, pathogen suppression, and abiotic stress mitigation.

Furthermore, we explore the transcriptomic responses of Arabidopsis/rice plants to microbial colonization, deciphering the molecular pathways involved in host-microbe crosstalk and physiological adaptation to environmental challenges. Validation of microbial inoculants derived from mangrove and Indigofera microbiomes initially on the model plant Arabidopsis, followed by application to rice, demonstrates enhanced crop resilience and productivity in arid regions. By harnessing the power of desert rhizosphere microbes, our study offers new insights and solutions for sustainable agriculture and ecosystem management in desert environments, contributing to resilient plant communities and thriving ecosystems in the face of climate change and environmental challenges.

Anthropocene and biogeochemical cycles

FT016

Mitigation of methane emissions from peatlands – a role for micro-propagated *Sphagnum*-associated methane oxidising bacteria?

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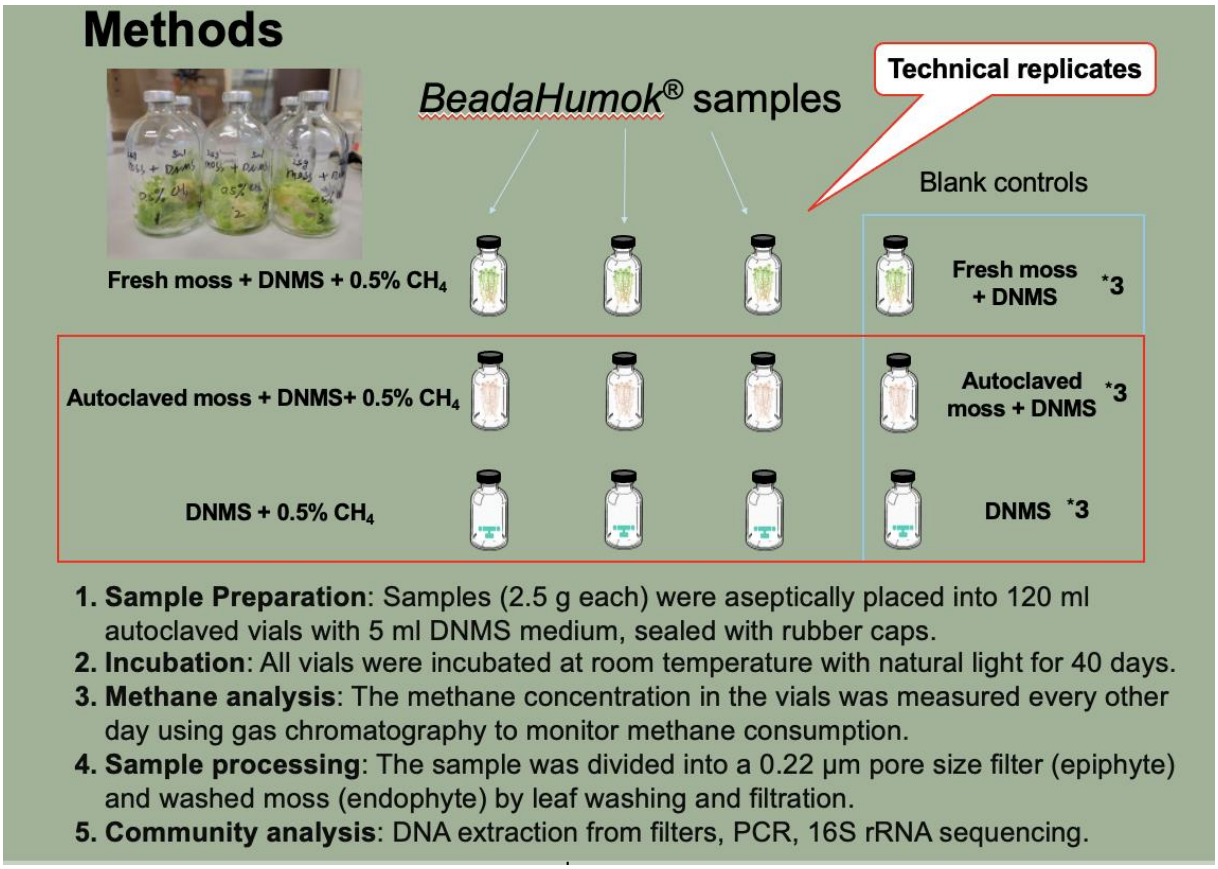
Peatlands, a significant source of atmospheric methane, rely on *Sphagnum* mosses and methane oxidising bacteria (MOB). However, over 15% of peatlands have been destroyed. In the UK, restoration projects aim to rewet peatlands and replant *Sphagnum* mosses. Greenhouse-grown *Sphagnum* mosses, especially Beadamoss® (BeadaHumok®), have been used for this purpose. This study shows BeadaHumok® hosting MOB before established into the wild damaged peatlands and investigates the interaction between greenhouse-grown *Sphagnum* and MOB. These findings support future peatland restoration efforts by enhancing understanding of *Sphagnum*-MOB interactions.

Fig. 1



Food-gut microbiome axis

AL009

Harnessing Probiotics for Gut Health: From Microbiome Modulation to Clinical Benefit

I. Klymiuk[1]

[1]Institut AllergoSan, Graz, Austria

Our knowledge about the human associated microbiome has grown dramatically during the last decade. The human gut microbiome plays a pivotal role in maintaining human health and preventing disease. Probiotics, defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, have emerged as a promising and sustainable tool to support human health.

Although the mechanisms of action remain the focus of ongoing research, preclinical and clinical trials have shown that inflammation reduction, immune system as well as metabolic interaction and pathogen inhibition are among the main mechanisms of action. Recent trials have confirmed its beneficial role in modulating the gut-brain axis, particularly with respect to depression and sleep.

This presentation will highlight findings from recent clinical trials and discuss challenges in translating microbiome science into effective indication specific interventions, including strain-specific effects and the need to bridge the gap between microbiome research and the development of next-generation probiotic therapies.

Food-gut microbiome axis

AL010

Microbiota of Urbanites – Double Blind, Randomized, Placebo Controlled Rewilding Trials

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Gut dysbiosis is linked to microbially poor living environment. Comparative studies have shown an inverse association between the greenness of living environment and the risk of atopy and type 1 diabetes. Since correlation does not mean causation, I – together with colleagues - did microbiologically oriented intervention trials with dirt.
First, we rewilded daycare yards with forest floor, planting boxes and lawn, while other yards were left intact. We also tested whether green walls enrich commensal microbiota. Thereafter we worked with double-blinded, placebo-controlled trials. In the first trial, we added microbially rich dirt or visually similar but microbiologically poor peat into sandboxes of daycare children. In the second trial, urban dwellers cultivated vegetables in microbially rich soil or placebo peat. The third trial consists of infants that are exposed to rich environmental microbiota via skin, gut and airways. The results of the trials are discussed.

Food-gut microbiome axis

AL012

Characterisation of novel Lacticaseibacillus species, isolated from various silages

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Six rod-shaped, non-motile, non-spore-forming, facultative anaerobic, Gram-stain-positive lactic acid bacteria, designated as HO 1656T, HO 0673, EB0058T, SCR0080, LD0937T and SCR0063T were isolated from different corn and grass silage. Analysis of 16S rRNA gene sequence of the strains indicated that they belong to the Lacticaseibacillus group. Based on genomic, chemotaxonomic, and morphological data obtained in this study, three novel species, Lacticaseibacillus huelsenbergensis, Lacticaseibacillus parahuelsenbergensis and Lacticaseibacillus styriensis with the type strains HO 1656T (=DSM 115425=NCIMB 15466), LD0937T (=DSM 116105=NCIMB 15471) and SCR0063T (=DSM 116297=NCIMB 15473) were proposed. These novel species are very closely related to each other and could be differentiated from each other by functional genomics. The two strains LD0937T and SCR0063T could be distinguished from HO 1656T and all their closely related type strains, because only these two strains possess the araA gene and therefore an L-arabinose isomerase. Furthermore, only SCR0063T owns the sequence for an alpha-mannosidase and the corresponding enzyme, in contrast to LD0937T and all related Lacticaseibacillus type strains. The differential utilization of the substrates was validated through phenotypic analysis in metabolic experiments.

Due to whole-genome sequence-based characterization, EB0058T and SCR0080 were separated into a distinct clade from Lacticaseibacillus zeae DSM 20178T, together with CECT9104 and UD2202, whose genomic sequences are available at NCBI GenBank. The average nucleotide identity (ANI) values within the new subgroup are 99.9% and the digital DNA-DNA hybridisation (dDDH) values are 99.3%- 99.9%, respectively. In contrast, comparison of the new subgroup with publicly available genomic sequences of L. zeae strains, including the type strain DSM 20178T, revealed dDDH values between 70.2%- 72.5% and ANI values between 96.2%- 96.6%. Based on their chemotaxonomic, phenotypic, and phylogenetic characteristics, EB0058T and SCR0080 represent a new subspecies of L. zeae. The name Lacticaseibacillus zeae subsp. silagei was proposed with the type strain EB0058T (=DSM 116376 =NCIMB 15474).

Food-gut microbiome axis

AL013

A scalable metagenomic approach for joint quantification of gut microbiome and dietary intake

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[2]University of Illinois Urbana-Champaign, Department of Food Science and Human Nutrition, Urbana, IL, United States
[3]AdventHealth Translational Research Institute, Orlando, FL, United States
[4]Intitute for Systems Biology, Seattle, WA, United States

Dietary intake and the human gut microbiome are intimately connected. Many of the foods we consume will eventually come in contact with 38 trillion microbes inhabiting the human intestinal tract. Microbial metabolism of dietary compounds dictates not only the composition but also the function of the human gut microbiome. For instance, short-chain fatty acids, indoles, and breakdown products of polyphenolic compounds, all dependent on the presence of their respective fermentation precursors, are important immune regulators.

Understanding the intricate interplay between diet and the gut microbiome is crucial for human health. However, accurately quantifying both simultaneously has remained a significant challenge. Current methods are limited by temporal variability, measurement inaccuracies, and incomplete understanding of digestive processes, hindering reliable joint quantification of food and microbial abundance in the distal gut.

Here we present an integrated and highly specific approach that quantifies microbial and remaining food DNA in metagenomic sequencing samples. Through a combined DNA-Nutrition database covering Bacteria, Archaea, the host, and food-related organisms, we leverage a decoy-aware detection approach to achieve high specificity for all taxa. We show that the derived food intake estimates correspond well with food frequency questionnaires and food diaries and validate their accuracy in two controlled feeding studies. We then show various examples of how this can uncover specific microbiome-diet interactions in large cohorts.

The high scalability and suitability for prospective and retrospective studies makes the presented strategy an accessible option to uncover microbial and dietary patterns in various settings, enabling deeper insights into microbiome-diet interactions.

Food-gut microbiome axis

AL014

Water kefir ecosystem (meta)genomics and metatranscriptomics – a stepping stone towards understanding water kefir grain assembly

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Water kefir grains (WKGs) are natural dextranous granules used to produce the fermented beverage water kefir (WK). When placed in a sugary solution with dried fruits, the WKG-associated microorganisms, such as lactic acid bacteria, yeasts, bifidobacteria, and acetic acid bacteria, carry out the fermentation resulting in the production of acids and flavour compounds, as well as the dextran and the biomass forming the WKGs. Whereas microbial compositions of many WK ecosystems and their response to technological factors have been unravelled, the functional traits of microbial species forming the WK community are understudied. Except for a handful of lactic acid bacteria-yeast interaction studies, studies addressing interactions between WK microorganisms are lacking, especially in light of their impact on gene expression. Moreover, molecular mechanisms driving the assembly of WKGs - the hallmark of WK - are not understood. Therefore, this study aims to unravel the WK ecosystem structure-function mystery by combining strain isolation and characterisation with long-read (meta)genomics, metatranscriptomics, and metabolite targeting analyses.

A near-complete collection of microorganisms forming the WK ecosystem under study has been isolated, comprising bacterial strains (from the species *Lacticaseibacillus paracasei*, four *Liquorilactobacillus* species, *Schleiferilactobacillus harbinensis*, *Bifidobacterium psychraerophilum*, and two *Acetobacter* species) and yeast strains (from the species *Brettanomyces bruxellensis, Hanseniaspora valbyensis, Zygotorulaspora florentina*, and [Candida] *boidinii*). In addition to isolate genome sequencing, metagenomics provided genomic information on species not yet isolated (*e.g.*, *Lentilactobacillus hilgardii*, *Oenococcus sicerae*, and a *Zymomonas mobilis* relative). The metatranscriptomic data suggested that *Liquorilactobacillus nagelii* was the most transcriptionally active bacterium, whereas *Z. florentina* and *B. bruxellensis* were the most transcriptionally active yeasts.

By quantifying transcriptional activity of genes involved in metabolism (*e.g.*, nutrient assimilation, biosynthesis of amino acids and cofactors), stress resistance, and microbial interactions (*e.g.*, extracellular polysaccharides production) of the WK microbial community the work reveals novel insights into the WK ecosystem function and the assembly of WKGs.

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Food-gut microbiome axis

AL015

Functionalizing probiotic *Bacilli* through triggering biofilm inspired antagonistic activity in response to plant-derived prebiotics

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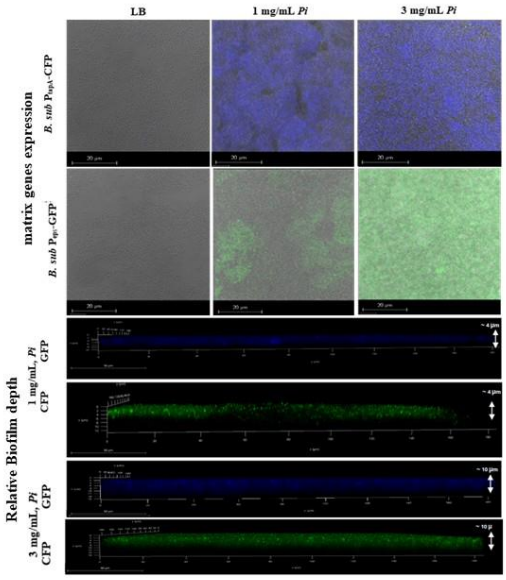
The biofilm forming probiotic *Bacilli* may significantly influence healthiness of mammalian host through generating functional postbiotic products. However, there are substantial challenges in achieving the persistence of probiotic cells during generating postbiotic products, including their survivability or production of antagonistic postbiotic molecules. We hypothesized that biofilm-inspired encapsulation using plant-derived prebiotics will enhance the resilience of probiotic bacteria against physiological stresses through promoting potent antagonistic activity against enteropathogenic species. Accordingly, the favorable plant derived prebiotic activity of *Pulicaria incisa* (*Pi*) infusion was characterized on probiotic *Bacilli*, such as *B. subtilis* and other related species. Our findings indicate that the *Pi* infusion affects significantly the beneficial bacterial physiology, specifically through triggering the biofilm formation process by the probiotic *B. subtilis*. It was further found that the induced biofilm formation is accompanied by a robust antagonistic activity triggered by the *Pi* infusion or one of its major soluble sugar molecules - myo-inositol. It is also apparent that combination of *Pi* infusion together with this alcoholic sugar molecule results in synergistic effect in terms of enhanced biofilm formation as well as antagonistic activity against pathogenic bacteria. Thus, it is conceivable that such constituent molecules may direct the probiotic cells to accelerate pathways associated with biofilm formation in conjunction with potent antagonistic activity against different enteropathogeic bacteria.

Fig. 1



Food-gut microbiome axis

FT017

The olive rhizosphere microbiome under different agricultural management across the Mediterranean Basin

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The complex and co-evolved interplay between plants and their microbiota is crucial for the health and fitness of the plant holobiont which also have implications on fruit production. At the same time, the plant microbiome is also influenced by the environmental conditions and the agricultural managements. The olive tree cultivation has economic, social and historical importance in the Mediterranean Basin since 5,000 years B.C. In this work, we have analyzed 52 olive orchards from 15 provinces in 5 countries of the Mediterranean Basin: Portugal, Spain, Morocco, Italy and Greece. The olive orchards were subjected to either traditional, organic or hedgerow cultivation systems, under different climatic and pedological conditions. All orchards were sampled on May 2023 at or just after full bloom. A total of 472 samples from olive root rhizosphere were subjected to MiSeq sequencing for ITS2 and 16S *rRNA* gene amplicons. We obtained more than 40 million raw reads for both bacteria and fungi which yielded 11,460 ASVs for bacterial and 5,411 ASVs for fungal communities. The beta diversity analyses established two clearly distinctive groups: a) samples from Spain and Morocco, and b) samples from Portugal, Italy and Greece. This picture was also present in the taxonomic analyses that showed a dominance of Proteobacteria in the rhizosphere of olive trees from Portugal, Italy and Greece while in Spain and Morocco Actinobacteria was the predominant bacterial phylum. A similar image was obtained for the fungal communities but with samples from Spain and Morocco in different groups. Although Ascomycota was the most abundant phylum in all samples, it was highly depleted in these latter countries. Moreover, the phylum Mortierellomycota had a relative abundance of 33 % in Morocco and the Glomeromycota had 7 % in Spanish soils, while both phyla were clearly diminished or absent in the other countries. At genus level *Pseudomonas* was highly abundant in samples from Portugal, Greece, Morocco and Italy while in the Spanish samples it was nearly absent. In the fungal communities, the genus *Fusarium* was highly abundant in samples from Portugal, Italy and Greece (above 30% in all cases), however in samples from Morocco and Spain the relative abundance of this genus was always below 10%.

Funding

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Food-gut microbiome axis

FT018

One Health perspective – Large-scale (meta)genomic analysis revealed the distribution and transmission of antimicrobial resistance genes from environment to human

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By the year 2050, it is estimated that drug-resistant pathogens will contribute to 10 million deaths annually. This concerning prediction underscores the need to understand the role of environmental microbiota in the rise of antibiotic resistance to develop effective counter-strategies. Natural environment, which often display high microbial prevalence and diversity, are viewed as significant reservoirs of antimicrobial resistance (AMR) genes. To comprehensively understand the evolution, emergence, and spread of antimicrobial resistance, it is essential to investigate the distribution of antibiotic resistance genes (ARGs) through interconnected processes that highlight the complex relationships among humans and the environment, as framed by the One Health approach. In this study, we utilize a large-scale dataset comprising over 19,000 metagenome-assembled genomes (MAGs) and 4,000 metagenomic samples from soil, food, oral microbiomes, and the human gut to examine the distribution and transmission of ARGs across these interconnected microbiomes. Specifically, we aim to address the following questions: 1) What are the primary carriers of ARGs, and which factors significantly shape the resistome? 2) Which taxa are likely to contribute to the emergence of antimicrobial resistance (AMR) that is transmitted across various biomes? 3) Do shifts in bacterial community structure account for changes in ARG profiles across different biomes? 4) What factors influence ARGs in various biomes? 5) Our study aims to provide a comprehensive overview of ARG distribution and transmission among interconnected environments, thereby enhancing our understanding of the complex pathways of ARG spread and identifying new targets for AMR surveillance and control efforts.

Food-gut microbiome axis

FT019

Understanding the effects of domestication on the sweet cherry microbiome

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Sweet cherry trees (*Prunus avium*) are extensively cultivated in many countries mainly for their edible fruits, which are rich in health-promoting nutrients, sugars, organic acids, and phenolic compounds. Research indicates that sweet cherries and their metabolites exhibit anti-inflammatory, antimicrobial, and anticarcinogenic properties. However, commercial exploitation of sweet cherry orchards is often impeded by yield losses due to both biotic and abiotic stresses. This situation presents a promising opportunity for microbiome management as a strategy to mitigate these challenges. The wild relatives of sweet cherries can be found in Austrian forests. Therefore, to understand the sweet cherry microbiome and the human influence on it, we collected from 17 sweet cherry trees, encompassing a range of specimens including wild-native trees, trees from private gardens, and Prunus avium Regina trees from an orchard to represent the spectrum of domestication. We analyzed the bacteria and fungi of ripe fruits, seeds, twigs, and bulk soil through amplicon sequencing and quantitative PCR (qPCR) of 16S rRNA and ITS fragments. In addition to the well-described effect of the sampling location, domestication also had a significant effect on the microbiome composition across all sample types. In the cherry fruit pulp, the bacterial diversity significantly increased and the fungal diversity significantly decreased with higher domestication. This domestication effect on the alpha diversity was confirmed by significant positive spearman correlation of bacterial diversity and significant negative spearman correlation of fungal diversity with fruit weight. Interestingly, the sweetness and bitterness of the cherries co-correlated with fruit weight, highlighting that human selection and management for larger, tastier cherries play an important role in shaping the microbiome of cultivated sweet cherries. However, these shifts in the cherry microbiome resulting from domestication may have adverse effects. A differential abundance analysis revealed that the fungal features exhibiting the most significant increase between wild and cultivated cherries are the well-known cherry pathogens *Monilinia laxa* and *Taphrina wiesneri*. With this study, we reveal microbiome features of sweet cherry trees along their domestication, potentially applicable as basis to develop strategies for biocontrol and post-harvest spoilage control.

Food-gut microbiome axis

FT020

Functional diversity of lettuce endophytes may help with lettuce resilience against pathogens

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The use of beneficial microbes aligns naturally with the pursuit of sustainable agricultural practices. This approach minimizes adverse ecological impacts and enhances microbial diversity, fostering a healthy environment for crop growth. Interactions between microorganisms and plants play a pivotal role in determining plants' responses to abiotic and biotic stressors. Crucially, these interactions are influenced by the plant genotype. In this study, we assessed the phyllosphere of 34 different organically grown lettuce cultivars by isolating potentially plant-beneficial endophytic bacterial strains. To obtain a diverse set of beneficial isolates, plants were cultivated in different media, including organic potting substrate and organic field soil, each with distinct microbiome profiles. The isolates were classified using enzymatic activity assays and 16S rRNA gene sequencing. Their effects on lettuce growth, marketability, and stress resilience are being evaluated in direct comparison to commercial plant growth-promoting bacteria (PGPR). In addition, these tests are performed across different lettuce cultivars to analyze cultivar-specific response variations to PGPRs. Additionally, we aim to enhance food safety by assessing the ability of our bacterial isolates to suppress the growth of *Salmonella enterica*, a human pathogen capable of colonizing lettuce and frequently associated with food-borne diseases caused by contaminated fresh produce.

Food-gut microbiome axis

FT021

The role of gut-liver axis in *Amanita* species mushroom poisoning

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Death cap (*Amanita phalloides*) poisoning leads to life-threatening acute liver failure, with treatment options limited to supportive care and liver transplantation. Mushroom picking is a popular leisure activity in Eastern Europe and each year, cases of mushroom misidentification lead to severe medical consequences. Anatomical connection between the gut and the liver, along with the route of poison delivery, and previously described connections between microbiota and liver disease suggest that gut microbiota might influence the degree of liver damage in death cap poisoning. We focused our study on the best-described and deadliest of death cap toxins, the bicyclic octapeptide α-amanitin. While cyclic peptides are considered very stable, there are reports of bacteria capable of efficiently degrading microcystin-LR, another important cyclic peptide. The goals of the project are to (a) test for a potential role of microbiota in α-amanitin poisoning, and (b) identify potential toxic cyclic peptide degrading bacteria that could help in devising novel treatments.

To test if microbiota plays role in the extent of amanitin toxicity we performed experiments in vivo in mouse models. We divided a mouse cohort into two groups, one was treated with broad-spectrum antibiotics to remove the microbiota, while the second remained with normal gut flora. Amanitin was administered to both groups by intraperitoneal injection. Blood samples were collected from each mouse, we assessed liver damage by measuring levels of liver enzymes ALT and AST in blood plasma. The level of ALT and AST was significantly higher in the control group than in the group that received antibiotics treatment. These results suggest that depletion of microbiota alleviated liver damage caused by α-amanitin poisoning. Currently, we are performing further experiments to formulate and verify the hypothesis explaining the mechanism of this process.

To identify potential α-amanitin degrading bacteria, we conducted a preliminary experiment in which we collected fresh mouse feces and cultured the gut microbiome in a medium supplemented with amanitin. The filtered medium from these cultures was then applied to Hep3B cell cultures to determine whether amanitin's effect on hepatocytes had been diminished. In one sample, the toxic effect of amanitin was significantly lower and we are currently verifying this result by quantifying the amount of the toxin before and after incubation with bacteria using MS and HPLC.

Food-gut microbiome axis

FT022

Impact of antibiotic use in cattle farms on the resistome of fecal *Acinetobacter* spp.

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Question: Cattle manure is widely used as a soil fertilizer; however, manure from antibiotic-treated animals may contribute to the spread of antibiotic-resistant bacteria and their resistance genes (ARGs) in agricultural soils. The bacterial microbiota of cattle feces and manure includes species of the taxonomically and ecologically diverse genus *Acinetobacter*. Some, such as *Acinetobacter baumannii*, can cause opportunistic infections in humans and accumulate horizontally acquired ARGs. Here, we investigated whether antibiotic use in cattle affects antibiotic resistance and the presence of horizontally acquired ARGs in fecal *Acinetobacter* species.

Methods: Our work integrates culture, genomics and long-read metagenomics, all conducted on composite fecal samples from 28 Czech cattle farms with contrasting levels of antibiotic use. Cultured *Acinetobacter* strains (n=284) were tested for susceptibility to 19 antibiotics. Genomes of chosen strains and metagenome-assembled genomes and contigs from *Acinetobacter* enrichment cultures were analyzed for the presence of acquired ARGs and their genetic context.

Results: *A. indicus* and *A. pseudolwoffii* were the most abundant species based on both isolated strains and metagenomic results. As many as 40% of the strains represented putative novel species, while the well-known opportunistic pathogen *A. baumannii* was scarce. Strain-specific decreased susceptibility to streptomycin, sulfonamides and trimethoprim were most common, with significantly lower streptomycin susceptibility in *A. indicus* and *A. pseudolwoffii* from antibiotic-using farms. Genomic and metagenomic analyses revealed the presence of streptomycin (mostly *strA-strB*), sulfonamide (*sul2*), chloramphenicol (*clmB1, floR*), tetracycline [*tet*(Y), *tet*(X), *tet*(39)] and carbapenem (OXA-58) resistance genes, associated with various combinations of insertion sequences, transposons and plasmids. While carbapenems are not administered to cattle, OXA-58 may have been co-selected by other antibiotics used in farms, as suggested by the co-occurrence of OXA-58, *strA-strB* and *sul2* on contigs.

Conclusions: Cattle feces harbor diverse *Acinetobacter* spp. with mobilizable ARGs, including the clinically relevant OXA-58 gene, potentially co-selected by aminoglycoside or sulfonamide antibiotics. This highlights the need for responsible antibiotic use in livestock and effective manure management practices.

Food-gut microbiome axis

FT023

Diversity and antimicrobial potentials of the microbiome of Nigerian medicinal plant, *Ficus thonningii* (BLUME)

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Question: The phyllosphere microbiome contributes to plant metabolism and protection from pathogens. Medicinal plants constitute a rich source of phytochemicals and antimicrobial drugs, but bioprospecting is yet to fully exploit the potentials of their microbiomes. This research characterised leaf microbiomes *Ficus thonningii* (BLUME), with the hypothesis that the microbiota produces antimicrobial compounds.

Methods: *F. thonningii* was sampled thrice annually for two years in Ibadan, Nigeria. A polyphasic approach combining confocal microscopy, strain characterisation, quantitative polymerase chain reaction, amplicon and shot-gun metagenome sequencing, and genome mining for biosynthetic gene clusters was used to characterise the microbiome. Data were analysed with publicly available bioinformatic tools.

Results: Bacteria, visualised in clusters on leaves of *F. thonningii*, were the most abundant phyllosphere inhabitants with Pseudomonadota being the most abundant phylum. Species of *Streptomyces*, *Pseudomonas* and *Xanthomonas* were abundant in the phyllosphere, and *Cladosporium* was the dominant fungal genus. Five previously unknown species of *Lysobacter* (3)*, Pseudoduganella* (1), and Weeksellaceae (1) were isolated. Culture-independent methods detected 65% of cultured bacterial genera and pointed to two independent microbiome perturbations which were attributable to antimicrobial-producing *Pseudomonas* and *Xanthomonas* at the second and fourth samplings, respectively. The viral and archaeal profiles were consistent across all samples. *Pseudomonas, Xanthomonas* and *Bacillus* isolates showed antimicrobial activity against tested clinical bacteria. Genomes of antimicrobial producing strains encoded non-ribosomal peptides, terpenes and polyketides, which could account for antimicrobial activity, with the molecule produced by the respective clusters being novel in 91.3% of instances. Plant-derived sugars, cellulose, pectin, xylans and starch were among substrates of the metagenome which also had capacity for methanogenesis, short chain fatty acid and alcohol conversion.

Conclusions: *Ficus thonningii* possess diverse and distinct phyllosphere microbiomes that are dominated by bacteria with vast metabolic capacity, including novel species. The microbiome includes antimicrobial-producing bacteria with previously unidentified biosynthetic gene clusters. Medicinal plants" phyllosphere microbiota should be considered as a potential source of novel antimicrobials.

Soil, plant microbiome and One Health

FT024

Genomic and metabolic potential of myxobacteria from disease-suppressive soil

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Background: Disease-suppressive soils harbor microbial communities that interfere with the life-cycle of soil-borne plant pathogens. Previously, we screened 28 agricultural soils for suppression of root rot in wheat caused by *Fusarium culmorum*. For one of the highly suppressive soils (S11), metagenomic analysis revealed an enrichment of Myxobacteria. To further investigate their role in disease suppression, we isolated 29 [GP1] Myxobacteria from S11 and explored their genomic and metabolic potential.

Methods: Whole-genome sequencing was performed using Illumina for all isolates, while six strains underwent additional long-read sequencing with Oxford Nanopore. The disease-suppressive potential of all isolates was assessed *in vitro,* volatile organic compounds and water-soluble metabolites were analyzed using GC-MS and LC-MS, respectively. Biosynthetic gene clusters (BGCs) were predicted from genome data to explore potential links between metabolic profiles and disease suppression. Selected Myxobacteria strains were tested *in planta* for their ability to protect wheat against *F. culmorum*

Results: One Myxobacterium strain (SBMx462) exhibited strong inhibition against *F. culmorum both in vitro and in planta*. Several strains demonstrated enhanced antagonistic activity when co-cultured, suggesting potential cross-feeding interactions that enhance biocontrol efficacy. Genomic analysis identified BGCs associated with siderophores, lipopeptides, and other secondary metabolites potentially linked to disease suppression.

Conclusions and future perspectives: Our findings highlight the genomic and metabolic potential of Myxobacteria in plant disease suppression. The observed interactions and BGCs provide insights into the mechanisms underlying their disease suppressive activity. Currently we are integrating bioactivity, genomic, transcriptomic and metabolic data to decipher key pathways involved in the biosynthesis of antifungal compounds.

Soil, plant microbiome and One Health

FT025

Putting the sub-Antarctic Kerguelen archipelago on the map – unveiling plant holobiont bacterial diversity and dynamics in the world"s most isolated land

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Plants interact with numerous microorganisms recruited in their environment and that colonize all of their organs, both below and aboveground. This biotic assemblage is referred to as a "holobiont". The associated microbiome, either transmitted vertically or recruited horizontally, plays crucial roles in the plant performance, by contributing to its nutrition, pathogen resistance, and stress tolerance.

The uninhabited (to the exception of a scientific base) sub-Antarctic Kerguelen Islands are an ideal open-air laboratory to study plant-microbiome recruitment, diversity and evolution due to their geographic isolation, being thousands of kilometers away from the nearest continent. Fellfield ecosystems are inland pristine elevated bare areas subject to frost and strong wind exposure, and are home to most of the archipelago"s endemic plant species. They represent key sentinel sites of biological heritage that have evolved under low anthropogenic influence and historically buffered climate. However, confronted to rapid rising temperatures and decreasing rainfall in the South Indian Ocean, interactions within the holobiont will probably undergo significant shifts.

We hypothesize that Kerguelen"s isolation led to low α-diversity in soil and plant-associated microbiomes, with limited microbial interactions for both endemic and introduced plant species. Additionally, we anticipate that climatic changes along altitudinal gradients influence bacterial diversity, with lower diversity at higher altitudes. We also expect nutrients availability to be a key driver of microbial assemblage in the oligotrophic fellfields" soils.

We investigated bulk soil and plant-associated (rhizosphere, root, leaf, phyllosphere) bacterial communities of the natives *Poa kerguelensis* (Poaceae) and *Pringlea antiscorbutica* (Brassicaceae), alongside the alien *Poa annua*, across altitudinal transects, using 16S DNA metabarcoding. Additionally, we explored the specificity and originality of soil microbiota on Kerguelen by comparing with topsoil samples part of the Earth Microbiome Project.

Our findings provide insights into the assembly rules of microbiomes in Kerguelen, with plant species recruiting distinct bacterial communities whatever the organ; and with soil ammonium content, elevation and wind exposure as the main drivers of soil bacterial assembly. Therefore, climate change could disrupt plant-microbiome interactions, leading to shifts in their composition.

Soil, plant microbiome and One Health

FT026

Modelling and fate analysis of manure and recycled water introduced microbiome, resistome and mobilome to soil, reveals persisting and dissipating taxa and genes

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Water recycling and manuring are mandates of contemporary good agricultural practices. They are associated with the concepts of circular economy and climate change impact amelioration. One Health zero tolerance to pathogen and antimicrobial resistance (AMR) dispersal, dictates deep understanding of the fate of microbial inputs along such practices. Here, we attempt to model the impacts of these inputs employing soil microcosms. We tested urban wastewater treatment plant influent/effluent and pig manure as inputs and included treatments with tetracycline and sulfamethoxazole at realistic concentrations. We then monitored the microbial biomass (qPCR) and diversity (16/18S rRNA gene and ITS) and the antibiotics via HPLC, at five timepoints (0, 0.5, 1, 4 and 25 days). Key samples were also shotgun sequenced. Out of the tested inputs, only manure specific taxa (not detected in soil prior manure application) were detected post application in soil at significant amounts (>20% of the total community). Recycled water specific taxa showed only sporadic appearances at near noise levels. Manure taxa comprised 6-34% of the total soil community right after amendment with the prokaryotic *Clostridium*, *Turicibacter*, *Terrisporibacter* and *Romboustia* being persistent, whereas streptococci, lactobacilli, bifidobacteria, corynebacteria dissipated relatively quickly (DT50, ≤5 days) according to the best fit exponential decay model. Manure-introduced *Pseudomonadota* and *Methanobacteriota* went below detection limits after 4 days. Exponential decay also governed the abundance of marker genes coding for tetracycline (*tetQ*) and erythromycin (*ermF*) resistance (which increased by 2 and 6 orders of magnitude compared with the background abundance at day 0), while *intI1* abundance was stimulated post manure addition. In nearly all exponential decay modelled responses, antibiotic application resulted in increase of tested-marker persistence according to estimated dissipation time values. Shotgun metagenomic AMR, rest biocide resistance, virulence, bacteriophage marker analysis showed a convergence of the amended soils with the controls after 25 days. Overall, our results demonstrate significant, yet, transient, perturbations for the bulk of the tested microbial biomarkers, with several introduced taxa persisting and horizontal markers like *intI1* being stimulated. This study deepens our understanding about the impact of agricultural inputs from the One Health perspective.

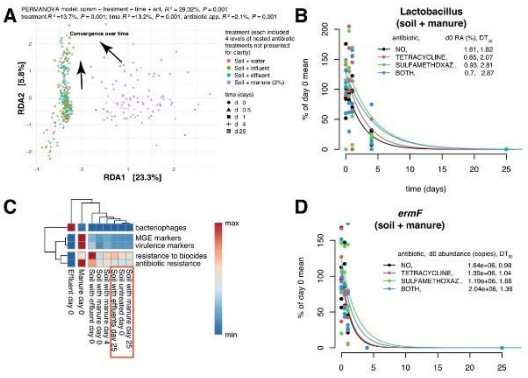

Figure 1 (A) Redundancy analysis and PERMANOVA testing the additive effects of substrate (water, wastewater influent/effluent, and manure), time and antibiotic fortification, on the soil microbial communities. (B & D) Exponential decay modelling of representative manure introduced microbial and resistance gene markers in the soil+manure environment. (C) Heatmap of the shotgun sequencing results regarding relative database hits to the 16S rRNA gene hits (min/max values based on sample comparisons are depicted).

Soil, plant microbiome and One Health

FT027

Development of an *in situ* monitoring system for tracking solutes, microbes and gas emissions for sustainable soil management

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Soil pollution caused by human activities presents a global challenge with adverse effects on public health and ecosystem functions and services. This is particularly evident in the context of nitrogen (N) fertilization, which, while enhancing plant growth, leads to soil degradation, air pollution through the release of reactive N gases (e.g., nitric oxide (NO) and nitrous acid (HONO)) and greenhouse gas emissions, and contributes to the eutrophication of freshwater and marine environments. To mitigate these environmental impacts, it is crucial to understand the complex biogeochemical processes that control N transformations in soils, such as nitrification and denitrification. Despite progress in the study of soil N cycling, there are still gaps in mechanistic insights and quantitative measurements, particularly with regard to N retention and transformation across various phases.

In this project, we will develop a setup that combines flux measurements of gaseous compounds and concentration measurements of soil solutes. The open flow microperfusion (OFM) technique will be used for continuous *in situ* monitoring of soil solutes such as contaminants and microbial metabolites, while the dynamic chamber (DC) will enable the measurement of soil gas emissions both in the laboratory and in the field. This hybrid method (OFM-DC) will allow us to simultaneously sample and analyze soil substances in the gas and liquid phases and thus assess soil and air quality and health.

By integrating these tools with mechanistic modeling, the project aims to optimize soil management practices, reduce food production costs and ultimately contribute to sustainable soil use and restoration in the face of global environmental challenges.

Soil, plant microbiome and One Health

FT028

How does dark resuscitation impact desert microbial communities?

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Drylands cover ~40% of Earth's surface and support diverse life despite water scarcity. Microorganisms, lichens, algae, and mosses form biological soil crusts (biocrusts), a few mm thick structures that prevent soil erosion and play a crucial role in ecosystem stability. Biocrusts host microbial communities that survive desiccation by entering a dormant state, reducing metabolic activity. Upon rehydration, the reactivation of these organisms, followed by energy-generating metabolisms and DNA repair, sustains their survival. Previously, we studied microbial activation after a rain event in the daytime at 27 °C1, while nighttime responses at cooler temperatures remained unexplored. This study investigates whether microbial reactivation differs depending on temperature and light. We hypothesize that the differing light and temperature regimes will affect microbial gene expression patterns, such as in phototrophs deprived of light for photosynthesis.

A rehydration experiment in the dark was conducted with biocrusts sampled at the LTER Avdat site in the Negev Desert, Israel. Biocrusts were rehydrated (to 75% water-holding capacity) in a climate-controlled chamber, followed by 12 h of night condition at 19°C and 10 h of day at 27 °C with a 2 h transition. Daytime conditions let to a gradual desiccation over 65 hours. Samples were collected at multiple time points during this experiment. The RNA was Illumina HiSeq 2500 sequenced, and reads were mapped to previously generated metagenome-assembled genomes1 and analyzed for differential gene expression.

Preliminary metatranscriptomics analysis revealed that after rewetting, photosystem I expression in *Cyanobacteria* decreased, and ribulose bisphosphate carboxylase gene expression increased expectedly. Moreover, about 60% of genomes showed significant differential gene expression within 15 minutes of rehydration, increasing to 93% of genomes between 15 minutes and 3 hours. This indicates that nighttime rewetting resulted in a delayed reactivation of some organisms, especially in the first 15 minutes, likely due to lower temperature and lack of light. We are comparing the responses of nighttime and daytime reactivation to identify temperature- and light-dependent patterns, advancing our understanding of microbial adaptation and biocrust resilience in dryland ecosystems.

1. Imminger, S. et al. Survival and rapid resuscitation permit limited productivity in desert microbial communities. Nat. Commun. 15, 3056 (2024).

Soil, plant microbiome and One Health

FT030

On the trail of bark-dwelling carbon-monoxide oxidizers

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Carbon-monoxide (CO) is a ubiquitous atmospheric trace gas that is toxic to most organisms but a readily available energy source to CO-oxidizing microorganisms. Playing a crucial role in global carbon cycling, microbial communities are estimated to remove >20% of total atmospheric CO mostly originating from anthropogenic emissions. *coxL*, the functional marker gene of aerobic CO oxidizers, encodes the large subunit of a CO-dehydrogenase enzyme catalysing the oxidation of CO into CO2. Aerobic CO oxidizers have been known from soils and marine ecosystems, and recently were also found in the epiphytic communities of tree leaves. However, the bark of trees, a more persistent phyllosphere environment (i.e. above-ground parts of plants), has never been investigated as their potential habitat. Here, we demonstrate for the first time the presence of CO oxidizers on the bark of two, commonly planted tree species, paper birch (*Betula papyrifera*) and black pine (*Pinus nigra*), using *ex-situ* CO degradation experiments with bark samples and bacterial strains isolated from bark, PCR-based *coxL* screening and sequencing, 16S rRNA gene-based microbial community analysis, and genome data of CO-degrading isolates. Gas chromatography analysis of the headspace of bark-containing vials of both tree species showed constant CO emission by dry bark, while moisturized bark degraded 100 ppm CO to below the detection limit (~5 ppm) indicating a humidity-dependent net CO flux in these systems. Repeated CO spiking after depletion primed the community resulting in increasing degradation rates from ~7.5 – 9 to ~50 – 100 ppm/day. *coxL* was present in all bark wash samples. Six different sequenced *coxL* types belonged to 4 different classes of bacteria. CO degradation experiments with three *coxL* positive isolates identified as species of *Caballeronia*, *Yersinia*, and *Rahnella* demonstrated similar priming effect as in the bark community. 16S rRNA gene-based community analyses of fresh and post-experimental bark samples indicate that up to 50% of the most prevalent identified genera are potential CO oxidizers that seem to be more abundant on trees exposed to high CO pollution at busy roads. Thus, with multiple decades of longevity, the tree holobiont is likely an important and efficient CO sink, especially in polluted urban areas. Metagenomics and metatranscriptomics analyses will further our understanding of the functional importance of CO oxidizers in the phyllosphere microbiota.

Fig. 1

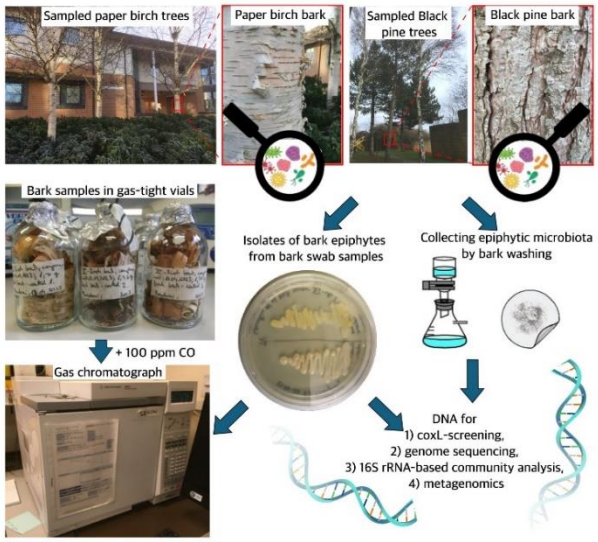
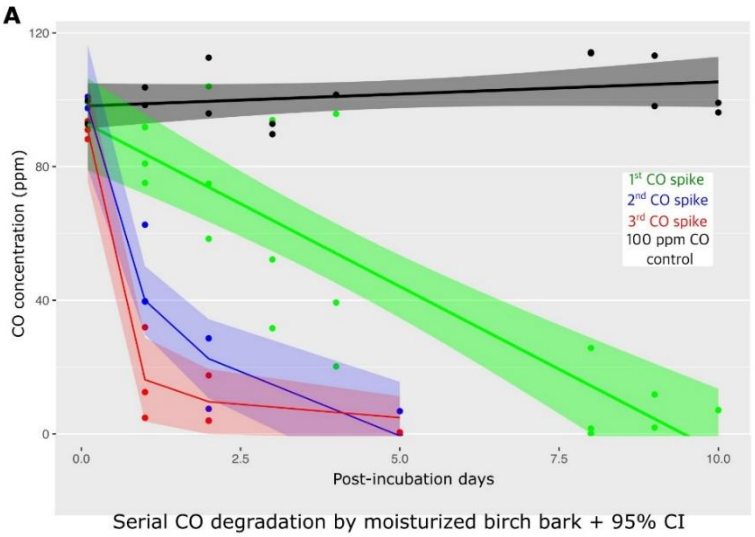
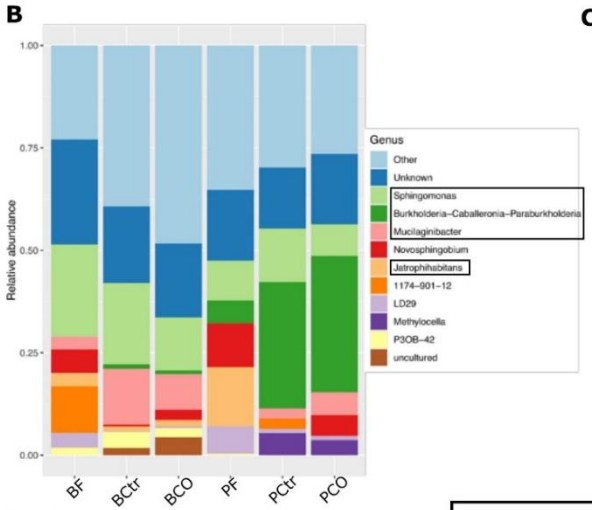


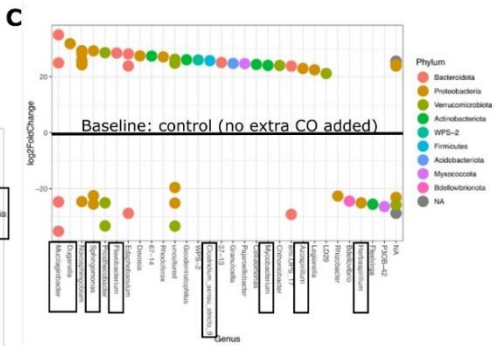
Fig. 2



Serial CO degradation by moisturized birch bark + 95% CI



Relative abundance of genera in fresh (F), control (Ctr), and CO-incubated (CO) birch (B) and pine (P) bark samples



Differential abundance analysis of genera of CO-incubated birch bark samples with apparent enrichment of potential CO oxidizers compared to control

Potential CO oxidizers:
Genera with *coxL*-possessing members found in the NCBI database

Soil, plant microbiome and One Health I

AL017

Concepts, innovations and challenges in exploiting the soil microbiome toward one health

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The soil microbiome has become central to pursue the One Health approach in agriculture. However, to fully exploit its potential it is necessary to develop innovations that shall address all factors that can affect its correct management. From a conceptual point of view, the integration of pre-, pro- and post-biotics could be the base for developing strategies suitable to be translated into agricultural practices. The production of bioinocula is another factor that could benefit from a circular economy approach, utilizing "wastes" or by-products as growing substrates, exploiting also new formulation methods. Registration for marketing purposes of new strains or species could benefit from data that are connecting various traits linked to the genomic, phenomic and metabolomic domains. Regulatory constraints derived from a "single function" approach, should be challenged by the widely demonstrated multifunctionality of microbial species. Challenges derived from the field application of microbial biostimulants could be tackled by developing devices allowing to track and monitor the strain, useful also to define methods and timing to assure high efficacy of the treatment. Indicators of soil quality that include microbiome-based features would be expected to further optimize the adoption of microbial products. It is believed that only such an integrated frame of mind could become a game changer for agricultural practices aiming at improving soil health. Practical examples of each of these factors will be presented and discussed in view of current researches.

This work was supported by the project EXCALIBUR funded by the European Union"s Horizon 2020 Research and Innovation Program under grant agreement no. 817946 and the SPIN-FERT project funded by the European Union"s Horizon Europe Research and Innovation Programme under grant agreement 101157265.

Soil, plant microbiome and One Health I

AL018

Distinct functional responses of root and rhizosphere microbial communities in intercropping systems under arid conditions

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Intercropping enhances soil nutrient availability and positively regulates soil microbiota, therefore improving crop yields in intensive agroecosystems. We conducted field experiments under arid conditions using intercropping with key crops (i.e., barley, mustard, alfalfa, broad beans, wheat yokara, and Egyptian wheat) and collected soil and root samples from amplicon sequencing and also isolated bacteria. We evaluated multiple PGPR tests in-vitro conditions under salinity and drought stress. Most exhibited PGP traits, enzymatic activity, and the ability to survive under osmotic stress (-132.0 MPa), high temperatures (45°C), and 5% NaCl. Rhizosphere bacterial isolates were more drought-tolerant and produced more EPS in the presence of glucose, while endophytic communities produced EPS with sucrose and more indole acetic acid. The functionality of endophyte and rhizosphere bacterial communities varied but was not dependent on the intercrop type. The 16S rRNA analysis revealed that mainly root endophytic isolates were *Proteobacteria*, *Bacillota*, *Pseudomonadota*, *Bacteroidota*, and *Actinomycetota*. While the rhizosphere isolates were *Proteobacteria*, followed by *Bacillota*, *Actinomycetota*, and *Bacteroidota*. The compatible strains were used to construct synthetic bacterial communities (SynComs). The results demonstrated that the eight root isolates were compatible, with seven SynComs consisting of two isolates and four SynComs comprising three isolates. Results suggest that bacterial strains could serve as plant probiotics, promoting growth and enhancing plant health under climate change, particularly in dryland areas.

Soil, plant microbiome and One Health I

AL019

Changing irrigation water quality affects the soil resistome and mobilome

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Untreated wastewater from Mexico City has been used to irrigate crops in the Mezquital Valley for more than 100 years. Starting in 2018, effluent of the new Atotonilco wastewater treatment plant has increasingly replaced untreated wastewater. To assess the impact of this shift on antibiotic resistance genes and mobile genetic elements in soils, we performed a four-week soil microcosm incubation experiment. Three different soil types frequently encountered in the area were selected for irrigation with wastewater treatment plant influent or effluent, with or without additions of antibiotics and disinfectants. Spiking of the irrigation water with antibiotics increased the water-extractable concentrations of sulfamethoxazole in soil. qPCR analysis showed that abundances of antibiotic resistance genes and mobile genetic elements in soil were significantly affected by spiking the irrigation water, while soil type and irrigation water quality had no impact. 16S rRNA gene amplicon sequencing revealed that soil bacterial communities were shaped only by soil type and incubation time. The relative abundance of two ASVs affiliated to the genus *Methylotenera* increased in soil after wastewater irrigation, suggesting introduction via the irrigation water or benefiting from the water addition. Our results confirm that antibiotics and disinfectants in irrigation water influence the relative abundances of antibiotic resistance genes and mobile genetic elements but interestingly not the overall soil bacterial community composition. In addition to the outcomes of the incubation experiment, results on relative abundances of selected antibiotic resistance genes and mobile genetic elements from a soil column experiment under controlled greenhouse conditions with two different soil types from the Mezquital Valley planted with cilantro and irrigated with untreated or treated wastewater will be presented.

Soil, plant microbiome and One Health I

AL020

Harnessing soil microbiome traits for climate-adaptive agriculture

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[3]Zhejiang University, College of Environmental and Resource Sciences, Hangzhou, China
[4]Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Microbiome Biotechnology, Potsdam, Germany
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The incredibly diverse soil microbiota mediates soil"s response to climate change and extreme weather. The soil microbial communities are selected by pedoclimatic factors and provide an important genetic reservoir for soil functionality. In particular, the turnover of soil microbial biomass and soil carbon are of vital interest because humus formation determines crucial soil functions. Soil organic matter content increases soil water retention, water infiltration capacity and soil structural stability; critical factors to prevent pluvial flooding and soil erosion. However, the bacterial traits favoring healthy soil structure and their ecological context remain poorly understood. How bacteria can improve the soil water balance, increase resilience to extreme weather, help retain nutrients to foster climate adaptation of agricultural practices is at the core of the CARA project [1]. Here we present first results from controlled experiments using a cutting-edge rainfall simulator capable of reproducing the rainfall characteristics (droplet sizes and velocity) of different climate scenarios. We use artificial soil columns with a gradient of organic matter contents to study how the soil microbiota responds and modulates soil structure for altering soil water infiltration and retention under present and future climate scenarios. Furthermore, we also treat soils with animal- and plant-based liquid fertilizers to study how nutrients, microbiota and their associated functions including antibiotic resistance genes can establish or leach from the soil columns. Our findings will provide insights into the controlling mechanisms of the soil microbiome for climate-adaptive agriculture and enhance our understanding on the persistence and dissemination of bacterial genetic traits across the soil-water interface.

[1] Climate change adaptation through flood-reducing agriculture (CARA); https://projekte.ffg.at/projekt/4754252

Soil, plant microbiome and One Health I

AL021

Utilizing microresp™ for assessing microbial respiration in maize rhizospheres under drought stress

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Drought stress hampers a plant's ability to absorb essential nutrients and maintain metabolic processes. Thus, the impact of such stress on microbial activity in the maize rhizosphere remains underexplored. This study examined how microbial respiration is affected by varying water levels (60%, 80%, and 100% water holding capacity) in the rhizosphere of two maize cultivars, Monsanto DKC 72-70 and CRN-3505.

Microbial activity and community-level physiological profiles were analyzed using the MicroRespTM method with 11 distinct C substrates to gauge microbial respiration rates and substrate preferences in the rhizosphere of each cultivar under the different water treatments. The results showed that the Monsanto DKC 72-70 cultivar consistently exhibited higher CO_2 production across substrates compared to CRN-3505, suggesting greater microbial activity and possibly a more robust soil-microbe interaction. Both cultivars demonstrated elevated microbial respiration from amino acids, particularly arginine and tyrosine, under 60% water conditions, with DKC 72-70 showing the highest rates. Microbial respiration of carboxylic acids was also enhanced under drought conditions, indicating microbial reliance on these compounds when water is limited (Figures 1 & 2).

These findings suggest that cultivar-specific interactions influence microbial nutrient cycling, demonstrating the role of cultivar selection in managing soil health and resilience in water-limited environments for optimizing microbial interactions to improve drought tolerance and sustainable crop productivity.

Fig. 1

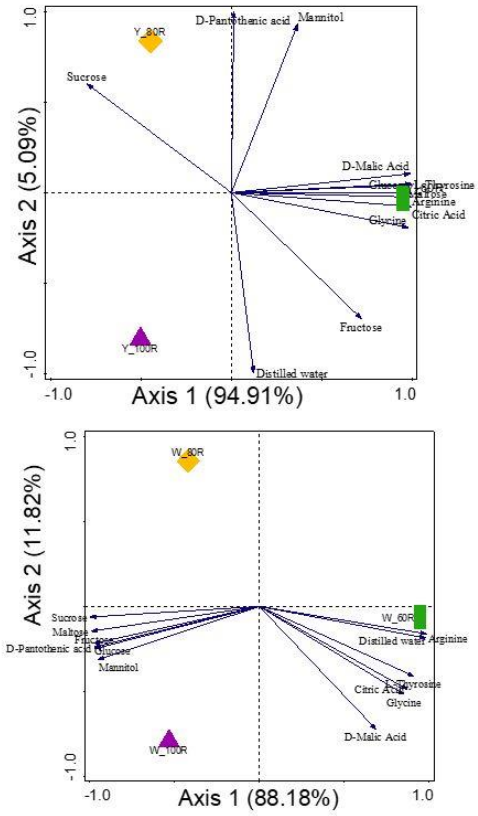
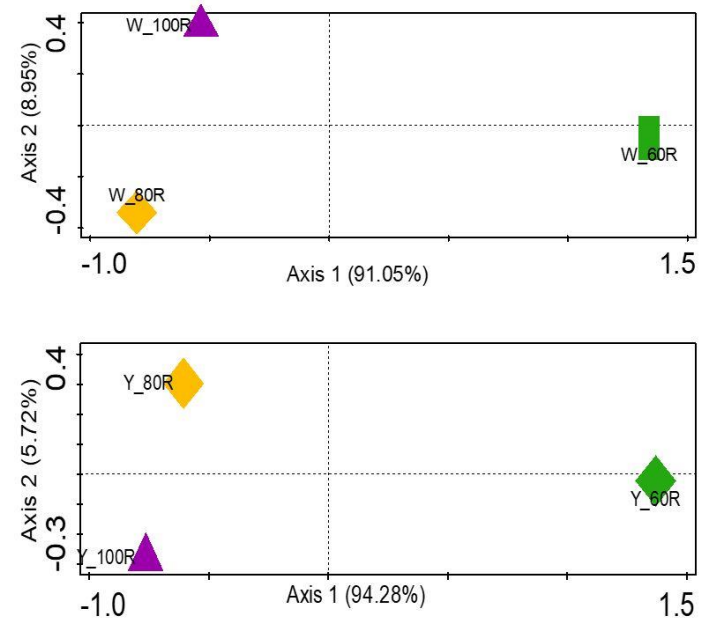

Y_80R
D-Pantothenic acid
Mannitol
Sucrose
D-Malic Acid
Arginine
Citric Acid
Glycine
Fructose
Y_100R
Distilled water
Axis 2 (5.09%)
Axis 1 (94.91%)
1.0
-1.0
W_60R
Sucrose
Maltose
D-Pantothenic acid
Glucose
Mannitol
Distilled water
Arginine
Citric Acid
Glycine
D-Malic Acid
W_100R
Axis 2 (11.82%)
Axis 1 (88.18%)

Fig. 2


W_100R
W_60R
W_80R
Axis 2 (8.95%)
Axis 1 (91.05%)
0.4
-0.4
-1.0
1.5
Y_80R
Y_60R
Y_100R
Axis 2 (5.72%)
Axis 1 (94.28%)
-0.3

Soil, plant microbiome and One Health I

FT031

Development of a custom replicon typing database for the comprehensive classification of plasmids in *Pseudomonas*

M. Shintani[1,2,3,4], Y. Nishimura[5], M. Tokuda[3], N. Isogai[1], H. Xie[6], C. Suzuki-Minakuchi[6], H. Suzuki[7], M. Suzuki[8]

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Plasmids are key drivers of bacterial evolution, facilitating the horizontal transfer of antimicrobial resistance genes (ARGs). Among them, plasmids carrying carbapenemase-encoding gene(s) pose a major clinical threat due to their ability to inactivate β-lactam antibiotics. *Pseudomonas* species, ubiquitous in clinical and environmental settings, serve as important reservoirs for these resistance plasmids. However, despite the widespread use of tools such as PlasmidFinder[1] and MOB-typer[2] for plasmid classification, their replicon databases have limitations in identifying plasmids in *Pseudomonas*.

To overcome this limitation, we developed a custom replicon typing database designed for the classification of plasmids in *Pseudomonas*.

Using PLSDB, the latest public plasmid database comprising 72,556 plasmids[3], we analyzed 988 *Pseudomonas* plasmids and identified 239 carrying carbapenem resistance genes, including 25 distinct *bla* genes. However, PlasmidFinder successfully classified only 10 plasmids, highlighting the need for a more robust comprehensive classification approach.

To address this, we constructed a custom replication initiation protein (RIP) library, incorporating sequences from IncP-1 to IncP-14, as well as previously unclassified plasmid groups (e.g., PromA, pSN1216-29-like, pQBR103-like, and pSTY-like groups)[4]. Additionally, we integrated data from the MOB-typer database. As a result, our system successfully classified 203 out of 239 carbapenem resistance plasmids (85%), making a significant improvement over existing methodologies. Notably, we identified IncP-2 (82/239), IncP-9 (11/239), and IncP-10 (34/239) as the dominant plasmid groups associated with carbapenem resistance in *Pseudomonas*.

Our study presents a novel custom-made replicon typing system that significantly enhances the classification and tracking of resistance plasmids in *Pseudomonas*. This advancement bridges critical gaps in plasmid classification, providing deeper insights into ARG dissemination mechanisms and laying the groundwork for improved antimicrobial resistance surveillance.

1) Carattoli et al., 2014, Antimicrob Agents Chemother, 58(7):3895-903.

2) Robertson et al., 2018, Microb Genom, 4(8):e000206.
3) Molano et al., 2025, Nucleic Acid Res, 53(D1):D189-D169.
4) Nishimura et al., 2014, BioRxiv, doi:10.1101/2024.09.03.610885.

Soil, plant microbiome and One Health I

FT032

Elevated CO_2 increased antibiotic resistomes in seed endophytes – evidence from a free-air CO_2 enrichment (FACE) experiment

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Climate warming affects antibiotic resistance genes (ARGs) in soil and the plant microbiome, including seed endophytes. Seeds act as vectors for ARG dissemination in the soil–plant system, but the impact of elevated CO_2 on seed resistomes remains poorly understood. Here, a free-air CO_2 enrichment system was used to examine the effects of elevated CO_2 on seed-associated ARGs and seed endophytic bacteria and fungi. Results indicated that elevated CO_2 levels significantly increased the relative abundance of seed ARGs and mobile genetic elements (MGEs), especially those related to beta-lactam resistance and MGEs. Increased CO_2 levels also influenced the composition of seed bacterial and fungal communities and the complexity of bacteria–fungi interactions. Fungi were more sensitive to changes in the CO_2 level than bacteria, with deterministic processes playing a greater role in fungal community assembly. Co-occurrence network analysis revealed a stronger correlation between fungi and ARGs compared to bacteria. The structure equation model (SEM) showed that elevated CO_2 directly influenced seed resistomes by altering bacterial composition and indirectly through bacteria–fungi interactions. Together, our work offers new insights into the effects of elevated CO_2 on antibiotic resistomes in the seed endosphere, highlighting their increased dissemination potential within soil–plant systems and the associated health risks in a changing environment.

Fig. 1

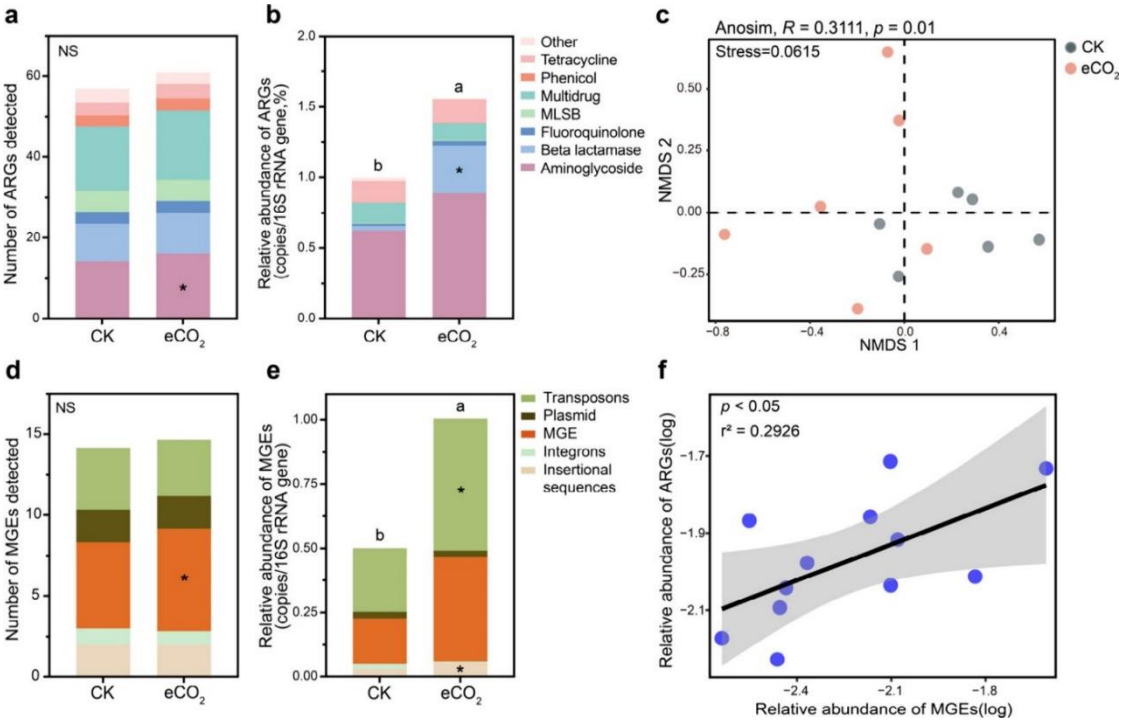
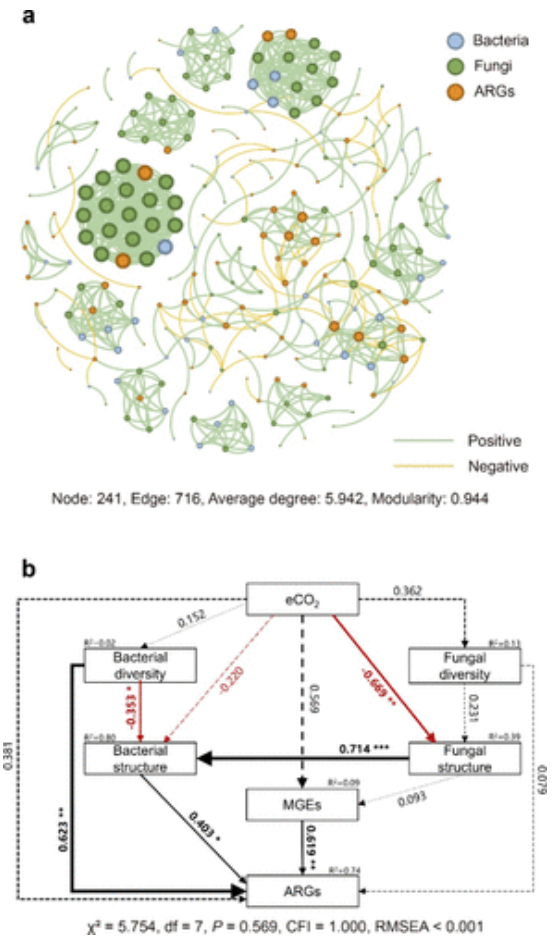


Fig. 2


a
Bacteria
Fungi
ARGs
Positive
Negative
Node: 241, Edge: 716, Average degree: 5.942, Modularity: 0.944
b
eCO2
0.362
0.152
Bacterial diversity
Fungal diversity
-0.353 *
-0.220
0.569
-0.669 **
0.231
0.381
0.714 ***
Bacterial structure
Fungal structure
0.079
MGEs
0.093
0.623 **
0.403 *
0.619 **
ARGs
χ² = 5.754, df = 7, P = 0.569, CFI = 1.000, RMSEA < 0.001

Soil, plant microbiome and One Health I

FT033

Gut microbiome composition correlates with drug resistance profiles in pulmonary tuberculosis

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Background

Individuals vary widely in their response to drugs. Pioneer studies explored the connection between variability in drugs metabolization to interpersonal differences in microbiomes, and discovered that microbial taxa can indeed modify drugs resulting in their activation, inactivation or toxification. Subsequent research aimed to uncover the mechanisms of the gut microbiome-drug interactions driving the inter-individual variation in drug metabolism, and identified drug-metabolizing gut bacteria, bacteria-produced drug metabolites, and drug-metabolizing, strain-specific gene products.

Material and Methods

Here, we explore the link between gut microbiome, and drug resistance profiles in the context of pulmonary tuberculosis (TB). We examined the co-variation between resistance profiles to anti-TB drugs, and gut microbiome composition in a cohort of adult patients with culture-confirmed pulmonary tuberculosis. We performed phenotypic and genotypic drug resistance testing, and sequenced the V3-V4 region of 16S rRNA gene from stool samples to define the gut microbiome composition.

Results

We found that resistance profiles significantly associated with certain gut microbiome taxa. Specifically, amplicon sequence variant (ASV) ASV_43 *Klebsiella* was enriched in patients resistant to the first-line, and in patients resistant to the second-line injectable drugs. Besides, ASV_14 *Phocaeicola* was more abundant in Levofloxacin- and Moxifloxacin-resistant patients. In contrast, we found ASV_58 *Bifidobacterium*, and ASV_111 *Ruminococcus* to be enriched in patients susceptible to Isoniazid, and Rifampicin, respectively.

Conclusion

These preliminary results emphasize the link between gut microbiome, and drug resistance in TB. Further deeper characterization of bacterial strains, and functional assays will highlight the mechanisms underlying drug-microbiome interactions in TB.

Key words: TB, gut microbiome, anti-drug resistance.

Soil, plant microbiome and One Health II

AL022

Plant disease management via *Microbiome* genes

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Plant diseases pose a significant challenge to global agriculture, calling for innovative strategies beyond conventional chemical treatments. The increasing prevalence of phytopathogens is not only facilitated by climate change, but also rapidly evolving resistances against conventional pesticides. Recent advances in microbiome research have highlighted the potential of *Microbiome* genes (*M* genes) in shaping plant-associated microbial communities to enhance disease resistance. This approach leverages the natural interactions between plants and their microbiota, offering a sustainable alternative to agrochemical-based plant protection.

Recent studies have demonstrated that specific microbiome-shaping host genes can influence microbial composition, leading to improved pathogen resistance. For instance, increased activity of phenylalanine ammonia-lyase (*PAL*) genes was shown to enrich antagonistic bacteria in the microbiome of different crop plants, reducing susceptibility to fungal and bacterial infections. Pseudomonadales and other *M* gene-responsive bacterial groups efficiently increase the defence repertoires of their host plants. These observations have provided an extended basis to explore microbiome engineering approaches to manipulate microbial communities in a targeted way, fostering beneficial interactions that support plant health.

The collective findings will be useful for integrating *M* genes into plant breeding programs to develop crops with enhanced disease resistance. By identifying and selecting plants with favourable microbiome-shaping gene variants, breeders will be able to cultivate varieties that naturally support beneficial microbial communities. This approach aligns with global initiatives aimed at reducing dependency on agrochemicals while maintaining high crop yields. However, challenges such as environmental variability and microbial competition must be addressed to ensure the efficacy of microbiome-based disease management strategies. In addition, the inter-connectivity of microbiomes has to be taken into account – engineering the plant microbiota is likely to not only affect microbial communities in the environment, but also in organisms that consume them.

Overall, *M* genes represent a promising frontier in plant disease management, offering a sustainable and nature-based solution. Continued research into microbiome engineering and plant breeding applications will be crucial for harnessing the full potential of emerging possibilities.

Soil, plant microbiome and One Health II

AL023

Disarming rather than killing: Transforming fungal lifestyle from pathogenic to saprophytic by inhibiting fungal virulence

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Under the concept of one health, soil microbiomes are recognized as reservoirs of microorganisms that can benefit agriculture and animal and human health. As an additional concept, I postulate that soil microorganisms may be exploited for disarming pathogens as a mild alternative to killing, to minimize niche occupancy loss, avoiding activation of the resistance selection process, and decreasing the chances of disabling biocontrol. I discuss the concept of reverting growth inhibition paradigm by considering interfering with the virulence process rather than killing the target pathogen. This concept exploits bacterial secondary metabolites to inhibit the virulence of a fungal pathogen rather than killing its cells and to transform a dangerous microorganism in a neutral saprophyte. Such hypothesis has been tested on *Magnaporthe oryzae*, a worldwide threat for rice, by inhibiting the production of the secondary metabolite melanin which is necessary to invade plant tissues. From the rice rhizosphere, three bacterial strains were selected, whose VOCs do not inhibit growth but reduce melanin pigmentation of *M. oryzae*. Fungal metatranscriptomics after exposure to bacterial VOCs indicated transcriptional inhibition of the melanin biosynthesis pathway, remodelling of MAPK signalling and enhanced autophagy. Indeed, VOC exposure inhibit sporulation and conidia germination, suppress appressoria formation both *in vitro* and *in vivo* on rice leaves. Among nine VOCs that were identified by head space solid-phase micro-extraction gas-chromatography mass-spectrometry and were shared by the three bacterial strains, 1-butanol-3-methyl reduces fungal pigmentation of 60% and completely inhibits conidia germination *in vitro*. Optical and scanning electron microscopy of rice leaves inoculated with *M. oryzae* conidia and exposed to VOCs documented the abolishment of appressoria formation and tissue invasion and showed that *M. oryzae* cells persist only by growing with vegetative filaments. The overall data shows that bacterial VOCs can switch off *M. oryzae* virulence, disarming the fungus, yet leaving it to grow saprophytically on the leaf surfaces.

Soil, plant microbiome and One Health II

AL024

Nitrifier homeostasis contributes to gaseous NO and HONO emissions from drying soils – a mechanistic modelling approach

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Ammonia-oxidizing microorganisms in soils are recognized as key producers of reactive nitrogen gases, including nitrogen oxides (NO_x = NO + NO_2) and nitrous acid (HONO), which adversely affect air quality by influencing near-surface atmospheric chemistry. Previous studies have revealed that NO and HONO emissions are dependent on soil hydration, exhibiting a single peak under relatively dry conditions. However, the underlying mechanisms for this emission pattern so far remained poorly understood, particularly concerning the soil nitrification process that links these gases. In this study, we combined novel gas flux experiments with a strongly controlled in vitro approach, supported by a mechanistic model of N transformation during drying of active ammonia-oxidizing bacteria embedded in artificial soil systems. The mechanistic modeling approach enabled us to assess the biotic and abiotic factors influencing the emissions. Our findings suggest extracellular NO and nitrite (NO_2^-) accumulation in a limited volume of pore water governing the emissions, with water loss during evaporation serving as the key driving factor. Additionally, we identified several previously overlooked but significant abiotic processes that scavenge oxygen and transform N compounds, thereby affecting the extracellular environment. Our results demonstrate that the drying process can obscure the distinction between aerobic and anaerobic processes in soils, highlighting the need to investigate microbial homeostasis within small pore spaces under varying soil hydration conditions. Based on our analysis, we suggest possible strategies for effectively managing the release of soil-derived reactive N gas.

Soil, plant microbiome and One Health II

AL025

Reference-free and culture-free study of environmental microeukaryotes using single-cell transcriptomics

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Single-cell transcriptomics is a key tool for unravelling metabolism and tissue diversity in model organisms but its potential for elucidating the ecological roles of microeukaryotes, especially non-model ones, remains largely unexplored. In the previous BAGECO, I presented a pilot study where we employed Smart-seq2 on Ochromonas triangulata, a microeukaryote lacking a reference genome, and showcased how single-cell transcriptomics could be used to taxonomically identify the microeukaryote, differentiate and elucidate its transcriptional states, and reveal distinct bacterial communities associated with individual cells. Here, I present a follow-up study where we expand this concept by going from one species to several. We designed an experiment where we sampled water from Lake Erken, Sweden, during an algal bloom in the autumn, acclimated it to daytime light treatment for 3 days, and then subjected half of the experiment to a shading treatment while we continued with the light treatment for the other half. We sampled the experiments before and during the treatments and sorted cells from the samples by chlorophyll autofluorescence and a food vacuole stain to select for mixotrophic microeukaryotes. We prepared single cell transcriptomic libraries from the sorted cells using Smart-seq3 workflow. Using the carryover rRNA, we have been able to identify 22 microeukaryotes among the >1500 single cells, as well as their associated bacteria belonging to 100s of different families. Of the 22 *de novo* assembled transcriptomes, all are representative of the reads isolated from the single cells and many contain transcripts for the most conserved genes in their taxa. Sequence homology as well as structure homology has been used to annotate the functions of the assembled transcripts. Downstream analysis combining this functional annotation with expression profiles of the cells clarifies the metabolism of these different microeukaryotes. The previous study underscored the power of single-cell transcriptomics for characterizing metabolic states in microeukaryotes without a reference genome, offering insights into a priori unknown physiological states and individual-level interactions with different bacterial taxa. This follow-up study further solidifies the method"s broad applicability to describe the ecological roles of environmental microeukaryotes in a culture-free and reference genome-free setting, surpassing alternative methods like metagenomics or metatranscriptomics.

Soil, plant microbiome and One Health II

AL026

Global insights into genome size and microbial carbon metabolism

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Understanding the relationship between genome size, genomic traits, and microbial carbon metabolism is critical for deciphering the complexities of global soil ecosystems. We investigated how genomic characteristics drive microbial strategies for carbon acquisition and utilization across diverse terrestrial biomes, with a focus on carbohydrate metabolism. Our findings show that larger microbial genomes exhibit enhanced capacities for polymer decomposition, driven by the expansion of genes encoding carbohydrate-active enzymes. These adaptations are often facilitated by horizontal gene transfer, enabling microbes to optimize carbon acquisition strategies under varying environmental pressures. Using genome-resolved metagenomics, we demonstrate that microbial motility—essential for rhizosphere colonization—is tightly linked to genomic traits supporting carbohydrate metabolism. Highly motile microbial genomes are enriched in genes related to carbon depolymerization and cycling, underscoring the critical role of genome-enabled traits in microbial niche differentiation. A global meta-analysis of 2,650 genomes reveals that genome size mediates trade-offs between catabolic and anabolic metabolism, shaping microbial contributions to carbon cycling and sequestration. Large-genome microbes prioritize carbon acquisition through extensive decomposition pathways, while small-genome counterparts emphasize biomass yield, collectively driving soil organic carbon dynamics worldwide. This integrative research underscores the global significance of genome size and genomic traits in shaping microbial carbon metabolism. These findings provide a framework for leveraging microbial genomics to enhance carbon sequestration strategies and promote sustainable soil management in the face of climate change.

Fig. 1

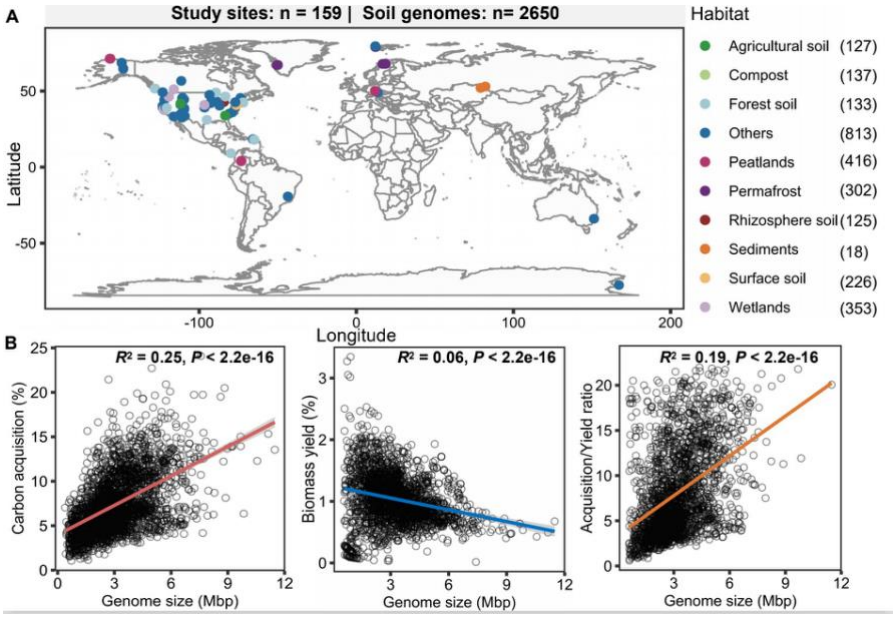
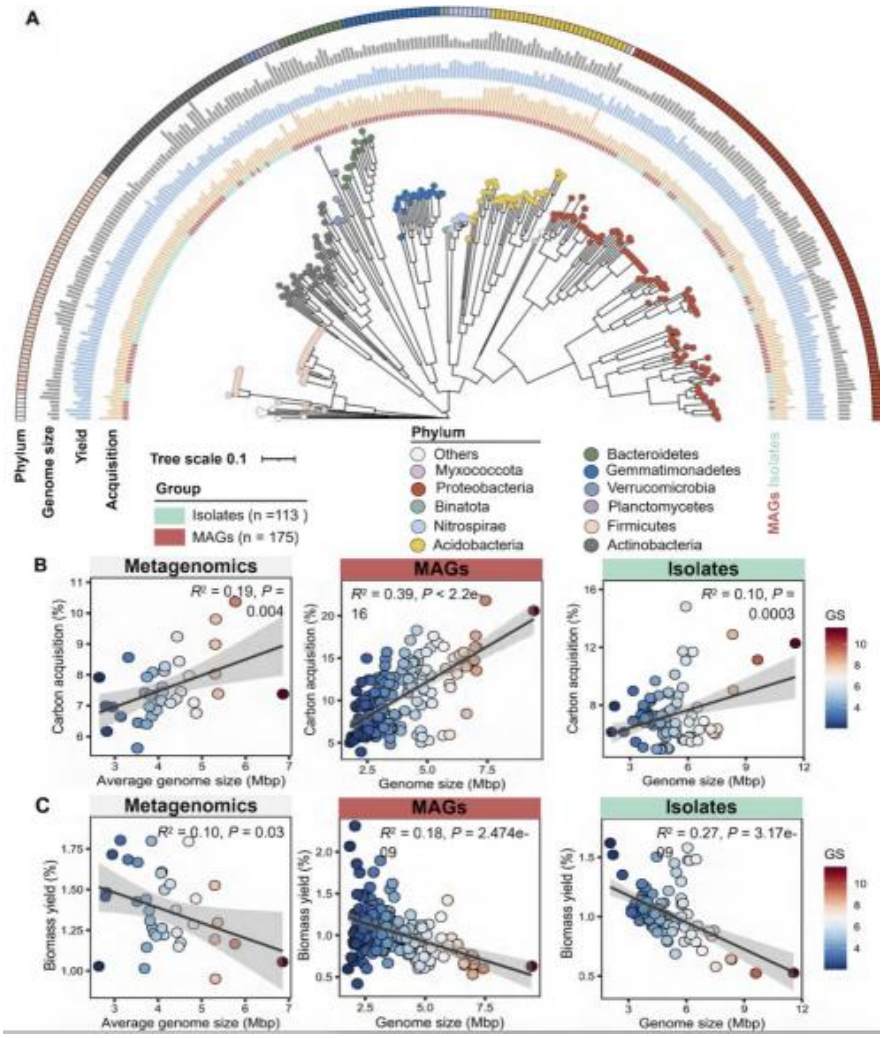


Fig. 2


A
Phylum
Genome size
Yield
Acquisition
Tree scale 0.1
Group
Isolates (n =113)
MAGs (n = 175)
Phylum
Others
Myxococcota
Proteobacteria
Binatota
Nitrospirae
Acidobacteria
Bacteroidetes
Gemmatimonadetes
Verrucomicrobia
Planctomycetes
Firmicutes
Actinobacteria
MAGs
Isolates
B
Metagenomics
MAGs
Isolates
Carbon acquisition (%)
Average genome size (Mbp)
Genome size (Mbp)
R² = 0.19, P = 0.004
R² = 0.39, P < 2.2e-16
R² = 0.10, P = 0.0003
GS
C
Metagenomics
MAGs
Isolates
Biomass yield (%)
Average genome size (Mbp)
Genome size (Mbp)
R² = 0.10, P = 0.03
R² = 0.18, P = 2.474e-09
R² = 0.27, P = 3.17e-09
GS

Soil, plant microbiome and One Health II

AL027

One Health Concept of Quorum Sensing in Microbiome-Host interactions and human health

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All plants and animals have evolved with microbial communities in holobiontic metaorganisms. Tight colonization and interaction of plants with microbes are known since more than 120 years, when symbiotic interactions of Rhizobia with legumes were identified and rhizosphere colonizing microbes were characterized to be mostly beneficial but occasionally also pathogenic. Among other metabolites, bacterial quorum sensing (QS) signals of the N-acyl-homoserine lactone (AHL) type play an important role in priming the innate immune system towards improved stress and pathogen resistance. Through quorum quenching (QQ) - modulation or degradation of QS-signals - pathogens can be controlled. This indicates an important role of QS-signals for plant health (Hartmann et al. 2024).

In aquatic animals, the best studied example for the important role of AHLs is the squid-Vibrio symbiosis, in which two distinct AHL-synthetases are essential for colonization and persistence of V. fischeri in the squid light organ. Molecular interactions with environmental bacteria play also a central role in the joint evolution of the innate immune system of the sweet water poly Hydra, in which associated bacteria promote the normal development of the nervous system. The host produces specific antimicrobial peptides supporting beneficial bacteria and inhibiting pathogenic ones. It turned out that Hydra polyps are able to modify the quorum sensing signal of the main colonising Gram-negative bacterium Curvibacter. An enzyme of Hydra reduces 3-oxo-HSL to 3-hydroxy-HSL. Thus, Curvibacter cells respond differentially to 3-OH-C12-HSL and are stimulated to colonize the epithelial surface of Hydra. In addition, 3-OH-C12-HSL induces specific transcriptional responses such as the activation of carbohydrate metabolism. In contrast, 3-O-C12-HSL results in downregulation of cell wall synthesis and the up-regulation of flagellum assembly. Thus Curvibacter switches its phenotype in the presence of Hydra (Pietschke et al. 2017, Bosch et al. 2024).

Further evidences from sponges, corals, insects and mammals will be presented, which demonstrate that AHLs or other bacterial QS-signals are involved in holobiontic interactions, indicating that indeed these omnipresent signals constitute a common language for microbe-host interactions.

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Soil, plant microbiome and One Health II

FT034

First insights into soil microbiome preservation – strategies, challenges, and future directions

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Microbiomes are complex communities of microorganisms that, together with their "theatre of activity", define a specific habitat and provide essential ecosystem services critical to the health of plants, animals and the environment. Among the use cases investigated by the EU-funded MICROBE (Microbiome Biobanking (RI) Enabler) project, the soil microbiome is particularly important because of its complexity and the wide range of functions and ecosystem services it provides. Furthermore, the soil microbiome plays a significant role in human health through the food system.

The MICROBE project addresses the critical challenges of microbiome preservation and current challenges faced by culture collections and biobanks that need new approaches for sample preservation and pre-analytical metadata management. By establishing standardised protocols and optimising preservation methods, the project aims to ensure the long-term stability of microbial communities and their functionality, as well as, the storage of metadata associated with samples, to enable future scientific research and industrial applications.

To this end, the project has conducted a comprehensive study evaluating different preservation strategies on well-characterised soil samples, including different storage temperatures, cryoprotectants and cooling rates. Preliminary results suggest that these methods successfully maintain bacterial and fungal culturability over time, while ongoing assessments explore their effects on community composition.

The insights gained from these efforts will provide a blueprint for microbiome biobanking across different systems, fostering collaboration between academic and industrial stakeholders while promoting best practices for data sharing and long-term resource accessibility.

Soil, plant microbiome and One Health II

FT035

Uncovering the carriers of antibiotic resistance genes in complex communities using methylation signals (Previous title: Sulfonamide resistance gene sul4 is hosted by common wastewater sludge bacteria and found in various newly described contexts and hosts including clinically relevant species)

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Question

Beyond detecting antibiotic resistance genes (ARG), it is crucial to understand the carriers and transmitters of these genes, as they play a key role in the ongoing resistance crisis. The introduction of the first broad-spectrum antibiotics, sulfonamide drugs fundamentally revolutionized medicine in the 1930s. Shortly after and ever since sulfonamide resistance genes (*sul* genes) have been widely detected. Still, the most recent variant of these genes, *sul4*, was first described only in 2017 and the host range and transmission mechanisms of this gene are still largely unknown.

Methods

Here we applied deep PacBio long-read metagenomic sequencing and an innovative bioinformatic methodology including methylation detection to uncover the bacterial hosts and the mobility potential of the recently described sulfonamide resistance gene *sul4*. This approach tackles the previous methodological challenges of metagenomic studies in linking functional genes such as ARG to their host bacteria. Furthermore, we extended our description of *sul4* carriers to previously published data sets.

Results

We demonstrate that *sul4* is present in both influent and effluent wastewater but is particularly enriched in sludge. Using methylation analysis and genome-resolved metagenomics, we identified three novel hosts for *sul4* in the sludge representing phyla Myxococcota, Desulfobacterota, and Chloroflexota. Our exploration of published data sets revealed additional *sul4*-hosting genera that had not been described earlier such as sludge-associated *Desulfobacillus* but also opportunistic pathogens such as *Aeromonas* and *Moraxella*. Using comparative genomics, we found that *sul4* is always associated with integrons, but not exclusively with class 1 integrons. Moreover, the *sul4*-flanking region consisting of the *fol*K gene and ISCR28 element demonstrated strong conservation.

Conclusions

We show that the sulfonamide resistance gene *sul4* is found in diverse bacteria including both common sludge bacteria and opportunistic pathogens. The conserved gene region containing mobile genetic elements and flanking *sul4* is suggested to explain the widespread occurrence of this gene across bacterial taxonomic and ecological boundaries. Therefore, our findings depict the dissemination of ARGs between environmental and clinically relevant bacteria. Our

study provides valuable insights and methods that enhance our understanding of ARG carriage and spread, leveraging metagenomics and novel bioinformatic analyses.

Soil, plant microbiome and One Health II

FT036

The octocoral microbiome – from function and host-microbial interactions to novel recombinant chitinases for the blue bioeconomy

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Octocorals are fundamental components of benthic marine ecosystems worldwide. Our research has shown that the octocoral microbiome is diverse, distinct from its surroundings, host genus-specific, and undergoes complex structural changes under dysbiosis [1]. To elucidate the metabolic capacities of octocoral symbionts, we examined 66 high-quality metagenome-assembled genomes (MAGs) of 30 prokaryotic species, recovered from the microbial metagenomes of *Eunicella verrucosa*, *Eunicella gazella*, *Leptogorgia sarmentosa*, and surrounding seawater [2].

Octocoral symbionts were dominated by the bacterial families *Endozoicomonadaceae*, *Metamycoplasmataceae* and *Candidatus* Thioglobaceae, amongst others. Phylogenomic analysis revealed that the *Endozoicomonadaceae* MAGs represent two species of a novel genus unique to temperate octocorals, designated *Candidatus Gorgonimonas*. *Candidatus Gorgonimonas* MAGs exhibited metabolic potential to thrive in suboxic conditions and possessed multiple traits related to host colonization and symbiosis establishment. All 11 *Gorgonimonas* MAGs encoded a chitinase, indicating their capacity to hydrolyse chitin—the most abundant polysaccharide in the ocean. Meta-analysis of >40 genomes from both cultured and uncultured *Endozoicomonadaceae* lineages demonstrated that chitinases are widespread within this family, suggesting that these bacteria play key roles in chitin turnover in marine animals and benthic ecosystems [3]. The chitinase genes of each *Gorgonimonas* species were found to be unique and species-specific.

Since *Gorgonimonas* symbionts remain unculturable, we utilized synthetic biology and heterologous expression to harness their enzymes. Two novel, active endo-chitinases were successfully produced and their kinetics deeply characterized. Despite originating from two closely related species, the two chitinases exhibited distinct responses to abiotic factors, with one displaying high tolerance to salinity and alkalinity. Such variability may contribute to the plasticity and adaptability of the *Endozoicomonadaceae* family across diverse environmental conditions and ecological niches. Additionally, the two novel chitinases have lower optimal temperatures compared to commercial enzymes, suggesting potential applications in recycling chitin-rich seafood waste.

[1]Keller-Costa et al DOI: 10.1186/s40168-021-01031-y

[2]Keller-Costa et al DOI: 10.1186/s40168-022-01343-7

[3]da Silva et al & Keller-Costa DOI: 10.1038/s43705-023-00316-7

Poster Session

P001

Unsupervised learning based definition of the microbial rare biosphere

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The microbial rare biosphere consists of low-abundance taxa that act as a genetic reservoir, enabling ecosystem adaptation to changing conditions [1]. Despite its ecological significance, defining the rare biosphere remains challenging due to the arbitrary thresholds commonly used to separate rare from abundant taxa. Most studies rely on fixed relative abundance cutoffs (e.g., 0.1%), which lack biological justification and can lead to inconsistent findings across methodologies.

To address this limitation, we developed Unsupervised Learning based Definition of the Microbial Rare Biosphere (ulrb) [2,3], an unsupervised machine learning approach that automatically classifies taxa into "rare," "undetermined," and "abundant" without relying on predefined thresholds. This method is independent of sequencing platforms and provides consistent abundance classifications across datasets. We validated ulrb using 16S rRNA gene amplicon (full-length and V4V5) and shotgun metagenomic sequencing data, demonstrating its robustness across varying sample sizes, taxonomic compositions, and sequencing depths [2].

Additionally, ulrb identifies ecologically meaningful abundance patterns. In coral-associated microbial communities, it correctly distinguishes rare and abundant taxa across healthy (eubiosis) and diseased (dysbiosis) states, capturing biologically relevant shifts that traditional methods may overlook [2].

By integrating machine learning into microbial ecology, ulrb provides a standardized, biologically grounded approach to defining the rare biosphere. This method enhances comparability across studies and datasets, offering a reliable tool for investigating microbial community dynamics.

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3. Pascoal, F., et al. (2025). Definition of the microbial rare biosphere through unsupervised machine learning. Communications Biology. [in peer-review]

Poster Session

P002

Predicting Plant Microbiomes from Phylogeny: Toward a Phylosymbiotic Framework

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Plants and their microbiomes form complex associations that are increasingly recognized as essential to host fitness, adaptation, and evolution. Recent studies increasingly support the concept of **phylosymbiosis**, where microbiome composition reflects the evolutionary history of the host. However, the predictive potential of this relationship remains underexplored. In this study, we introduce a machine learning framework that leverages plant phylogeny to forecast the seed microbiome composition across plant families. By integrating amplicon data from diverse plant lineages, we evaluate the extent to which microbial community structure can be predicted based on host evolutionary relationships. We employ supervised learning models—including k-nearest neighbors, random forests, and Gaussian processes—to test phylosymbiotic signals in the seed microbiome. Our results reveal consistent phylogenetic imprints in specific microbial groups, suggesting that evolutionary constraints and selective recruitment jointly shape the plant microbiome. This work lays the foundation for a predictive, evolutionary-informed microbiome ecology with applications in agriculture, conservation, and synthetic community design.

Poster Session

P003

Mapping the microbial dark matter – relevance of *Patescibacteria* in petroleum hydrocarbon contaminated groundwater

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Question

Despite continued efforts by environmental microbiologists in the last decades, only small numbers of bacteria can be thoroughly investigated in complex environments like groundwater and soil. One of the prime examples is *Patescibacteria*, a newly proposed bacterial phylum, formerly member of the Candidate Phyla Radiation (CPR). This group of Bacteria have been found to exhibit remarkable diversity and distribution in wide range of environments. On the other hand, as they have a small genome and cell size coupled with restricted metabolic capacities, they are considered to thrive only in symbiotic or parasitic lifestyles.

Methods

Our investigation focused on a peculiar environment: we studied the diversity and distribution of *Patescibacteria* in a petroleum hydrocarbon contaminated aquifer in Hungary. The bacterial community was investigated by cultivation-free molecular methods such as traditional and *Patescibacteria*-specific 16S rRNA gene amplicon analysis. Additionally, we used metagenome-informed microscopy to identify potential hosts of symbiotic *Patescibacteria*.

Results

As expected, the most dominant phylum across all samples was *Proteobacteria*, followed by *Bacteroidota*, *Firmicutes* and *Patescibacteria*. With the application of the novel *Patescibacteria*-specific PCR primer pairs, we aimed to reveal the real composition of the community since the abundance and diversity of *Patescibacteria* is often underestimated. Based on our results, we could state that the application of this primer pair does not always increase the abundance of *Patescibacteria*, only in cases where we could formerly observe a higher abundance. At the same time, in cases where *Patescibacteria* was detected by traditional 16S rRNA gene amplicon analysis, increased abundances were revealed for *Ca*. Shapirobacteria, *Ca*. Woesebacteria, *Ca*. Kaiserbacteria, *Ca*. Moranbacteria and *Saccharimonadaceae* using *Patescibacteria*-specific amplicon analysis.

Conclusions

An integrated CARD-FISH and SEM approach was proven to be adequate tool for visualizing cell-to-cell interactions in environmental samples. Additionally, we found that the application of *Patescibacteria* specific amplicon primers could lead to the distortion of the real bacterial community structure.

Poster Session

P004

Exploration of carbon monoxide oxidation by phyllosphere bacteria reveals unexpected functional diversity of carboxidovores

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Carbon monoxide (CO) is a colourless, toxic gas that influences atmospheric chemistry by extending the lifetime of major greenhouse gases such as methane. Microbial utilisation of CO as a carbon and/or energy source contributes significantly to global carbon cycling. Recent studies indicate that tree phyllosphere microbiomes harbour diverse CO-degrading bacteria; however, the physiological role of CO degradation and the enzymes and genes underpinning CO oxidation in these bacteria remain unclear. To address these gaps, we employed both culture-based and culture-independent approaches. Holly leaf microbiome samples were enriched under different conditions, with CO concentrations of 100 ppmv and 10,0000 ppmv, to examine the phylogenetic and functional diversity of CO-oxidizing bacteria. Several CO-degrading strains were isolated from the enrichments and their genomes sequenced, including a strain belonging to a putative novel genus containing only form II CODH, which was capable of CO oxidation. Additionally, we identified a *Pseudomonas* strain (SB113) that oxidised CO across a wide concentration range (100 ppmv – 10,000 ppmv) despite lacking any known carbon monoxide dehydrogenase (CODH)-encoding genes. Metagenomic analysis revealed that CO concentration significantly influenced microbial community composition and *coxL* gene diversity. CO oxidation by strain SB113 in the absence of a detectable *coxL* gene suggests the presence of a hitherto unidentified enzyme degrading CO, a hypothesis further supported by proteomic data. This study provides novel insights into the metabolic diversity and genetic basis of CO oxidation in phyllosphere bacteria. These findings suggest that our current understanding of microbial CO cycling is incomplete and highlight the need for further exploration of alternative CO oxidation pathways.

Poster Session

P005

Does time make a difference? Studying plastic biofilm formation in search for plastic degraders

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Plastic in soil is known to attract a diverse array of microorganisms that colonize its surface forming a biofilm, often referred to as the plastisphere. Although the plastisphere community and related biofilm formation has been studied for quite some years in marine environments, research in terrestrial ecosystems is lacking. It can be expected that the biofilm formation over time differs compared to marine environments and depends on soil type, weather circumstances and the polymer type. The mature biofilm can potentially serve as a source of plastic-degrading bacteria, which could be useful for future soil remediation.

A controlled pot experiment with agricultural soil was conducted aiming to (1) monitor biofilm formation, (2) study the plastisphere bacterial and fungal community and (3) identify and isolate plastic-degraders. Three different polymer types commonly found in agricultural soils were selected: polyethylene (PE), polyvinylchloride (PVC) and polylactic acid (PLA) including both a soft and a hard type of plastic. These plastics were added to two agricultural soils differing in soil texture, pH, soil organic matter and total nitrogen content. The pot experiment runs for one year with weekly sampling during the first 2 months, biweekly sampling in month 3-4, and monthly sampling from month 5 onwards. The evolution of the biofilm will be monitored by scanning electron microscopy for visualization combined with a study of the plastisphere community through 16S rRNA and ITS gene metabarcoding. The microbial profiles of these samples will be used to select samples for the isolation of potential plastic-degrading microorganisms. By exploring the dynamics of the biofilm formation and the role of soil microorganisms in plastic degradation, this research contributes to a better understanding of the factors driving biofilm development in soil and highlights the microbial potential for plastic degradation.

Poster Session

P007

Harmonizing soil biodiversity monitoring across pedoclimatic regions and land uses

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Healthy soils are essential for food production and play a crucial role in maintaining biodiversity, regulating water cycles, storing carbon, and supporting ecosystem resilience. However, soil degradation threatens these functions, and despite the availability of various biological indicators, no international consensus exists on the most effective metrics or optimal monitoring approaches for both scientific rigor and policy relevance. Monitoring is further complicated by landscape heterogeneity, shifting soil properties, and variations in organismal activity.

The HORIZON 2020-funded project *Soil Biodiversity into Ecosystem Services* (SOB4ES) is addressing these challenges by harmonizing soil and soil organism sampling protocols across different climatic zones and land uses. The project assesses soils in urban, agricultural, forest, (semi)-natural, wetland, dryland, industrial, and mining environments, with a key focus on evaluating the impacts of sustainable versus high-input management on soil health.

Here, we present the SOB4ES protocol for soil biodiversity monitoring, which integrates a comprehensive set of soil parameters with biodiversity assessments across the trophic web. This approach ensures the generation of comparable datasets across diverse ecosystems, enabling robust evaluations of soil condition in relation to land use and management intensities. Our sampling and processing techniques are designed to minimize methodological bias while aligning with existing methodologies, data resources, and monitoring efforts. We assess multiple dimensions of biodiversity, including species, genetic, phenotypic, trophic, and functional diversity, as well as ecological interactions.

Poster Session

P008

Microcosm study of chlorpyrifos degradation by *Dietzia* sp. AUC11 isolated from chlorpyrifos applied agricultural field

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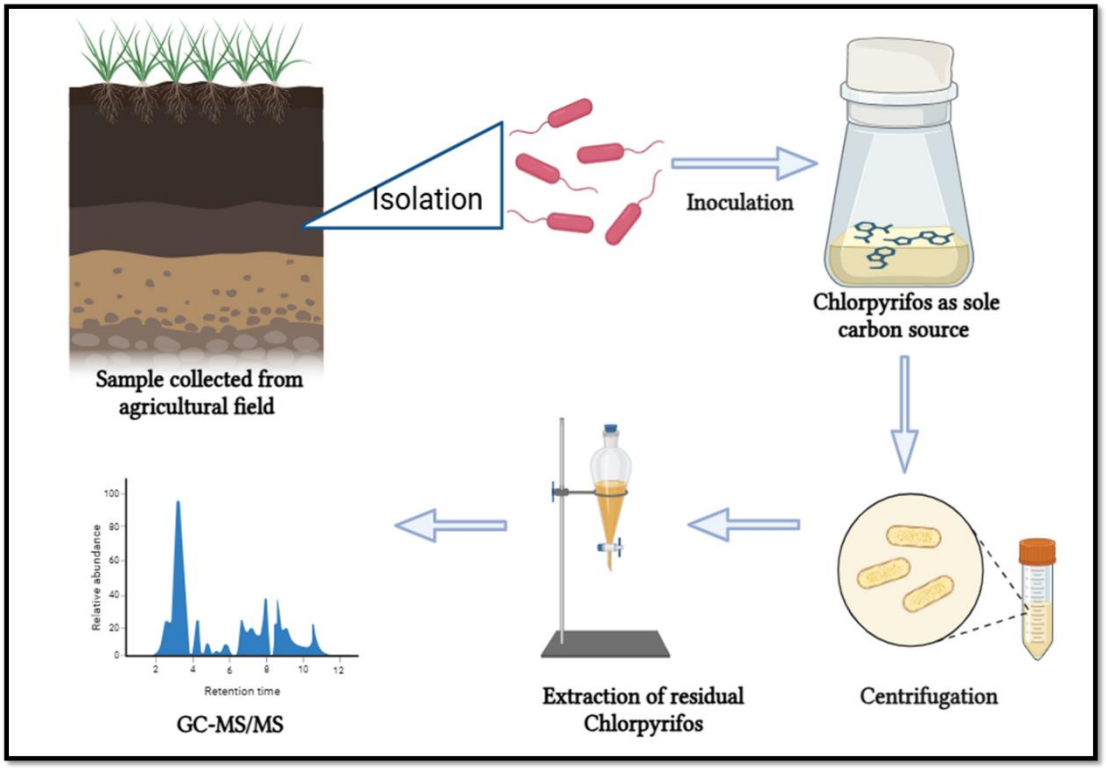
An organophosphate insecticide chlorpyrifos is primarily used to control foliage and soil-borne insect pests on variety of food and feed crops. As per reports, half-life of chlorpyrifos is ranged between 7 - 120 days and thus it shows its stability in soils. It can persist in soil for varying periods, depending on factors such as soil type, temperature, moisture, and microbial activity. Thus, it affects rhizospheric microbes and possibly damage beneficial microbial flora present in soil. Hence, in present study a newly isolated bacterium *Dietzia* sp. AUC11 was explored for its ability to degrade chlorpyrifos. Isolate was identified using 16S rRNA and sequence was submitted to GenBank. Environmental conditions were optimized for biodegradation. Microcosm study revealed that Isolate degrade 99.07 % chlorpyrifos via various mechanism. Metabolites were identified by GC-MS. Biosurfactant production and emulsification activity suggested that isolate emulsify hydrophobic molecules via production of biosurfactant and increased its availability for metabolism. Moreover, seed germination revealed that intermediate metabolites produced during biodegradation were less toxic compared to parent molecule. Thus, indigenously isolated *Dietzia* sp AUC11 can be effectively used for biodegradation of chlorpyrifos contaminated sites.

Fig. 1



Poster Session

P009

Establishing *Dyadobacter fermentans i*noculation in soil – investigating impacts on native microbial community and N_2O emissions reduction

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Over the past decades, soil microorganisms have been widely used as environmentally friendly solutions in agriculture. However, most microbial-based applications have focused on enhancing plant growth, pathogen defence, and nutrient maintenance, such as adding plant growth-promoting bacteria (PGPR) to fertilizers. In recent decades, greenhouse gas emissions have posed a significant global threat, leading to severe environmental consequences for ecosystems, wildlife, and human life. Nitrous oxide (N_2O) emissions from agriculture constitute a substantial portion of total greenhouse gas emissions. In this situation, microbial inoculation, considered a promising nature-based climate change mitigation strategy, might be a sustainable solution for the future.

Dyadobacter fermentans is a non-denitrifying N_2O reducer that has shown the ability to reduce N_2O emissions from soil. In this study, we conducted a greenhouse experiment to evaluate the effects of *D. fermentans* inoculation on native microbial communities, soil functions, and greenhouse gas emissions by varying the inoculation time point (early vs late) and inoculation frequency (unique vs recurrent). The establishment of *D. fermentans* and its presence might influence potential N_2O production and consumption. Meanwhile, soil microbial community composition and functional niches were significantly altered, indicating that *D. fermentans* plays a role when it presents with local microbial interactions. This shift in microbial dynamics affected soil nutrient cycling and plant growth, highlighting the broader ecological impact of *D. fermentans* inoculation. This experiment gives a possible insight into utilizing microbial-based strategies that can optimize agricultural practices to enhance soil functions, support plant growth, and mitigate greenhouse gas emissions, thereby contributing to climate-smart and environmentally sustainable agricultural systems.

Poster Session

P010

Microbial infallibility to degrade persistent organic pollutants

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The microbial infallibility hypothesis suggests that microorganisms can evolve to degrade any organic compound (1). The spill of crude oil into the Gulf of Mexico, highlighted the remarkable metabolic versatility and adaptive capacity of microbiomes in response to chemical perturbations (2). Yet the question remains. Does this hypothesis extend to synthetic chemicals with no natural analogs?

Atrazine was considered poorly biodegradable. However, the prolonged atrazine exposure exerted selection pressure on soil bacteria, driving the evolution of the *atzA* gene encoding the enzyme atrazine chlorohydrolase (AtzA) that enables rapid atrazine biodegradation (3). A similar process has been observed with polychlorinated compounds, originally designed for their resistance to biodegradation. Today, several mechanisms are known for the microbial degradation of these compounds, including the reductive dehalogenation by enzymes (4). These findings suggest that nature"s diverse microbiomes harbor yet undiscovered enzymatic functions capable of degrading synthetic pollutants.

A recent concern in chemical pollution research are per- and polyfluoroalkyl substances (PFAS) that are highly resistant to degradation, and researchers are actively seeking ways to prevent their bioaccumulation while developing cost-effective removal strategies. PFAS compounds are characterized by strong C-F bonds and often include a carboxyl, sulfate or hydroxyl functional group. Their degradability largely depends on the alpha carbon. A free alpha carbon or a double bond between alpha and beta carbon increases the likelihood of breakdown. In contrast, fully saturated PFAS, where all carbon bonds are occupied by fluorine, are the most resistant to degradation (12). Despite their chemical complexity, multiple bacterial species have been discovered that can break down PFAS, releasing fluoride and metabolic by products.

At Graz University of Technology, the lead project DigiBioTech is dedicated to finding new enzymes enabling the breakdown of "forever chemicals" like PFAS. Our multidisciplinary team integrates biotechnology, data science and artificial intelligence to tackle this challenge. We will apply bioinformatic approaches such as metagenomics, metatranscriptomics and functional analysis together with machine learning to optimize experimental research, accelerating targeted discovery of novel microbiome function. By analyzing microbial compositional shifts in different habitats and contaminated sites, we will identify the key taxa involved in yet unknown biodegradation pathways of persistent pollutants.

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Poster Session

P011

Order and frequency shape multiple stressor effects on freshwater benthic microbial biogeochemical cycling.

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Introduction

Benthic microbial communities underpin vital aquatic ecosystem functions like decomposition, biogeochemical cycling and primary productivity, both threatened by climate change-driven stressors such as salinisation and nutrient enrichment. These stressors often co-occur, either simultaneously or in sequence during extreme weather events, yet the influence of stressor timing—particularly order—on microbial function remains poorly understood. Improving our understanding of how stressor interactions are influenced by temporal characteristics of exposure is critical to our ability to predict changes to biogeochemical cycling in the Anthropocene.

Objectives

We tested three hypotheses: (i) repeated exposures have stronger effects on biofilm functions than single exposures, (ii) stressor interactions vary with order of application, and (iii) effects weaken over time, with effects from salinity change (a press stressor) persisting longer than nutrients (a pulse stressor).

Methods

A manipulative experiment was conducted using 1000-L semi-natural freshwater ponds in the field. Biofilms were grown on ceramic tiles for 30 days, then transferred into treatment ponds and exposed to elevated salinity and/or nutrients individually, repeatedly, or in combination in different sequences. Sampling at 1, 21, and 81 days post-treatment assessed 31-carbon-source metabolic profiles, respiration, primary productivity, photosynthetic efficiency, chlorophyll a, and microbial community structure via 16S rRNA sequencing.

Findings

The order of stressor application significantly altered functional outcomes. When nutrient enrichment preceded salinity, gross primary productivity was halved, and carbon metabolic activity increased by 50% compared to the reverse sequence.

Conclusion

Stressor order can critically shape the effects of multiple stressors. As global change accelerates, freshwater microbial functions including carbon cycling may become more variable due to the timing and sequence of interacting stressors.

Poster Session

P012

Deciphering the olive microbiome – bacterial diversity, functional potential, and impacts of storage on community composition

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Aims

The conditions of fermented olives might support the dominance of highly diverse lactic acid bacteria (LAB) with potential probiotic properties. Despite the numerous LAB-related studies, our understanding of the microbial composition of fermented olives remains limited. With this study, we aimed to compare the effects of different storage practices on the brined olive´s microbiome.

Methods

Kalamata olives from Greece were stored in glass jars and vacuum-sealed plastic bags at 4, 8, and 15°C, and samples were collected after 9, 30, and 54 days of storage (T1, T2, and T3, respectively). Baseline samples were collected as well. Extracted DNA was used for qPCR, targeting the bacterial 16S marker gene and amplicon sequencing of the bacteria 16S gene V4 region. The results of this part will be supported with metagenomics data from baseline samples. Gene annotations of the constructed bacterial genomes are performed using DRAM v.1.4.6 (Distilled and Refined Annotation of Metabolism). The analysis will include adhesion and gut colonization genes, antimicrobial production, immune modulation, carbohydrate metabolism, and vitamin synthesis.

Results

Total bacterial loads were significantly affected by storage time ($F = 26.42$, $p < 0.001$) and temperature ($F = 9.50$, $p < 0.001$). Marker gene abundances were lower at T1 compared to T2 ($p < 0.001$) and T3 ($p < 0.001$), and higher at 15°C compared to 8°C ($p = 0.017$) and 4°C ($p < 0.001$). Packaging did not significantly affect total bacterial abundances ($p = 0.162$). Except for the difference between samples, stored at 8°C and 4°C at T1 ($p = 0.008$), no significant effect on alpha diversity was observed. However, the beta diversity of stored olives was affected by packaging ($F = 3.42$, $p = 0.017$), temperature ($F = 3.22$, $p = 0.007$), and storing time ($F = 3.08$, $p = 0.007$). Interestingly, 31% of bacterial reads were assigned to non-LAB taxa at the baseline, but this number dropped below 2% on average after storage.

Conclusions

The results of this study will enhance our understanding of how storage practices influence the olive microbiome's diversity, structure, and function. Investigating the bacterial genomic

potential will provide the functional context of storage-induced taxonomical shifts and their possible contributions to human health. These insights could unveil new possibilities for the food sector aimed at increasing product quality and improving preservation methods on fermented olives.

Poster Session

P013

Microbial diversity and food safety risks in indigenous dried plant products from urban informal markets in South Africa

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Indigenous plant-based products provide considerable benefits for human nutrition and health. However, all plants harbor diverse microbial communities that can be influenced by various processing methods from the field to the consumer. In urban areas of developing nations, indigenous foods are frequently available at informal markets, where food safety can be a significant concern. This study examined the bacterial communities present in five indigenous dried plant products (baobab, masau, nyii, morogo dinawa, and morogo lude) sourced from urban informal markets in South Africa. Bacterial abundance, diversity, and community composition showed significant variation among the products, with abundance ranging from 10^3 gcn in masau to 10^9 gcn. Bacterial richness, which reached up to 1460 ASVs and was highest in baobab fruits, correlated with beneficial taxa such as Bifidobacterium and Prevotella. In contrast, the highest bacterial abundance was found in dried leafy greens and correlated with potential pathogens like Salmonella, Vibrio, and Acinetobacter baumannii. Additionally, the opportunistic pathogens Stenotrophomonas maltophilia and A. baumannii were prevalent members of the core microbiome. Cultivation-dependent assays identified bacterial isolates with beneficial traits, including protease activity, bile salt tolerance, and antagonism toward human pathogens; however, the presence of a diverse range of antimicrobial resistance, primarily against sulfadiazine and ampicillin, raises potential health concerns. These findings highlight the dual nature of indigenous dried foods at the point of sale, acting as reservoirs for both beneficial and potentially harmful bacteria. In particular, in settings where food safety standards may not consistently be upheld, enhanced food handling and processing practices are essential to preserve their health benefits.

Poster Session

P014

PGP bacteria as key drivers for cadmium mitigation in agricultural ecosystems

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Cadmium (Cd) contamination in agricultural ecosystems poses a critical challenge due to its toxicity, impact on human health, and threats to environmental sustainability (Massa *et al*., Microorganisms 2022). As a highly mobile and bioavailable metal, Cd readily migrates from soil and water into the edible portions of crops. Current management strategies to mitigate Cd levels focus on reducing its bioavailability in soil and minimizing its transfer to plant tissues. Among these, the use of Cd-tolerant plant growth-promoting (PGP) bacteria has emerged as a promising solution offering a sustainable strategy for mitigating Cd contamination in agricultural systems (Xiao *et al*., Ecotoxicology and Environmental Safety, 2023).

The aim of this study is to characterize Cd resistant bacterial strains with PGP activity towards edible plants in the presence of the metal in order to assess the possibility to use bioinoculants to reduce Cd uptake.

Within the screened collection, *Serratia plymuthica* strains As3-5a(5) (Cd MIC > 3 mM) and SC3-I2 (Cd MIC > 4 mM), and *Pseudomonas koreensis* strain 69RS (Cd MIC > 2 mM) exhibited PGP activities also in the presence of Cd. Particularly, their ACC deaminase activity was higher in the presence of 0.4 mM Cd(II). In plant growth pouches experiments, root and shoot biomass and seed germination of *Oryza sativa* (L.) and *Solanum Lycopersicon* (L.) increased in inoculated plants in the presence of 0.1 and 0.5 mM Cd(II) (Fig. 1).

The present work highlighted the ability of Cd-resistant PGP bacteria to increase Cd resistance of plants and ongoing experiments will ascertain their effect on metal uptake in plant tissues.

Acknowledgments: This work was supported by PSR 2021 Linea 6 1H_HUB project. A.M. is awarded by a PhD fellowship by the University of Milan - Food Systems PhD Program

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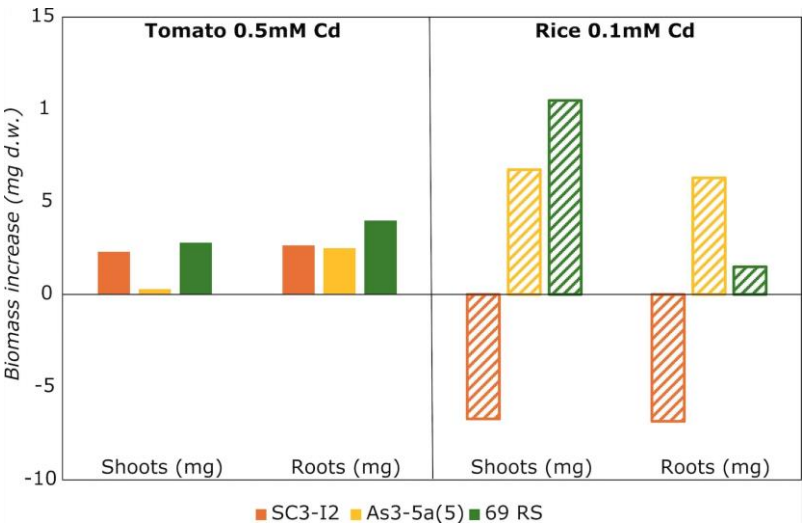
Fig. 1



Poster Session

P015

Streptomycete actinomycetes exhibiting ACC deaminase activity alleviates heavy metal stress in corn in the United Arab Emirates

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Lead (Pb) contamination presents a significant ecological hazard by diminishing soil fertility and hindering agricultural yield. Actinomycetes that promote plant growth (PGP) can improve plant resilience to heavy metal (HM) contamination. The current work assessed the impact of rhizosphere-competent (RC) and non-rhizosphere-competent (NRC) actinomycete strains on maize growth and lead (Pb) tolerance by the 1-aminocyclopropane-1-carboxylate deaminase (ACCD) enrichment method. A pot experiment was performed under lead stress (1000 mg/kg soil as $Pb(NO_3)_2$) to compare two actinomycetes isolates. Inoculated plants exhibited superior performance compared to uninoculated controls, especially in uncontaminated soils. Under Pb stress, ACCD-producing isolates #22 and #30 considerably (P<0.05) improved plant height, dry weight, and chlorophyll content, with isolate #30 demonstrating the most substantial effects. This isolate diminished electrolyte leakage by 26.7% and H_2O_2 levels by 23.7%, while enhancing antioxidant enzyme activities, in comparison to plants cultivated in Pb-contaminated soils. Isolate #30 also activated essential plant growth regulators (PGRs), such as auxins and polyamines, hence enhancing stress resistance. A principal mechanism of isolate #30 entailed reducing endogenous ACC levels in plant tissues by 67.2-72.8% during Pb stress, thus alleviating ethylene-induced growth suppression. Isolate #30 significantly reduced Pb deposition in shoots and roots by 51.1% and 63.0%, respectively. Isolate #30 enhanced urease, invertase, and catalase activity in rhizosphere soils, alongside increased microbial activity, underscoring its potential to improve phytoremediation and soil health. RC strain #30, classified as *Streptomyces mirabilis* UAE2, exhibited enhanced Pb tolerance and bioavailability management, positioning it as a viable candidate for sustainable bioremediation approaches.

Poster Session

P016

Rhizosphere-competent actinobacteria exhibiting plant growth-promoting characteristics and ACC deaminase activity, alleviate salinity stress in tomato in the United Arab Emirates

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Actinobacterial strains isolated from the rhizosphere of tomato (*Solanum lycopersicum*) plants were assessed for their capacity to produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (ACCD) and/or plant growth regulators (PGRs). This study sought to evaluate the impact of rhizosphere-competent (RC) and non-rhizosphere-competent (NRC) consortia on the growth of tomato seedlings subjected to elevated salinity (200 mM NaCl) by analyzing their morphological, physiological, and biochemical mechanisms. In our greenhouse experiment, the application of any consortium of isolates considerably enhanced the length and biomass of shoots and roots compared to control plants; however, RC consortia were comparatively superior to NRC under both normal and salt stress conditions. This indicates that the elevated rhizosphere-competence of inoculants is believed to significantly enhance plant development and salt tolerance. While the application of isolate #36 (the PGRs-producer) alone led to substantial improvements in seedling growth and biochemical photosynthetic parameters (net photosynthesis and chlorophyll content index), the synergistic use of RC isolates #36 and #53 (the ACCD-producer) markedly augmented tomato growth under saline stress conditions. Specifically, inoculation with RC consortia of actinobacteria positively influenced plant growth and photosynthetic parameters, inducing a primed state in the plants that enabled a more vigorous response to salt stress, likely through the efficient activation of antioxidant metabolism, detoxification of Na+, and significant enhancement of polyamine, auxin, gibberellin, and cytokinin levels in planta. Tomato seedlings exhibited enhanced salt stress tolerance after treatment with isolates #36/#53, which correlated with a threefold drop in endogenous ACC levels compared to control plants subjected to salinity stress. We determine that RC consortia (*Streptomyces violaceus* UAE1 and *Streptomyces levis* UAE1) serve as effective bioinoculants/biofertilizers to alleviate the adverse effects of salt stress on tomato plants in greenhouse experiments.

Poster Session

P017

Unveiling the impact of selenium nanoparticles on the wheat microbiome – a gateway to enhanced soil health and crop resilience

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The employment of selenium nanoparticles (SeNPs) in agriculture has gained substantial attention due to its potential to boost crop development and stress tolerance. This study examines the impact of SeNPs on the wheat microbiome, elucidating their function in altering microbial communities and fostering sustainable agricultural practices. Utilizing high-throughput sequencing and bioinformatics analysis, we investigated the alterations in bacterial and fungal diversity, composition, and functional potential within the rhizosphere and endosphere of wheat plants subjected to SeNPs treatment. Our results demonstrated that SeNPs considerably affected the wheat microbiome, enriched beneficial microbial taxa such as *Pseudomonas*, *Bacillus*, and *Trichoderma*, which are renowned for their plant growth-promoting and pathogen-suppressing characteristics. Additionally, SeNPs reduced the number of harmful fungi, including *Fusarium* and *Alternaria*, suggesting a preventive role against soil-borne illnesses. Functional profiling revealed augmented metabolic pathways associated with nutrient cycle, stress response, and selenium metabolism in SeNP-treated microbiomes, underscoring their capacity to boost soil fertility and plant resilience. Notably, SeNPs enhanced the bioavailability of selenium in the soil, leading to its uptake by wheat plants and subsequent improvement in their antioxidant capacity and general health. These findings highlight the dual effect of SeNPs in improving both plant performance and microbial activity. This study presents compelling evidence that SeNPs can function as an innovative instrument for microbiome engineering, providing a sustainable approach to enhance crop output and soil health amid environmental difficulties.

Poster Session

P018

Isolation of a novel *Sphingomonas* strain able to degrade the pleuromutilin tiamulin – omic analysis reveals its transformation pathway

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Tiamulin (TIA) is a commonly used veterinary antibiotic. It is persistent in the animal digestive system, its excreta and receiving environments, posing environmental and public health concerns due to potential selection for microbial TIA resistance. Within the context of gaining insights regarding natural attenuation mechnanisms like that of antibiotrophy, and the potential of employment of antibiotrophs for bioaugmentation, we isolated a TIA-degrading bacterial strain and characterised its capaciy to biotransform TIA. In liquid cultures, the isolate was able to biotransform TIA in very short time-periods (1-10 days depending on TIA dose and rest conditions), at concentrations of up to 100 mg ml-1 of TIA, at a range of pH values between 5.5 and 9, and at cool (16 ˚C) and warm (25 ˚C) temperatures. Phylogenomic analysis deemed the isolate to be a new *Sphingomonas* species (83.87 % ANI with *S. laterariae*, ≤ 95 %), which was named *Candidatus* Sphingomonas perruchonii. Genomics and transcriptomics revealed the TIA mediated induction of features conducive with the antiotrophic character for the microbial isolate, including (i) ABC-F transporters and efflux pumps able to protect the ribosome and microbial cells from TIA, and (ii) oxygenases (e.g. P450 cytochrome) and hydrolases (e.g. alpha/beta-hydrolases and amidohydrolases) possibly contributing to its degradation. LC-MS/MS analysis detected putative transformation products (TPs) of TIA, leading to a proposed transformation pathway. This involved a primary oxidation of the tricyclic moiety of TIA to a mono-hydroxylated derivative (OH-TIA), potentially mediated by the highly upregulated monoxygenases, which was either further oxidized to di-OH-TIA or hydrolysed, by the upregulated hydrolase or amidohydrolases, to 2-diethylamino-ethyl-thio acetic acid, both not degrading further. This study enforces the prospects of exploitation of antibiotrophy in treating anthropogenically enriched environments with antibiotics, such as those associated with livestock manure.

This work is under review for publication and a preprint of it can be found at https://www.biorxiv.org/content/10.1101/2024.12.16.628731v1

Poster Session

P019

Alterations in the belowground microbiota and metabolome of *Pinus pinaster* trees affected by forest decline

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Pine forests are experiencing generalized deterioration and loss of vigor due to recurrent episodes of forest decline worldwide. It is already known that biotic and abiotic perturbations result in alterations of the plant associated microbiota and metabolome, which can affect negatively the plant host fitness. Nevertheless, there is a knowledge gap regarding the shifts in the microbial communities and metabolome profiles of maritime pines affected by forest decline. Thus, here we unraveled the alterations in the metabolome and in the diversity and taxonomical profiles of the microbiota associated to the roots of *Pinus pinaster* (maritime pine) trees with symptoms of forest decline and affected by *Matsucoccus feytaudi* in the National Park of Sierra Nevada (Granada, Spain). We also aimed at deciphering potential correlations between the metabolome and microbial communities.

Trees infected by *M. faytaudi* and asymptomatic individuals showed differences in the taxonomical profiles and associative patterns of root microbiota. While unhealthy pines were enriched in several plant growth promoting microorganisms (i.e., *Streptomyces* and certain ectomycorrhizal fungal genera such as *Clavulina*), other beneficial microbes were more abundant in unaffected trees, namely the actinobacteria *Micromonospora*. The rhizosphere of unhealthy trees was richer in secondary metabolites involved in plant defense than unaffected trees, and the opposite trend was detected in root samples. The abundance of several microorganisms was significantly and positively correlated with different antimicrobial metabolites, thus, being all of them worthy of further isolation and study of their role in forest decline.

Poster Session

P020

Functional diversity and environmental distributions of IncP/P-1 plasmids across subgroups

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Plasmids play a crucial role in bacterial adaptation and evolution by horizontal gene transfer. Among them, IncP/P-1, a group of broad-host-range plasmids, frequently carry antimicrobial resistance and/or degradation genes and are isolated from various environments including soil, water and clinical samples. The IncP/P-1 group is classified into 15 subgroups (α, β, γ, δ, ε, η, ζ, ι, κ, ο, λ, θ, μ, ρ, τ) based on nucleotide sequence variations1). In this study, we investigated the functional diversity of these subgroups by comparing their features and environmental distributions.

First, we assessed their replication ability, transfer frequency and stability using *Escherichia coli* and *Pseudomonas putida* as host strains. The results revealed distinct subgroup-specific behaviors: (i) IncP/P-1η, -μ, and -ρ were unable to replicate in *P. putida*, whereas other subgroups could. (ii) IncP/P-1κ and -λ exhibited lower transfer frequencies to *P. putida* than other subgroups. (iii) IncP/P-1γ, and -ο were not stably maintained in *E. coli*, while IncP/P-1θ, -λ and -κ were unstable in *P. putida*.

Next, we analyzed metagenomic data using MGnify2) to determine the environmental distribution of IncP/P-1 plasmids, focusing on their replication initiation protein, TrfA. The results revealed subgroup-specific environmental distributions: for instance, α was predominant in compost, β, γ and δ in wastewater, ε in activated sludge, θ and ζ in marine water, ο in food production environments, κ in urban fresh water, τ in rhizosphere, and η, μ, and ρ in clinical samples. These results suggested that each IncP/P-1 subgroup might have diversified by modifying its features to adapt to different environmental niches.

1) Hayakawa M et al., 2022 Appl Environ Microbiol. 88(18):e0111422

2) Gurbich TA et al., 2023 J Mol Biol 435(14):168016.

Poster Session

P021

Improving resilience and yield of tomato in saline soils – a sustainable method utilizing biogenic silicon nanoparticles in the United Arab Emirates

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Salinity is a significant environmental stressor that restricts tomato development and yield, especially in dry and semi-arid locations, such as the United Arab Emirates (UAE). It interferes with critical physiological functions, including photosynthesis, nutrition absorption, and metabolic pathways. This research utilized silicon nanoparticles (SiNPs), biosynthesized by *Streptomyces* sp. (UAE1), on salt-stressed tomato plants through foliar application and soil drenching. The impacts on oxidative stability, photosynthetic efficiency, physicochemical characteristics, and yield were evaluated. Spherical silicon nanoparticles (SiNPs), averaging 52 nm in size and possessing a negative charge of -25.9 mV, demonstrated a 95% capability for free radical scavenging. Biogenic SiNPs (80 mg/L), primarily via drenching, markedly improved chlorophyll content, net photosynthesis, stomatal conductance, and water use efficiency by 45%, 71%, 27%, and 94%, respectively. Proline elevated by 20% and gibberellic acid by 35%, whilst abscisic acid and malondialdehyde diminished by 39% and 73.25%, respectively, in comparison to stressed controls. The activity of antioxidant enzymes reverted to levels approaching normal, exhibiting decreases of 25-36%. Physicochemical enhancements comprised a 45% rise in weight and a 77.2% improvement in overall yield. The carbohydrate and protein composition rose by 15% and 34%, respectively. The utilization of biogenic SiNPs is a sustainable method to enhance tomato yield in saline environments, hence fostering resilient agricultural practices in salt-affected soils in the UAE and beyond. This study is the first research demonstrating the ability of biogenic SiNPs sourced from actinobacteria to mitigate salt stress in tomatoes.

Poster Session

P022

Wastewater-borne pollutants affected the soil resistome and mobilome and shaped the microbial community composition in cilantro rhizosphere

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For decades, untreated wastewater from Mexico City has been used to irrigate crops in the Mezquital Valley. With the construction of the Atotonilco wastewater treatment plant, treated wastewater will gradually replace the untreated wastewater. To assess how this transition affects the abundance, diversity, and dissemination of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), we conducted a soil column experiment. Monolithic soil columns of two soil types (Leptosol and Vertisol) from the Mezquital Valley were planted with cilantro and irrigated for eight weeks with untreated or treated wastewater with or without spiked antibiotics and biocides. At the end of the experiment, total community DNA was extracted from soil, rhizosphere, and phyllosphere. qPCR and 16S rRNA gene amplicon sequencing analysis were performed.

Soil collected from preferential flow paths (stained with a non-toxic dye) exhibited a significantly higher relative abundance of erythromycin, sulfonamide, tetracycline resistance genes, class 1 integron integrase gene and IncP-1 plasmids compared to unstained soil, less exposed to infiltrating wastewater. The distribution patterns of ARGs and MGEs in stained, unstained soil, and phyllosphere were significantly affected by the spiked contaminants in the irrigation water. Spiking increased the relative abundances of the class 1 integron integrase and sulfonamide resistance genes across all sampled compartments, with significant increases in stained and unstained soils and cilantro rhizosphere.

The soil type influenced the ARG and MGE profiles in the rhizosphere and the microbial community composition of stained flow paths and rhizosphere. Specifically, higher relative abundances of ARGs and MGEs were observed in rhizosphere samples from Leptosol columns. However, in the rhizosphere, the microbial community composition was also influenced by the concentration of pollutants in the irrigation water.

Our findings confirm that antibiotics and disinfectants in irrigation water influence the abundance of ARGs and MGEs in soil and shape rhizosphere microbial communities. Future studies integrating chemical analyses of antibiotic accumulation in soils and plants, along with exogenous plasmid isolation experiments, will further elucidate the role of MGEs and wastewater pollutants in the spread of environmental antimicrobial resistance

Poster Session

P023

A snapshot of an organic apple orchard belowground biodiversity as affected by landscape management at farm level

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The introduction of flowering plants or mixtures of flowering plants into an orchard is considered a practice that enhances the biodiversity, by widening the plant species, and can provide other ecosystem services or benefits such as supporting pollinating insects, sheltering beneficial insects that can help reducing pests incidence, support soil nutrients level, provide additional income and, overall, positively affect the fruit yield. . However, it is important to design these mixtures to avoid resources competition, and thus it is necessary to shed light on their impact on soil functions.

In the present study, the effects of introducing two plant mixtures as living mulches on the soil nutrient status and microbiome functionality was investigated. A simple consociation of white clover (*Trifolium repens*) and sheep fescue (*Festuca ovina*) or a mixture composed of 40 different species of grasses and flowering plants were introduced into an organic 12-year-old apple cv. Gala/M9. Natural cover was considered as control. The orchard was drip irrigated, and localized fertilization was provided with organic fertilizers (dry bovine manure and stillage), applying a total of 12 g N/tree. The evaluation of the treatments was carried out after the full establishment of the living mulches, i.e. in the season following their sowing.

The greatest effect of both mixtures on soil macronutrient content ($N\text{-}NO_3$, P, K, Ca) was observed in spring, while no changes were induced in autumn and only potassium resulted affected by them during summer. Differences in the soil metabolic activity evaluated from Biolog analysis were also observed in spring and summer, but bacterial biodiversity was not affected by the presence of the living mulches mixtures. This result was confirmed by a detailed analysis of the bacterial community during summer, which showed a consistent composition among the treatments at phyla level, including among the most abundant ones (>1%) Proteobacteria, Actinobacteriota, Acidobacteriota, Verrucomicrobiota, Bacteroidota, Chloroflexi, Firmicutes, Planctomyceota and Myxococcota. In-depth analysis of the interaction between metabolic and biodiversity data will be provided to better understand the functioning of the soil bacterial communities and the potential of influencing the soil microbiome through soil management practices that are known to improve organic horticultural cropping systems.

This study was supported by AgroBioConnect (grant n. SUSCROP/AgroBioDiv/I/42/AgroBioConnect/2023) funded from NCBR under the SusCrop ERA-NET programme, within FACCE-JPI.

Poster Session

P024

Identification and characterization of plasmids carrying carbapenem resistance genes in the Philippines

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The emergence and spread of antimicrobial-resistant bacteria has been facilitated by the horizontal transfer of antimicrobial resistance genes via plasmids. Especially, the spread of carbapenem-resistant bacteria poses a serious threat to global health, as carbapenems represent the last resort in the treatment of infections. To address this issue, it is essential to monitor plasmids conferring carbapenem resistance and elucidate their behavior in the environment. The problem is especially prevalent in regions with inappropriate antimicrobial usage and poor sanitation[1)].

In this study, we aimed to identify and characterize plasmids carrying carbapenem resistance genes in the Philippines. Microbial samples were collected from hospital wastewater treatment facilities and river water in Tacloban city, Philippines. Carbapenem (meropenem)-resistant bacteria were isolated from them. Additionally, exogenous plasmid capture was performed using *Metapseudomonas resinovorans* CA10dm4RGFP as a recipient. The complete nucleotide sequences of meropenem-resistant isolates and transconjugants were determined with both long-read and short-read sequencing.

A total of eight meropenem-resistant bacteria were isolated from hospital samples, and three complete genome sequences were successfully determined. Among these, *Acinetobacter towneri* harbored a plasmid named pPT23-B2_1, with the co-occurrence of $bla_{\text{NDM-1}}$ and *tet*(X7), conferring resistance to carbapenems and tigecycline[2)]. Another isolate, *Pseudomonas inefficax*, harbored a plasmid named pPT23-C1_1, a member of pSTY-like plasmid group[3)]. This plasmid contained a carbapenemase gene $bla_{\text{VIM-2}}$, within a class 1 integron, which was also embedded in a Tn*3*-family transposon. In addition, mating assay showed that pPT23-C1_1 was transferrable to *M. resinovorans* CA10dm4RGFP.

Furthermore, we obtained five transferable plasmids from hospital wastewater and seven plasmids from river water by exogenous plasmid capture. One of these, named pPTBM23_1, was identified as a member of IncP-9 plasmid group. It carried $bla_{\text{IMP-26}}$, which was integrated into a Tn*402*-like class 1 integron. Currently, we are analyzing plasmids carrying carbapenem resistance gene from river water in the Philippines.

Reference: 1) Hounmanou *et al*., 2025. *Front Public Health*, 12:1525873. 2) Mallonga *et al*., 2025. *J Glob Antimicrob Resist*, S2213-7165(25)00024-4. 3) Köhler *et al*., 2013. *J Biotechnol*, 168(4):729-30.

Poster Session

P025

Persistence of *Acinetobacter* spp. in manured fields

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Question: *Acinetobacter* spp., including pathogenic *Acinetobacter baumannii*, occur in cattle feces and manure that are used as soil fertilizers. However, nothing is known about their fate after manure deposition to agricultural fields.

Methods: We monitored *Acinetobacter* abundance and species composition on farmers' fields over 3 months after cattle manure application. In two Czech conventional dairy farms, we collected manure (fresh or composted) and bulk soil samples from five plots per field before manuring and at 5–6 time points afterward. Additionally, rhizosphere soil was sampled at Farm 1 at three months. *Acinetobacter* abundance and species composition were assessed in total DNA using 16S rRNA qPCR and *rpoB* metabarcoding, respectively.

Results: The average *Acinetobacter* abundance in fresh manure (at Farm 1) was ~1E+10 16S copies/g dry weight (6% of total bacteria), while it was only ~7E+07 16S copies/g dry weight (0.07%) in composted manure (at Farm 2). Before manuring, acinetobacters were below the detection limit (~1E+06 copies/g dry weight) in both farmer's fields. Following manure application, *Acinetobacter* abundance fluctuated over time in both fields. At Farm 1, acinetobacters were detectable over three months, reaching a peak of ~1E+08 16S copies/g dry weight at four weeks. At Farm 2, however, they declined below the detection limit by that time. *Acinetobacter* species composition in manured field soil was therefore monitored only at Farm 1. Within the first two weeks, *Acinetobacter* species composition exhibited high spatial heterogeneity, with plots dominated either by manure-borne *A. pseudolwoffii* or by potentially soil-borne, unknown *Acinetobacter* spp. After 4-8 weeks, the community became spatially homogeneous, with manure-borne species prevailing in all plots. At three months, when crops (mixture of wheat and legumes) were grown, the spatial heterogeneity increased again. At this stage, some plots were dominated by *A. pseudolwoffii* while others by *A. terrae*, both in bulk soil and rhizosphere. Additionally, *A. guillouiae* and *A. calcoaceticus* were prevalent in several rhizosphere samples. Importantly, *A. baumannii* was not detected in any of the manured fields.

Conclusion: Acinetobacters persist in field and rhizosphere soil over three months following fresh cattle manure application, with their species composition changing over time. Further analyses will focus on their potential to spread of antibiotic resistance genes to the field.

Poster Session

P026

Antibiotic Mixtures Show Variable Effects on Soil Bacterial Activity, but Enhance Resistance Gene Proliferation Compared to Single CompoundsC. Ejikeugwu[1], Q. Hinkel[1], A. Worrich[1]

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Manure application introduces antibiotic residues into soils, potentially disrupting soil bacterial activity. However, most studies examine the effects of individual antibiotics rather than mixtures, despite manure containing multiple antibiotics simultaneously. In this study, we investigated shifts in soil microbial activity following treatment with veterinary antibiotics from three classes - tetracyclines, macrolides, and sulfonamides - applied individually and in all possible binary and tertiary combinations at a concentration of 12 mg/kg. Samples were incubated for 3, 7, and 14 days at ambient temperature, and bacterial activity was assessed by measuring CO_2 release using the MicroResp™ method. For single antibiotics, a moderate effect on soil bacterial activity was observed as evidenced by decreased bacterial respiration, except for doxycycline (DOX), which unexpectedly enhanced respiration, as indicated by increased CO_2 emissions compared to the control. In contrast, antibiotic mixtures had a more pronounced and significant impact, leading to reduced respiration and greater disruption of bacterial activity than single compounds. Notably, combinations of two or more antibiotics, regardless of their class similarity, had stronger and more persistent effects on soil bacterial activity over time, with the most pronounced impact observed in tertiary mixtures. These findings provide insights into microbial responses, potentially affecting the dynamics of antibiotic resistance genes (ARGs) and microbiome composition, which will be explored further. Our work highlights the critical need to incorporate antibiotic mixtures into ecotoxicological assessments of antibiotic pollution in soil, as current risk evaluations neglect their combined effects.

Poster Session

P027

Harnessing disease legacy effects in the soil microbiome for crop health

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This research leverages legacy effects in the soil microbiome to reduce the dependance on harmful agri-chemicals. Healthy plants from historically infected and uninfected potato fields were investigated to reveal a reservoir of microbial legacies associated with disease outbreaks, and these legacies were found to fortify the plants against pathogens.

Amplicon sequencing of 1953 plant and soil samples, spanning various growth stages & soil types, facilitated a comprehensive microbiome profiling of the fields. It was supplemented with large-scale isolation, identification, and screening of the microbiome, resulting in a well-characterized biobank of 2620 unique bacteria associated with these potato fields. Finally, biochemical assays and greenhouse experiments were performed test if the leads from bioinformatic analysis can enhance plant protection when applied as a microbial consortia.

We found that healthy plants grown in historically infected soils recruited a more complex, diverse and better-connected microbiome. Bacterial hubs from the microbial co-occurence networks were found to synergize in biofilm formation and antagonize common plant pathogens *in-vitro,* when applied together as a consortia. These microbial consortia show ehanced plant growth promotion and disease tolerance in greenhouse experiments, when compared to the individual strains. We further generalized their impact on plant health by testing them in a closely-related *Solanaceae* host, tomato.

In conclusion, this study supports the investigation of social interactions within the soil microbiome, and the subsequent transition from single strain bioinoculants to consortia. It further suggests that microbial legacies can serve as a resource for developing tailored bioinoculants that can contribute to resilient food systems and healthy soils.

Poster Session

P028

Acquired antibiotic resistance determinants of *Pseudomonas aeruginosa* isolates cultured from hydrocarbon-contaminated environmental samples

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Crude oil and its derivates are among the most important environmental pollutants, where *P. aeruginosa* strains producing AlkB1 and AlkB2 alkane hydroxylases are often involved in their biodegradation. The aim of this study was to analyze the acquired antibiotic resistance determinants of a *P. aeruginosa* isolate cultured from a hydrocarbon-contaminated soil sample from Ogoniland, Nigeria, and to compare its characteristics with other *P. aeruginosa* isolates cultured worldwide from hydrocarbon contaminated environmental samples. Using the ResFinder reference database, a catB7 chloramphenicol acetyltransferase gene, an ampC-type PDC β-lactamase gene and an OXA-50 type β-lactamase gene were detected in all *P. aeruginosa* strains analyzed in this study. In some of these *P. aeruginosa* strains loss-of-function mutations were detected in the regulatory genes mexR, nalC or nalD, predicting an efflux-mediated acquired antibiotic resistance mechanism. Several *P. aeruginosa* sequence types that were obtained from oil contaminated environmental samples have also been cultured from human clinical samples worldwide, including sequence types ST532, ST267, ST244 and ST1503. Our findings provide further evidence that environmental *P. aeruginosa* may serve as the source of human infections. This research was funded through the Hungarian National Laboratory Project, grant number RRF-2.3.1-21-2022-00007 and by OTKA grant number NKFI K 132687.

Poster Session

P029

Rooted in symbiosis – exploring bacterial dynamics in soybean

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Legumes play a crucial role in enhancing soil health through their symbiotic relationship with nitrogen-fixing bacteria. This legume-rhizobia interaction, which occurs in root nodules, is renowned for its high selectivity. Despite this, molecular surveys and cultivation have revealed a diverse range of other nodule-associated bacteria (NAB) in natural settings. The diversity, abundance, and roles of NAB in symbiosis, as well as their potential impact on plant growth and nutrient availability, remain understudied.

In 2024, we sampled soybean (*Glycine max*) nodules from four farms in Austria throughout the growing season to investigate the diversity and function of NAB in individual root nodules. Corresponding soil samples were also collected to test how environmental factors shape nodule microbial community composition. Amplicon sequencing of 16S rRNA and *nifH*, a marker gene for nitrogen-fixing bacteria, revealed that early in the growing season, root nodules contained more diverse communities with up to 10% NAB (*Streptomyces, Nocardia*, *Pseudomonas and Glycomyces*). Nearing harvest, a shift occurred towards consistently rhizobia-dominated communities (>99%). The massive dominance of host and rhizobia DNA in nodules make functional investigation of NAB, e.g., by metagenomics, particularly challenging. We are currently testing Nanopore adaptive sampling as a new way to access low-abundance NAB genomes.

Enhancing plant growth and increasing resilience will be essential in securing the future of agriculture. One possible step towards achieving this goal is to optimize nitrogen fixation by symbiotic plant-associated bacteria. Our research will contribute to a deeper understanding of plant-microbe interactions and offer potential strategies for improving crop yields and restoring soil health through (micro)biotechnological and agronomic practices.

Poster Session

P030

Bacterial community responses to antibiotic- and fungicide-modulated VOCs in soil

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Volatile organic compounds (VOCs) mediate microbial interactions, influencing bacterial community composition. However, the extent to which different concentrations of individual VOC types shape bacterial communities in adjacent soils remains unclear. This study investigates how VOCs emitted from antibiotic- and fungicide-treated soils affect bacterial communities in receiver soils over time. A split-compartment Petri dish system was used to expose untreated receiver soils to VOCs emitted from soils treated with Ampicillin (AMP), Bacitracin (BAC), or Cycloheximide (CYCL). These treatments were selected to modulate different VOC profiles by selectively targeting bacterial (AMP, BAC) or fungal (CYCL) populations in the source soil. Control soils (NO) remained untreated. Bacterial communities in receiver soils were analyzed after 7 and 14 days using high-throughput sequencing. Each treatment was replicated four times, and constrained ordination analyses, including Canonical Analysis of Principal Coordinates (CAP), were performed to assess treatment effects. At 7 days, CAP analysis showed significant shifts in bacterial communities (R^2 = 7.22%, P = 0.001), with Cycloheximide-treated soils forming the most distinct cluster, indicating that fungal suppression leads to unique VOC emissions with strong bacterial effects. Ampicillin- and Bacitracin-treated soils also influenced bacterial composition, though their clustering patterns overlapped, suggesting a more gradual VOC-driven shift. At 14 days, treatment effects remained significant (R^2 = 5.88%, P = 0.003), with clearer separation among treatments. Cycloheximide continued to drive the strongest divergence, reinforcing the role of fungal-derived VOCs in shaping bacterial interactions. Ampicillin and Bacitracin effects became more distinct over time, suggesting cumulative VOC-mediated impacts. Control soils remained separate, confirming that observed shifts were treatment-specific. These findings demonstrate that antibiotic- and fungicide-modulated VOCs lead to distinct, time-dependent shifts in bacterial communities.The greater explanatory power at 7 days suggests early VOC-mediated effects are stronger, while community differentiation stabilizes over time. These results highlight the role of VOC concentration and composition in structuring bacterial communities in soil environments.

Fig. 1

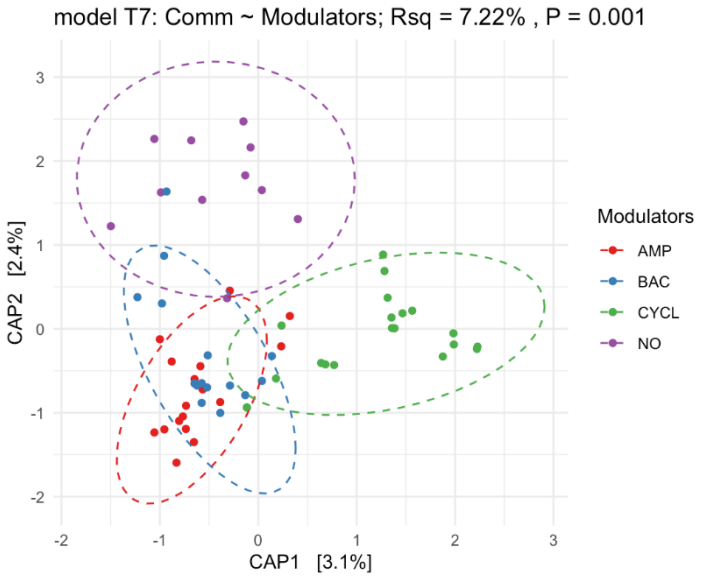
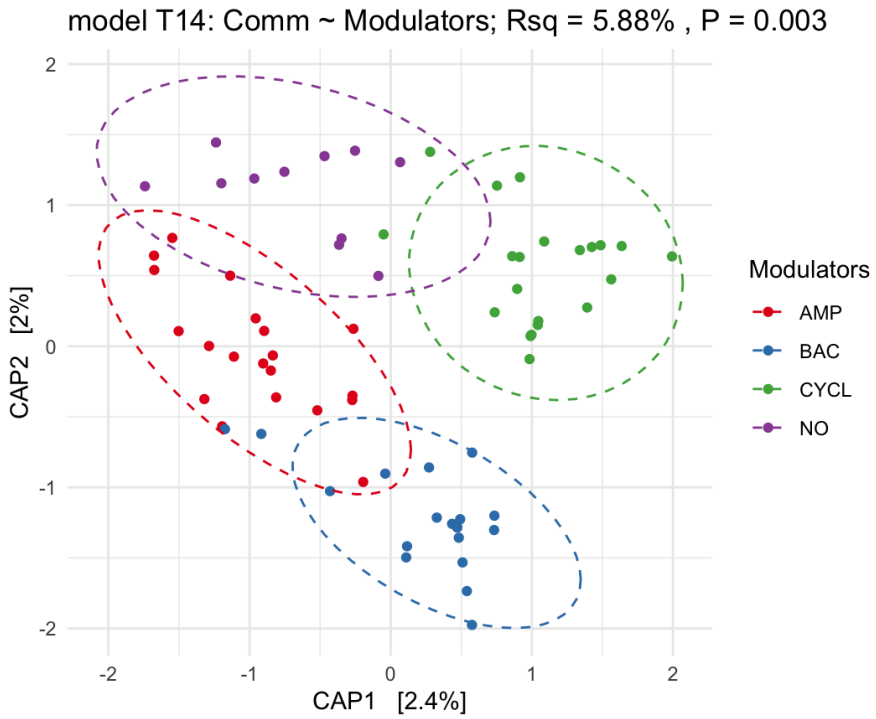

model T7: Comm ~ Modulators; Rsq = 7.22% , P = 0.001
CAP2 [2.4%]
CAP1 [3.1%]
Modulators
AMP
BAC
CYCL
NO

Fig. 2


model T14: Comm ~ Modulators; Rsq = 5.88% , P = 0.003
CAP2 [2%]
CAP1 [2.4%]
Modulators
AMP
BAC
CYCL
NO

Poster Session

P031

Exploring the antimicrobial power of *Streptomyces* sp. ATHUBA1179 against carbapenem resistant human pathogens

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Antimicrobial resistance constitutes a top global public health threat, characterized as "silent pandemic" and it"s expected to be the leading cause of death in a few years. It"s estimated that in 2019 it was directly responsible for 1.27 million deaths. To combat this the World Health Organization adopted the Global Action Plan on AMR in 2015, based on the One Health approach. A key point of the Action Plan is the support of research for the development of new antimicrobial drugs, as the production of new, innovative drugs has slowed down in recent years. Historically, the genus *Streptomyces* has been the source of two thirds of clinically used antibiotics, making it an important pull for novel products. *Streptomyces* are Gram-positive, filamentous, spore forming bacteria with a rich secondary metabolism, that can be enhanced in conditions of stress. Whole genome sequencing has revealed many silent biosynthetic gene clusters, which, if stimulated, can lead to the production of novel antibiotics. This study investigates the optimal culture conditions for enhancing the antimicrobial activity of *Streptomyces* sp. ATHUBA1179 against carbapenem resistant *Klebsiella pneumoniae* ST307 and *Stenotrophomonas maltophilia*. To investigate this, the strain was cultivated in liquid AGS medium for 7 days under varying salinity and pH stress conditions and the antimicrobial capacity of the supernatant was tested using the broth microdilution method. It was observed that the optimal NaCl concentrations for antimicrobial production were 0.1 % (w/v), 0.5 % (w/v) and 1 % (w/v). Regarding the pH the two pathogens showed different inhibition patterns, with the antimicrobial activity for the *Klebsiella* strain being greatest at pH 7, while for the *Stenotrophomonas maltophilia* strain it was greatest at pH 5. These findings highlight the potential of optimizing environmental conditions to unlock silent gene clusters, offering a promising avenue for developing novel antimicrobial therapies to combat antibiotic resistance.

Poster Session

P032

Unraveling the membrane vesicles' cargo of *Streptomyces* sp. strain ATHUBA1179 – An approach to drug delivery

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Antimicrobial resistance (AMR) is a natural process, but its uncontrolled spread has become a public health emergency, largely driven by antibiotic overuse. The World Health Organization has warned that the world is "running out of antibiotics" and has ranked antibiotic resistance among the top 10 global health threats. Multidrug-resistant (MDR) bacteria, including carbapenem-resistant strains, pose a serious risk to public health by worsening patient outcomes and increasing healthcare costs. The widespread use of broad-spectrum antibiotics further fuels MDR prevalence, creating a cycle of resistance that complicates infection control and limits effective treatment options. Therefore, it is essential to develop both new antibiotics and advanced therapeutic strategies, including functionalized antibiotic delivery systems that operate independently of conventional treatments. Bacterial cell-derived natural nanomaterials have recently gained significant attention. *Streptomyces*-derived membrane vesicles (MVs) are nano-sized vesicles (20-400 nm) capable of encapsulating and delivering antibiotics. They play key roles in microbial communication and metabolite transport, making them promising candidates for advanced antibiotic delivery. Their ability to encapsulate and deliver antibiotics provides a novel strategy to overcome drug resistance and enhance treatment precision. The purpose of this work is to unravel the membrane vesicles cargo of *Streptomyces* sp. strain ATHUBA1179 and explore their role in transferring antibiotic secondary metabolites against multidrug-resistant and carbapenem-resistant human pathogens, such as *Klebsiella pneumoniae* strain ST307 and *Stenotrophomonas maltophilia*. Four- to seven-day liquid cultures of *Streptomyces* sp. strain ATHUBA1179 in AGS medium were used for MV isolation and purification. The process involved ultracentrifugation, followed by TEM microscopy for MV observation. The isolated MVs exhibited the capacity to inhibit the growth of multidrug-resistant and carbapenem-resistant human pathogens. Proteomic analysis revealed that *Streptomyces* sp. strain ATHUBA1179 can produce distinct populations of MVs, each characterized by different protein and metabolite cargos. These findings suggest that *Streptomyces* MVs can serve as potential carriers for the targeted delivery of antibiotic compounds, offering an alternative therapeutic approach.

Poster Session

P033

The influence of heavy metals on antibiotic production in *Streptomyces* sp. strain ATHUBA1179

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Antibiotics have long been the cornerstone of treating bacterial infections, yet their overuse and misuse have led to the emergence of antimicrobial resistance (AMR), posing a critical threat to global public health. Of particular concern are infections caused by carbapenem-resistant *Klebsiella pneumoniae* (ST307) and *Stenotrophomonas maltophilia*, which are associated with high mortality rates and limited treatment options. The "One Health" approach emphasizes interdisciplinary collaboration across human health, animal health, agriculture, and environmental sectors to combat AMR and promote the responsible use of antibiotics. The genus *Streptomyces*, a prolific producer of bioactive secondary metabolites, holds promise for discovering novel antimicrobial agents. Notably, exposure to abiotic stressors, such as heavy metals, can enhance the antimicrobial potential of *Streptomyces* strains. This study explores the effect of heavy metal stress on the antimicrobial capacity of *Streptomyces* sp. strain ATHUBA1179 against carbapenem-resistant *K. pneumoniae* (ST307) and *S. maltophilia*. To investigate this, 7-day AGS liquid cultures of *Streptomyces* sp. strain ATHUBA1179 were exposed to various heavy metal salts (NiSO4, CoCl3, $K_2Cr_2O_7$, MnCl3, ZnCl2, FeCl3 and LiCl) at concentrations ranging from 0.1 mM to 2 mM. The antimicrobial activity of the resulting supernatants was tested using the broth microdilution method. Among the tested metals salts, potassium dichromate ($K_2Cr_2O_7$) significantly enhanced the antimicrobial efficacy against both human pathogens, as demonstrated by statistically significant inhibition data. These findings underscore the potential of exploiting abiotic stress to enhance the antimicrobial capacity of *Streptomyces* strains. Further investigation of the bioactive compounds produced under heavy metal stress may pave the way for the development of novel therapeutics to combat multidrug-resistant bacterial infections.

Poster Session

P034

Revealing the untapped potential of *Streptomyces* – co-Culture as a pathway to new antibiotics

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Antimicrobial resistance (AMR) has become a growing global health crisis, with resistant pathogens spreading at an alarming rate and rendering widely used conventional antibiotics ineffective. The demand for new treatments is heightened by this significant public health concern. Despite that, novel antibiotic research has stalled in the past few decades, while excessive antibiotic use contributes to the growing rate of resistant pathogenic strains. A potential solution may lie in the widely renowned bacterial genus of *Streptomyces*, the main producer of clinically used antimicrobial agents. Even though *Streptomyces* antibiotics production may seem heavily researched, it appears that the full potential of their secondary metabolite arsenal is limited under standard monoculture conditions. Co-culturing *Streptomyces* with other members of their genus and other microorganisms is an emerging strategy in activating silent genes and biosynthetic gene clusters (BGCs), resulting in increased secondary metabolite secretion. For this purpose, we isolated *Streptomyces* strains from the rhizospheres of *Mandragora officinarum* and *Ebenus sibthorpii*, both of which are indigenous Greek plants. Initially, we evaluated the antibiotic capacity of the isolated strains against six indicator strains, including two Gram-positive, two Gram-negative, and two yeast strains. Additionally, we co-cultured the *Streptomyces* strains, both within the same genus and across different genera, and re-evaluated their ability to inhibit indicator strains. The results show that co-culturing enhances secondary metabolite production with antimicrobial activity, even in initially non-producing strains. This suggests that intermicrobial interactions may activate previously silent biosynthetic pathways. Future research in co-culturing holds great promise for promoting the expression of previously unexpressed secondary metabolites, opening new avenues for the discovery of innovative antibiotics and aiding in the battle against antimicrobial resistance (AMR).

Poster Session

P035

Defining microbial diagnostic parameters to evaluate agricultural soil quality

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Growth, health and yield of agricultural crops highly depend on the in situ soil microbiome. The multitude of various microbes in the rhizosphere does not only deliver essential nutrients to the plant, but also increases its tolerance to environmental stress and resistance to pathogens. The intricate plant promoting effects of the soil microbiome has been subject of intense research for many years. Despite a growing awareness of the essentiality of soil microbes, the standard diagnostic of agricultural soil quality remains largely based on physical and chemical parameters. However, as these parameters are primarily the result of microbiological activity in the soil, a more direct measure of soil quality would be to evaluate microbial diversity, abundance and activity directly.

In the project *AgriBiom* we are tracking the microbiome of several agricultural fields in the region of Western Pomerania (Germany) over a period from 2023 to 2026. These fields are characterized by differences in crops and fertilization methods (mineral *vs*. manure) and conventional *vs*. ecological farming.

Microbial composition of more than 130 samples is determined via 16S/18S rRNA amplicon sequencing and, most importantly, the functionality of these complex microbiomes is analyzed by comprehensive metaproteome analyses. The latter is based on a tailor-made protein sequence database generated from Illumina whole genome sequencing data of six selected soil samples. Extracting proteins from soil for mass spectrometric analyses is most challenging due to interference from humic acids. To overcome this, we established an optimized protocol that enables the high-resolution identification and quantification of thousands of proteins from complex soil samples. The valuable information on actively expressed genes uncovers metabolic and regulatory functions of the soil microbiome involved in plant-microbe interactions.

By correlating metaproteomic data with amplicon-based data, physico-chemical soil parameters and farmer provided meta-data including crop type, yield, fertilization and pesticide use we aim to uncover effects of agricultural methods on the soil microbiome. Furthermore, the identification of key microbial functions will facilitate the development of standardized assays for rapid and reliable assessment of the microbial constitution of agricultural soils to support the development of sustainable farming strategies that promote environmental health and resource efficiency.

Poster Session

P036

Streptomycetes from the rhizosphere of historical trees in Greece – eco-Friendly biocontrol and antimicrobial agents

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The rhizosphere of historical plants represents a unique and rich ecological niche that supports a variety of microbial communities, including a wide range of antibiotic-producing Actinobacteria. These microbes are particularly valuable due to their ability to produce secondary metabolites with potent antimicrobial properties. *Ulmus carpinifolia*, *Cupressus sempervirens* var. horizontalis, and *Quercus ilex* are long-lived tree species that have developed complex and diverse root microbiomes, which could potentially serve as a rich reservoir of bioactive microorganisms. In this context, Actinobacteria, especially streptomycetes, are well known for their secondary metabolites, which have been harnessed for their antimicrobial activity against a wide range of plant and human pathogens. The aim of this study is to explore the rhizosphere of these historical trees in Greece, isolating and identifying streptomycetes capable of inhibiting both phytopathogenic bacteria and fungi, as well as human pathogens. Moreover, the study aimed to evaluate the potential of these streptomycetes for the development of eco-friendly biocontrol strategies for plant disease management, as well as novel antimicrobial agents for human pathogen control. Soil samples were collected from the rhizosphere of the selected trees, and Actinobacteria were isolated using selective culture methods. The antimicrobial activity of the isolates was screened using agar diffusion and broth microdilution assays indicated bioactivity against both phytopathogenic and human pathogen indicator strains. Our results demonstrated that several streptomycete isolates produced bioactive secondary metabolites, which effectively inhibited the growth of both phytopathogenic and human pathogenic bacteria and fungi. These findings underscore the significance of the rhizosphere of historical trees as a promising and untapped source of Actinobacteria with potential applications in sustainable agriculture. Further research on their secondary metabolites could lead to the development of natural, eco-friendly alternatives for plant disease management and human pathogen control.

Poster Session

P037

Leaves of *Alfalfa* and *Cilantro* plants from Mexican fields irrigated with different water qualities are colonized by antibiotic resistant *E. coli* and enterococci

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Irrigation water quality plays a key role for the spread of antimicrobial resistant potential pathogenic bacteria within agricultural systems. Especially wastewater derived potential pathogenic bacteria are able to colonize the internal plant tissues using (pathogenicity) mechanisms that are also responsible for the colonization of the human gut. In an intensive field sampling campaign performed by the PARES DFG research consortium in the Mezquital Valley in North of Mexico City, Mexico, we studied the occurrence of culturable ESBL producing and carbapenem resistant (CR) *Escherichia coli*, vancomycin resistant enterococci (VRE) and total enterococci in irrigation water of different quality as well as in leaves of Alfalfa and Cilantro plants which were irrigated with those water types. Leaves were washed with sterile buffer to remove loosely attached bacteria from the leaves surface, and to culture only those bacteria which were either strongly attached to the leave surface (biofilm forming bacteria) or colonized the internal plant tissue. A total of 105 potential ESBL *E. coli*, 59 potential CR *E. coli*, 137 potential VRE, and 177 enterococcal strains were cultured under selective and non-selective cultivation conditions from irrigation waters and leaves of the irrigated plants. Neither potential ESBL or CR *E. coli* nor VRE were cultured from spring and groundwater samples but all were cultured from treated and untreated wastewater used for irrigation and from Alfalfa and Cilantro leaves collected from all differently irrigated fields. An in-depths characterization of the isolates obtained, will provide further insights weather the type of irrigation water affected the occurrence of multidrug resistant strains and strains harboring virulence factors in leaves of plants irrigated with different water qualities

Poster Session

P038

Shifts in bacterial community and plasmid transfers in *Arabidopsis thaliana* rhizosphere induced by water deficit

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The rhizosphere, soil influenced by plant roots, is considered as a hotspot for bacterial activity and horizontal gene exchange between bacteria, particularly plasmid transfer. However, under climate change scenario, how adverse climatic events (e.g., drought) affect plasmids transfer within plant microbiomes is still poorly understood. In this study, we hypothesize that shifts in the bacteria community caused by water deficit in the rhizosphere limit the plasmid transfer between bacteria. We used *Pseudomonas putida* as donor of a conjugative *gfp*-tagged plasmid (pKJK5) and culture-independent techniques (amplicon sequencing and flow cytometry) to determine the rhizobacterial community, transconjugants and plasmid transfer efficiency in the rhizosphere of *Arabidopsis thalian*a under well-watered (500 mL every 24 h) and water deficit (500 mL every 72 h) treatments during 3 and 7 days. We observed shifts on the structure, richness and diversity of rhizobacterial community associated with *A. thaliana* under water deficit compared with controls, particularly after 7 days. Coincidently, the plasmid transfer efficiency was also affected by water deficit, decreasing the plasmid transfer rates (1.4×10-4) across the rhizosphere recipients and influencing the transconjugant fractions. Particularly, we observed a higher relative abundance (82%) of transconjugants belonging to gram-positive genus *Glutamicibacter* and *Bacillus* under water deficit treatment at 7 days. Thus, this study represents the first report of plasmid transfer from a gram-negative (donor) to gram-positive bacteria (recipients) "*in plant*" influenced by water deficit. Our study suggests that water deficit may significantly affect the conjugative plasmid transfer between bacteria in the rhizosphere with the concomitant implications on the composition and interactions of plant microbiomes, which is highly relevant for plant fitness.

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Poster Session

P039

Insights into Gram positive (*Rhodococcus* and *Microbacterium)* and Gram negative (*Variovorax* and *Delftia*) isolates in terms of plant-growth promoting, biocontrol, stress tolerance and genomic attributes

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The use of plant growth-promoting (PGP) bacteria as biostimulants is an attractive alternative in agriculture. In this context, the use of PGP bacteria consortia are commonly proposed, combining well-studied Gram-positive and Gram-negative isolates. However, members of less known taxa are being isolated by modern culture-based methods, and our knowledge on their PGP attributes as part of plant microbiome is still scarce. In this study, we chosen two Gram positive (*Rhodococcus* sp. S1 and *Microbacterium* sp. P77) and two Gram negative (*Variovorax* sp. 14.7 and *Delftia* sp. I31) plant-associated isolates, which were firstly characterized based on their phenotypic PGP, biocontrol and stress tolerance attributes. Then, whole genomes were sequences, gene annotated on basis to their PGP phenotypic attributes. A higher germination and plant growth promotion was achieved by *Variovorax* sp. 14.7 inoculation compared with other isolates. A higher biocontrol activity was also observed by *Variovorax* sp. 14.7 and *Delftia* sp. I31 against phytopathogens. All tested strains showed PGP attributes, such as phosphorus mobilizing, ACC deaminase activity and production of auxin, siderophore and exopolysaccharide. Both *Variovorax* sp. 14.7 and *Delftia* sp. I31 also exhibited high stress tolerance to salinity (5-15% NaCl) and acidity (pH 3-4.5). Whole genome sequencing revealed size of 11.3, 3.1, 7.2 and 6.6 Mbp for *Rhodococcus* sp. S1, *Microbacterium* sp. P77, *Variovorax* sp. 14.7 and *Delftia* sp. I31, respectively. Comparative analysis showed a core genome with 758 shared protein-coding genes. The isolate that contained a large number of unique genes was *Rhodococcus* sp. S1 with 658 unique protein-coding genes, followed by *Variovorax* sp. 14.7 with 190 unique protein-coding genes, whereas *Delftia* sp. I31 and *Microbacterium* sp. P77 showed 169 and 97 unique protein-coding genes, respectively. Coincidently with phenotypic, *Variovorax* sp. 14.7 and *Delftia* sp. I31 showed a higher number of genes involved in PGP attributes. The use of both phenotypic and genomic attributes represents a useful approach to evaluate the complementarity and specificity of PGP bacteria consortia for agriculture, particularly in those less known taxa.

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Poster Session

P040

Exploring the microbial diversity of the Dictean cave, Crete, Greece and evaluation of essential oils as a mild cleaning method

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Naturally occurring caves are sites of significant cultural value, while also displaying unique biodiversity of associated microbiomes which may provide an untapped source of potentially beneficial organisms. However, the touristic exploitation of show caves may ultimately result in the biodeterioration of speleothems, primarily through the introduction and establishment of alien microbiota or the uncontrolled growth of indigenous species, exacerbated by the use of artificial lighting. Such habitat characteristics are present in the Dictean cave, also known as "Diktaion Andron", a highly visited cave in eastern Crete, Greece, which was regarded in the ancient Greek mythology as one of the putative sites of the birth of Zeus. An efficient way of controlling these ecological niches without irreversibly disturbing microbial diversity is therefore necessary, and essential oils are currently being explored as a mild cleaning method. Herein, using 16S and 18S rRNA gene amplicon sequencing and methods for quantitative metabolic activity estimation, the microbial diversity of the Dictean cave was explored and the application of a formulation containing specific essential oils was evaluated as a mild cleaning method. The amplicon sequencing analyses revealed distinct profiles among the different sample sites, with species of the genera *Pseudomonas, Sporosarcina, Butiauxella, Glutamicibacter, Paenibacillus, Mortierella* and *Jenufa* being most abundant, while uncharacterized microorganisms were also detected. The single simultaneous application of 0.2 % (v/v) oregano and 0.4 % (v/v) cinnamon essential oils was effective in significantly reducing the microbial metabolic activity by up to 89.2% within 24 hours, without adversely affecting the coloration of speleothems.

Poster Session

P041

Neonicotinoids in agricultural soils – persistence, enzymatic activity, and microbial community disruptions

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Neonicotinoid (NNI) pesticides are widely used in modern agriculture due to their effectiveness in controlling insect pests. However, their extensive application raises concerns about their impact on non-target organisms, particularly soil microbiomes. These systemic insecticides persist in the environment, potentially disrupting microbial communities essential for soil health, nutrient cycling, and plant growth. Studies indicate that NNIs can alter microbial diversity, reduce beneficial bacteria, and impair key ecological functions. Understanding these impacts is crucial for developing sustainable agricultural practices that preserve microbial biodiversity and soil fertility. This study evaluates the effects of imidacloprid (IMI), thiamethoxam (THM), and clothianidin (CLO) on agricultural soil with high organic matter content. Soil microcosms were incubated under controlled laboratory conditions for 30 days. Samples were collected from an agricultural field with no prior NNI application to minimize background contamination. Treatments included Control (C), untreated soil; Low-Dose (LD), soil treated with a field-relevant concentration; and High-Dose (HD), soil treated with a concentration exceeding typical field applications to assess potential threshold effects. Pesticide removal was analyzed at the start and after 30 days. Removal rates in LD were 32%, 64%, and 24% for IMI, THM, and CLO, respectively, while in HD, removal was 24%, 73%, and 14%. IMI and CLO were less degradable in the studied soil. Enzymatic activity was also affected, with urease activity decreasing from 5.34 to 2.86 µmol NH_4^+ g^{-1} h^{-1} in IMI-HD, and a similar trend was observed for dehydrogenase activity. The pesticides reduced *nif*H gene abundance and increased *amo*A gene abundance, suggesting a significant impact on nitrogen-cycling microorganisms. These findings indicate that NNIs can alter microbial community structure and function, potentially reducing soil resilience and recovery after pesticide exposure. Studies like this are essential for assessing pesticide risks from a One Health perspective.

Poster Session

P042

Priming and toxic effects of biostimulants on microbial functional diversity of seeds

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A seed's microbiome is relevant as a reservoir of microorganisms that can provide special growth traits for the survival of plants. The microbiome of seeds is often considered as initial inoculum of plant host impacting the key plant growth stages, especially germination rate. Microbes associated with seeds are moved horizontally or vertically across plants, influencing the behaviour, metabolism and physiology of plants and can improve the adaptation of plants to climate change and (a)biotic stresses.
Therefore, one critical innovative goal for the practical use of seeds' microbiome is the recognition of its changes under treatment with biostimulants in order to develop tools for bioassay of biostimulant quality via evaluation of the response of seeds' microbial functional diversity to the treatments of selected biostimulants.
This step of the study aimed to develop an effective procedure for seed treatments or inoculation using biostimulants. The priming and inhibiting effects of biostimulants on seeds' microbiome functionality were assessed using Biolog Ecoplates. The results connected with growth intensity and metabolic stress were determined using intensively and slowly utilised substrates for microbial growth.

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Fig. 1

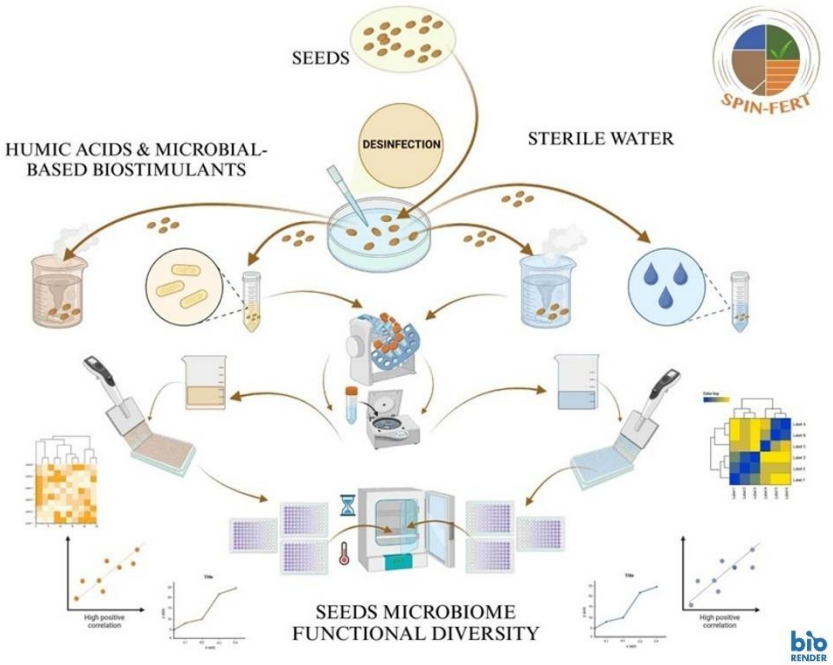
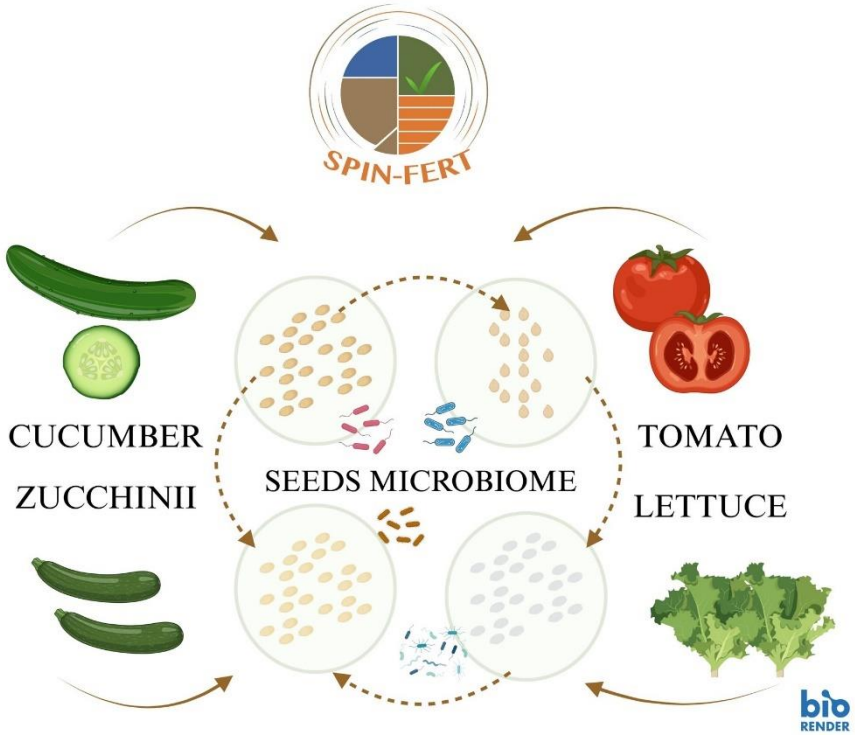

SEEDS
SPIN-FERT
DESINFECTION
HUMIC ACIDS & MICROBIAL-BASED BIOSTIMULANTS
STERILE WATER
SEEDS MICROBIOME FUNCTIONAL DIVERSITY
bio RENDER

Fig. 2


SPIN-FERT
CUCUMBER
ZUCCHINII
SEEDS MICROBIOME
TOMATO
LETTUCE
bio RENDER

Poster Session

P043

Impact of PAH Pollution on Environmental and Commensal Microbial Communities: Exploring Connections with Human Health

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Background and aim: Soil quality and microbial diversity are essential for well-functioning urban ecosystems and human health. Urban pollutants, such as polycyclic aromatic hydrocarbons (PAHs), can alter microbial communities and interfere with endocrine and immune signaling. Our aim was to estimate the connections between biodiversity, PAH pollution, and environmental and commensal microbial communities associated with health.

Methods: First, we estimated PAH induced bacterial shifts in several urban landscaping materials, and whether environmental exposure to PAHs affects commensal bacterial communities on the skin and in the gut. The study participants included elderly people living in urban and rural environments, and daycare children living in urban environment. Secondly, we measured the microbial communities and PAH concentrations from doormats placed indoors in the homes of children with type 1 diabetes and those in the control group. Results from the pollution studies were combined with publicly available satellite data.

Results: In the PAH experiment, the magnitude of shifts in bacterial communities, including shifts in Gammaproteobacteria, depended on landscaping material. High playground soil PAH contamination was associated with proteobacterial communities on the children's skin, whereas high coverage of forests was associated with decreased PAH levels in ambient air. Interestingly, PAH concentrations were higher and microbial taxonomies differed in the doormats of children with type 1 diabetes compared to the control group.

Conclusions: The PAH pollution studies indicate that PAH concentrations considered safe may still induce shifts in environmental and commensal bacterial communities linked to human health and type 1 diabetes. Our studies also demonstrate that it is possible to design landscaping materials that are more resilient to bacterial shifts induced by pollution. Urban biodiversity appears to be an important determining factor in the context of pollution-induced disturbances within commensal microbiota.

Keywords: Biodiversity, Microbiota, PAH pollution, Environmental health, Type 1 diabetes

Poster Session

P045

Early Changes in Core Rhizosphere Microbiota and Their Functional Variations Under Environmental Stress Potentially Determine Final Plant Health

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Many studies have highlighted the essential role of the core rhizosphere microbiome in mediating plant health, particularly under abiotic (e.g., herbicide pollution) and biotic (e.g., soilborne pathogens) stresses. However, most of these studies have focused on links between the final microbial profile and plant health, without fully understanding how changes in core rhizosphere microbiome and their functions over time might influence the plant"s condition. In addition, most studies focus on a single stress, overlooking the fact that abiotic and biotic stressors often occur together in complex soil environments. As a result, their combined effects on plant–microbiome interactions are still poorly understood.

To address these gaps, we conducted a time-series pot experiment to investigate how core microbiota and their functions respond to herbicide exposure during the soybean seedling stage, particularly their potential role in soilborne disease (*Fusarium oxysporum*) onset. Amplicon sequencing was first applied to explore core taxa and their potential links with soybean disease outcome, followed by metagenomic analysis of their functional roles using KEGG-based annotations and taxonomic origin tracing. Core taxa were identified through microbial network analysis, and their early-stage (before 7d-planting) microbial (*Streptomyces*) and functional responses (Biosynthesis of multiple antibiotics and siderophores) to herbicide treatments were observed to be highly associated with disease risk.

This study underlined the ecological significance of early core microbiota in modulating plant-pathogen interactions under environmental stress. Furthermore, we specifically defined the critical time when changes of key rhizosphere microbial taxa can influence disease development and plant health outcomes. During this period, particular attention should be given to herbicide residues and other agrochemical pollutants, as they may negatively impact the abundance and disease-suppressive functions of core microbiota. At the same time, this stage represents a critical period for regulating core microbiota to enhance crop resilience and manage soilborne diseases more effectively.

Poster Session

P046

Harnessing PGPR and AMF to Enhance Stress Resilience and Productivity in Durum Wheat Cultivation

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Durum wheat (*Triticum turgidum* L. var. *durum*) is a globally significant staple crop, especially in Mediterranean and semi-arid regions, where climate unpredictability and recurrent drought threaten productivity. Sustainable strategies such as inoculation with plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) offer promising solutions to mitigate drought stress, disease severity and to enhance nutrient uptake and productivity.

This study evaluated the effects of inoculating two durum wheat varieties, 'Svevo' and 'Puro', with selected PGPR and AMF strains, individually and in consortium, under semi-controlled greenhouse and open field environments. The 'Svevo' variety was monitored under both normal irrigated and drought-induced conditions using a Plant Array (PA) phenotyping platform, which allowed real-time, high-resolution monitoring of key physiological traits, including daily transpiration and water-use efficiency. Following the phenotyping phase, plants were grown until harvest maturity, after which biomass accumulation and yield components, including thousand kernel weight (TKW), were evaluated. The 'Puro' variety was assessed under field conditions in terms of TWK and *Fusarium* sp. mycotoxins (i.e., deoxynivalenol (DON)) production.

For 'Svevo', all treatments except PGPR improved daily transpiration, water-use efficiency, and overall biomass production. Notably, both treatments with AMF and PGPR alone resulted in the highest TKW production, indicating effective energy allocation toward productivity, under drought conditions, especially for bacterial inoculated wheat plants. In the open field trial with 'Puro', AMF alone showed superior TKW performance, whereas the PGPR-AMF consortium significantly reduced DON levels, contributing to the plant pathogen control, likely through enhanced root colonization and competitive exclusion mechanisms that reduce the prevalence of soilborne diseases.

These findings highlighted the distinct advantages of PGPR in a more stable and controlled experimental environment and the resilience of AMF under heterogeneous and fluctuating field conditions. Overall, beneficial microorganisms such as AMF and PGPR demonstrated substantial potential for improving durum wheat resilience and productivity under drought conditions, promoting sustainable agricultural practices.

Key words: drought stress, bioinoculants, Plant Array phenotyping, thousand kernel weight (TKW), biological control, sustainable agriculture

This study was conducted within the framework of the ROOTEM project (Understanding the Role of Root Exudation in Drought Stress Response of Wheat and Its Associated Microbiota) – PRIN, 2022.

Poster Session

P047

Community-level dynamics of spore-forming and other ethanol resistant taxa in the human gut

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The successful colonization of commensal bacteria in the human gut depends on their capacity to spread between hosts and persist within a host. In theory, survival strategies such as sporulation enhance both transmission between individuals and long-term prevalence within a host. More than 50% of bacteria in the human intestine possess genes enabling sporulation. We analyzed the ecological dynamics of spore-forming bacteria within and between individuals.

Our study involved nine healthy male participants, whose stool samples were collected every 14 days over 6 months. DNA was isolated using two protocols: a "bulk microbiota" and an "ethanol and ethidium monoazide (EMA) treatment" protocol. Ethanol shock and EMA treatment selectively removed DNA from ethanol non-resistant cells. By analyzing the By analyzing the presence and abundance of OTUs in ethanol-treated samples, we were able to distinguish between ethanol-resistant and ethanol non-resistant microbial fractions in the bulk microbiota. OTUs classified as Bacillota, which include known intestinal spore forming species were separated from other phyla in which survival strategies are poorly understood for the purpose of discussing biological relevance.

Microbiota composition was more similar within individuals over time than between unrelated and non-cohabiting individuals. When examining different microbial fractions, ethanol resistant Bacillota exhibited lower community variability than ethanol non-resistant Bacillota, both within and between individuals. A similar but weaker trend was observed for other ethanol resistant taxa compared to other non-ethanol resistant taxa. While ethanol-resistant Bacillota showed the least variability within individuals, variability increased over longer time intervals (six months). In contrast, ethanol non-resistant Bacillota and other taxa displayed consistently high variability, regardless of the sampling interval (two weeks or six months).

Our findings suggest that ethanol-resistant Bacillota are more likely to be transmitted between individuals in a population. Within individuals, the acquisition of new OTUs appears to follow non-random processes, in contrast to the more random patterns observed in ethanol non-resistant and other ethanol-resistant fractions.

Poster Session

P048

Gut microbial modulation of acute stress reactivity in healthy adults

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Acute stress describes the deviation from homeostasis caused by an immediate threat or challenge. Stress engages the hypothalamic-pituitary-adrenal axis triggering cortisol release, increasing stress reactivity and aiding recovery in the aftermath of stress. Stress appears modulated by the gut microbiome. Previous work showed exaggerated stress reactivity in mice without a conventional gut microbiome. In humans, depressed patients exhibit altered gut microbiome composition, and probiotics improve stress-related cognition. Whether gut microbes modulate the acute stress response in healthy adults is unclear. We hypothesized that an individual"s reactivity to, and recovery after acute stress would be associated with their gut microbial composition. Also, microbes produce metabolites such as short-chain fatty acids (SCFAs) that are known to alleviate the detrimental effects of stress. We thus expected that a higher microbial capacity to produce SCFAs would be tied to lower stress reactivity and faster recovery.

To test this, 78 healthy human participants (aged 18-35 years, 48 females) underwent an acute stress induction or a non-stressful control condition (n= 39 per group). Stress reactivity (rise in cortisol) and recovery (return to baseline) were assessed by repeated sampling of salivary cortisol, paralleled by subjective stress ratings. Stool samples (collected at baseline) were used to characterize gut microbiome profiles with 16S rRNA amplicon sequencing. The stress intervention significantly increased cortisol (stress: 1.336 ± 0.088 log(nmol/L), control: 1.084 ± 0.068 log(nmol/L), p = 0.026) and perceived stress in the stress group (p = 0.002). Females showed significantly higher stress reactivity than males (robust linear model: pseudo-R^2= 0.126/r=0.290; p < 0.001), while males exhibited faster stress recovery (pseudo-R^2= 0.256/r=-0.127*; p = 0.005).* Gut microbial alpha diversity (Shannon index, pseudo-R^2= 0.126/r=-0.110, p = 0.012) and richness (observed ASVs, pseudo-R^2=0.153/r=-0.141*; p* = 0.044) were significantly associated with lower stress reactivity, but not stress recovery (Shannon, p = 0.239; richness, p = 0.065). These findings are the first to highlight that more diverse gut microbial profiles are linked to lower stress reactivity in healthy adults. Ongoing data analysis (PICRUSt2 & metagenomics) focuses on individual microbial SCFA production capacity and its association with acute stress, and results will be presented at the meeting.

Poster Session

P049

Immunomodulatory activity of membrane vesicles derived from an aerobically cultured lactic acid bacterium

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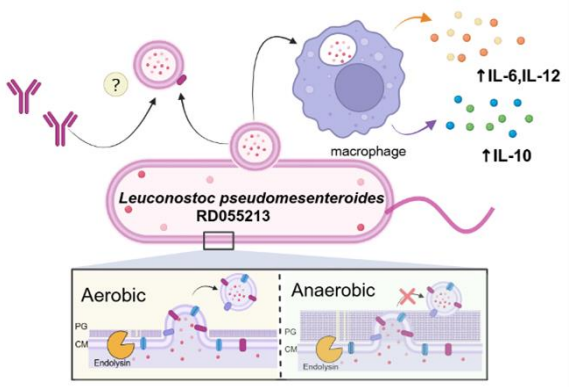
Bacterial membrane vesicles (MVs) offer significant potential for medical applications, including vaccines and drug delivery. However, MVs derived from Gram-negative bacteria, contain lipopolysaccharides (LPS) , which can trigger inflammation. A viable alternative may be the development of MVs from probiotic lactic acid bacteria, providing a safer and more effective platform for medical applications. Here, we found that *Leuconostoc pseudomesenteroides* RD055213, a lactic acid bacterium, produces abundant MVs under aerobic conditions. We characterized their structures and uncovered their potent immunomodulatory properties (Fig. 1).

High MV-producing strains were screened from 86 lactic acid bacterial strain under aerobic, microaerobic, and anaerobic conditions. While most lactic acid bacteria exhibited high MVs production under anaerobic conditions, *Leuconostoc pseudomesenteroides* RD055213 was the only strain that showed remarkably high MVs production under aerobic conditions. Transmission electron microscopy (TEM) analysis revealed that RD055213 formed a thinner cell wall under aerobic conditions. When peptidoglycan, was labeled with NADA-green, synthesis was localized to the specific areas of bacterial surface under aerobic conditions, in contrast to the uniform distribution observed under anaerobic conditions. These results suggest that thinner cell wall under aerobic condition promotes production in this lactic acid bacterium. Furthermore, MVs derived from aerobic cultures induced the secretion of the pro-inflammatory cytokines IL-6 ,IL-12, and IL-1β, as well as the anti-inflammatory cytokine IL-10, in the macrophage-like cell line J774. Highlighting that MVs derived from RD055213 exhibit immunomodulatory properties. Ongoing, whole-genome analysis and the identification of MV-associated proteins will provide a comprehensive understanding of the molecular basis governing MV biogenesis in RD055213.
Fig. 1 Graphical abstract of this study.

Fig. 1



Poster Session

P050

Metagenomic analysis of the spectacle microbiota – preliminary results from a comparative study of clinical and non-clinical environments

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QUESTION: Spectacles accumulate various microorganisms, which may lead to eye infections and other health issues. Previous cultivation and molecular studies conducted by our group have largely focused on the structure (species inventory) of the bacterial spectacle microbiota [1,2]. However, non-bacterial taxa as well as the genetic potential of the spectacle microbiota remained largely understudied. To close this gap and elucidate whether particularly spectacles from clinical environments might serve as fomites, we used shotgun metagenomics on two sample pools.

METHODS: We collected 25 spectacle samples from a university and a clinical environment, respectively. Total DNA was extracted using the ZymoBIOMICS DNA Mini Kit and quantified with a Qubit 2.0 Fluorometer. After quality control, 20 clinical and 18 university samples were sequenced on an Illumina MiSeq platform. Reads were trimmed and filtered using Fastp (v0.23.4), and taxonomic classification was performed with Kraken 2 (v2.1.3-1) to identify phyla and estimate relative abundances.

RESULTS: In total, 63,534,070 high quality DNA sequences were obtained, ranging from 124 to 196 per sample. In both environments, Actinobacteriota was by far the most dominant phylum, with a relative abundance of approximately 80 %, nicely aligning with our previous, PCR-based research findings [2]. Bacillota was the second most prevalent phylum, with a slightly higher proportion in university samples (14.4%) compared to clinical samples (13.6 %). Pseudomonadota ranked third, with a marginally greater presence in clinical samples (5.0 %) than in university samples (4.2 %). Bacteroidetes and Fusobacteria were detected in both settings at low relative abundances (< 1 %). In addition, some unclassified viral sequences were detected in both environments, at very low relative abundances (< 0.1 %).

CONCLUSIONS: The taxonomic community composition appears very similar in both environments, which was expected. Functional analysis, which is ongoing, may reveal variations, such as differences in the relative abundance of antibiotic resistance genes, which is of great importance to evaluate the hygienic relevance of spectacles and indicate whether they might contribute to the spread of resistance genes.

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[2] Fritz, B. et al. (2020). Sci Rep 10, 5577

Poster Session

P051

Towards quantitative trait analysis in complex microbial communities

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Recent studies suggest the importance of the microbiome genetic variation for the host health status. Nevertheless, most of the studies were done based on the reference databases and deposited isolated genomes. From the genomic point of view, microorganisms are characterized by the high level of intra-species heterogeneity coupled with frequent horizontal gene transfer events which limits the resolution and lead to reference bias. To overcome this limitation, a pangenomic approach consisting in aggregation of the entire set of genes within a species was proposed. However, most existing methods for pangenome construction from metagenomes focus on protein-coding sequences which limits the search for potential regulatory variants in non-coding sequences.

Here we propose a novel framework for variant calling and gene expression analysis on metagenome assembled genomes (MAGs) which supplements the reference with the sample-specific genetic information or could work in reference-free mode. The tool consists of four principal modules. The first module provides preprocessing and quality checking of MAGs based on estimation of completeness, contamination, and heterogeneity with consecutive taxonomic classification. The second module combines quality-filtered MAGs of the same lineage with representative genomes as an optional step to generate nucleotide-resolved sequence-graph based pangenomes of studied species. Pangenomes further are used to generate a cohort-specific reference by using the longest supported path through the pangenome graph. The third and fourth modules execute variant calling and gene expression counting on the cohort-specific reference. The tool provides a basis for the genome-wide association and quantitative trait loci (QTL) analysis taking into account sample-specific variance.

Poster Session

P052

Amplicon Sequencing Reveals JAK Inhibitor-Driven Changes in the Skin Mycobiome of Autoimmune Patients.”

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Janus kinase (JAK) inhibitors are widely utilized for the treatment of autoimmune skin diseases, including atopic dermatitis, vitiligo, and alopecia areata, by modulating inflammatory pathways and alleviating clinical symptoms. However, recent studies have reported dermatological adverse effects such as acneiform eruptions and folliculitis, which may vary depending on the specific JAK inhibitor and individual skin conditions. Although some clinical case studies have examined JAK inhibitor-associated skin reactions, research on the skin microbiome and its role in skin homeostasis remains limited. This study aims to comprehensively assess the impact of JAK inhibitors on the skin microbiome by analyzing both bacterial and fungal communities using a multi-omics approach. Skin samples were collected from patients receiving JAK inhibitor therapy, and microbial DNA was extracted for community profiling. Amplicon sequencing of the 16S rRNA and internal transcribed spacer (ITS) regions was conducted to characterize taxonomic shifts, while metagenomic shotgun sequencing enabled deeper resolution of microbial composition and functional potential. Bioinformatics analyses included quality control, taxonomic classification, functional annotation, and comparative assessments of microbial diversity and relative abundance. Comparative analysis of pre- and post-treatment microbiome profiles revealed significant shifts in both bacterial and fungal populations following JAK inhibitor administration. These findings underscore the broader impact of JAK inhibitors on the skin microbiome beyond bacterial communities, highlighting the importance of bacterial-fungal interactions in maintaining cutaneous health. A more comprehensive understanding of JAK inhibitor-induced microbiome alterations could facilitate the development of microbiome-targeted interventions and personalized treatment strategies to mitigate adverse effects in patients undergoing JAK inhibitor therapy.

Poster Session

P053

Comparative analyses of genomes obtained from long- and short-read metagenomic DNA sequencing of the dorsal tongue microbiome, with implications for genome recoverability from short-read data

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Question: Long-read sequencing methods are increasingly being used for the recovery of metagenome assembled genomes (MAG), with improvements in completeness and contiguity compared to genomes obtained from short-read data. These developments also permit further insights into the limitations of short-read derived MAG sequences, by undertaking comparative analyses of cognate MAGs obtained from each sequencing modality.

Methods: We studied three representative samples of the dorsal tongue microbiome, sequenced with both short-read (SR) and long-read (LR) sequencing. A total of 147 MAGs were recovered from the LR data, of which 47 were confirmed high quality (HQ) under MIMAG, and 224 from the short-read data (14 confirmed HQ). We adapt our previously developed methodology (Arumugam et al, 2019; 2021) to identify fractionation artefacts in short-read MAG data, where multiple low- or medium-quality genomes appear to arise from the same population genome or pan-genome, but appear as distinct genomes under current metrics.

Results: We develop a procedure for identifying and correcting such artefacts, estimating that around 20% of microbial populations recoverable at the MAG level are affected. Additionally, when applied to analysing conspecific short read MAG sequences from the background cohort (n=473 samples), we find evidence for a high degree of strain-level selection at the individual host level.

Conclusions: This analysis highlights 1) the advantages of joint analysis of MAG sequences obtained from both modalities; 2) cautions against over-reliance on extant genome quality metrics 3) indicates that MAG recovery still remains a dualistic task, with complementary roles for both algorithmic and data analytic approaches.

References:

Arumugam K, Bagci C, Bessarab I, Beier S, Buchfink B, Gorska A, Qiu GL, Huson DH, Williams RBH (2019). Annotated bacterial chromosomes from frameshift-corrected long read metagenomic data, *Microbiome* **7**: 61.

Arumugam K, Bessarab I, Haryono MAS, Liu XH, Zuniga-Montanez RE, Roy S, Qiu GL, Drautz-Moses DI, Law YY, Wuertz S, Lauro FM, Huson DH, Williams RBH (2021). Recovery of complete genomes and non-chromosomal replicons from activated sludge enrichment microbial communities using Nanopore long read metagenome sequencing, npj Biofilms and Microbiomes 7(1), 23.

Poster Session

P054

Dynamics of the airway microbiome in response to exposure to particulate matter 2.5 in patients with chronic obstructive pulmonary disease

B. Y. Choi[1], S. H. Heo[2], J. Kang[3], J. Y. Jung[4], H. C. Kim[5], S. J. Lee[6], W. J. Sul[1], S. W. Lee[2]

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Background: Particulate matter (PM) and air pollution have been suggested to be associated with chronic obstructive pulmonary disease (COPD), contributing significantly to global respiratory disease-related mortality. This study aimed to investigate whether seasonal exposure to PM influences dysbiosis in the respiratory microbiota of patients with COPD. Methods: Sputum samples were collected four times over 1 year from 102 patients with COPD, and 16S rRNA sequencing was performed. The dynamics of the airway microbiota were analyzed depending on PM exposure levels and season. Results: The PM-low exposure group had higher α-diversity compared to the PM-high exposure group, particularly noted in spring. Some bacterial groups, including seven species such as Treponema socranskii, were more abundant in the low exposure group. Additionally, the bacterial community structure in summer significantly differed from that in other seasons, with significantly increased α-diversity in this season. The difference in the airway microbiome due to PM exposure was prominent in patients with moderate COPD. Conclusions: PM exposure may influence changes in the sputum microbiome depending on exposure levels and seasonal variations. Our results suggest that airway microbiomes could vary with PM exposure according to seasonal trends.

Fig. 1. The seasonal structure of microbial communities. (A) Seasonal α-diversity of the sputum microbiome. The Shannon index in summer had a significantly higher value than in any other season. Chao1 and phylogenetic diversity in summer were significantly higher than in spring. (B) Principal coordinate analysis based on the unweighted UniFrac metric showed significant dissimilarity between summer and other seasons.

Fig. 2. Bacterial diversity according to the severity of chronic obstructive pulmonary disease (COPD).

Fig. 1

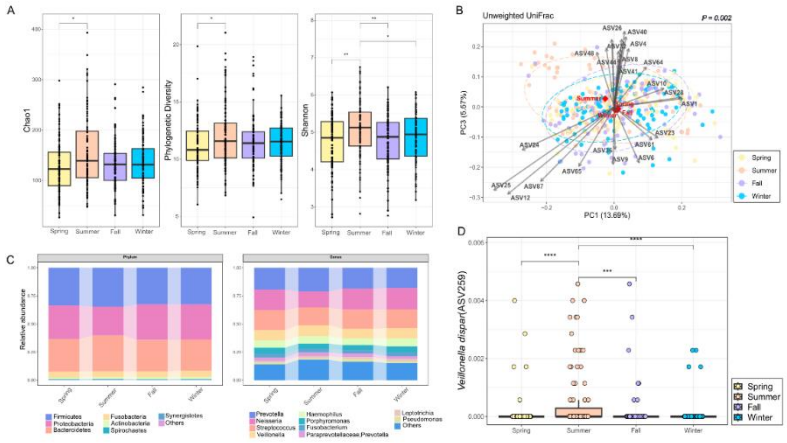
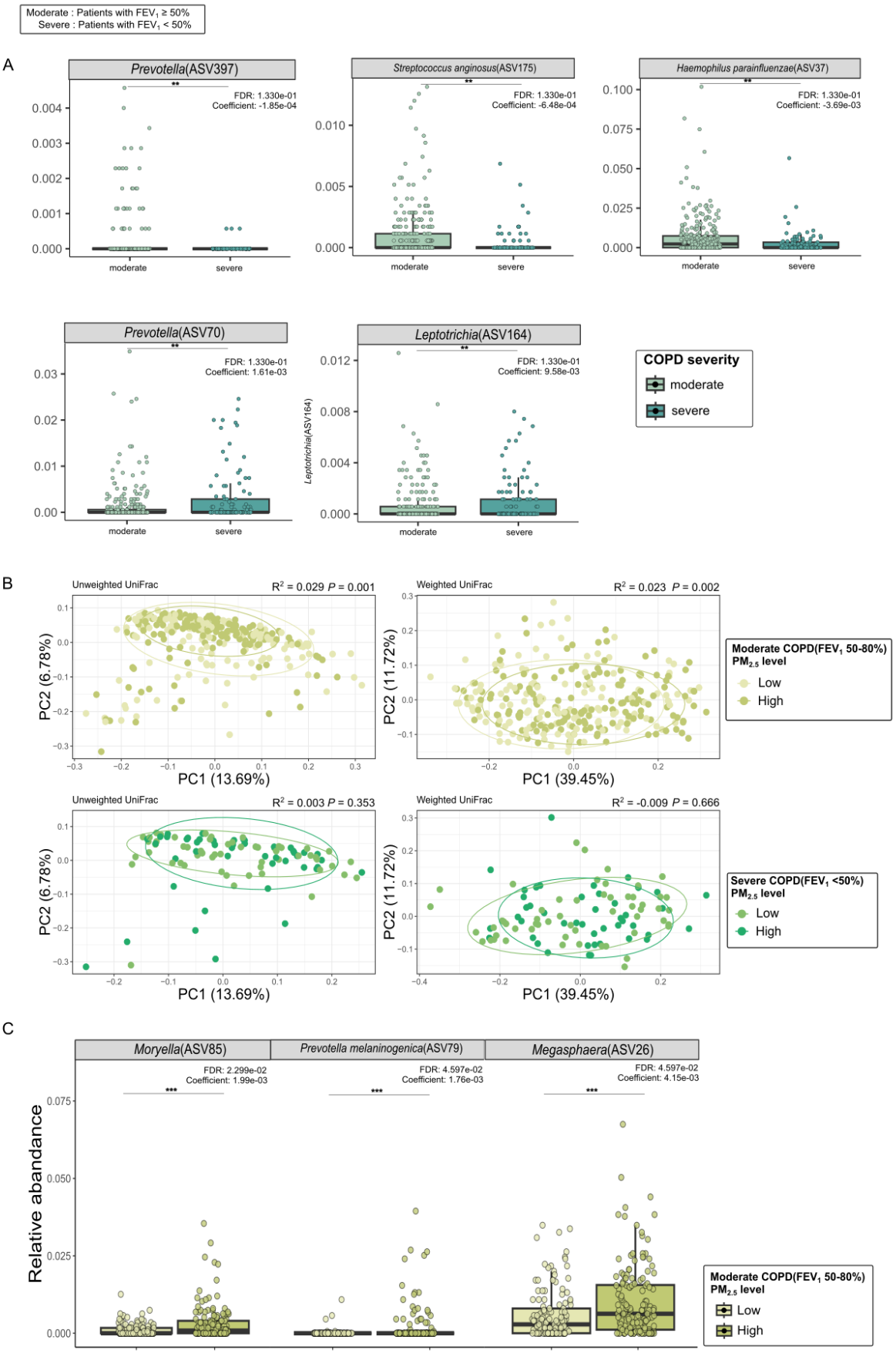

A
Chao1
Phylogenetic Diversity
Shannon
Spring
Summer
Fall
Winter
B
Unweighted UniFrac
P = 0.002
PC1 (13.69%)
PC3 (6.97%)
C
Relative abundance
Firmicutes
Proteobacteria
Bacteroidetes
Fusobacteria
Actinobacteria
Spirochaetes
Synergistetes
Others
Prevotella
Neisseria
Streptococcus
Veillonella
Haemophilus
Porphyromonas
Fusobacterium
Paraprevotellaceae;Prevotella
Leptotrichia
Pseudomonas
D
Veillonella dispar(ASV259)

Fig. 2


Moderate : Patients with FEV1 ≥ 50%
Severe : Patients with FEV1 < 50%
A
Prevotella(ASV397)
FDR: 1.330e-01
Coefficient: -1.85e-04
Streptococcus anginosus(ASV175)
FDR: 1.330e-01
Coefficient: -6.48e-04
Haemophilus parainfluenzae(ASV37)
FDR: 1.330e-01
Coefficient: -3.69e-03
Prevotella(ASV70)
FDR: 1.330e-01
Coefficient: 1.61e-03
Leptotrichia(ASV164)
FDR: 1.330e-01
Coefficient: 9.58e-03
moderate
severe
COPD severity
moderate
severe
B
Unweighted UniFrac
R2 = 0.029 P = 0.001
Weighted UniFrac
R2 = 0.023 P = 0.002
Unweighted UniFrac
R2 = 0.003 P = 0.353
Weighted UniFrac
R2 = -0.009 P = 0.666
PC1 (13.69%)
PC2 (6.78%)
PC1 (39.45%)
PC2 (11.72%)
Moderate COPD(FEV1 50-80%)
PM2.5 level
Low
High
Severe COPD(FEV1 <50%)
PM2.5 level
Low
High
C
Moryella(ASV85)
Prevotella melaninogenica(ASV79)
Megasphaera(ASV26)
FDR: 2.299e-02
Coefficient: 1.99e-03
FDR: 4.597e-02
Coefficient: 1.76e-03
FDR: 4.597e-02
Coefficient: 4.15e-03
Relative abandance
Moderate COPD(FEV1 50-80%)
PM2.5 level
Low
High

Poster Session

P055

Mechanisms of FMT-induced remission at the IBD mucosa

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[2]MU Graz - Dvision of Gastroenterology and Hepatology, Department of Internal Medicine, Graz, Austria

Fecal microbiota transplantation (FMT) is a powerful clinical platform for studying the interactions between the gut microbiome and the human host and their clinical indications, in our case Inflammatory Bowel Disease (IBD). Our longitudinal FMT cohorts, that include end stage patients with refractory chronic active ulcerative colitis (UC), receive repeated transplantations that provide an extensive sample library that include fecal material, biopsies and histology. Although the high potential of treatment, we lack understanding that predicts susceptibility of patient-donor pairs. Recently, the perceived role of donor microbiota composition has been downplayed, based on observed engraftment and lack of signals at endpoint studies. Nonetheless, taxonomic abundance data, MICOM-modelling and functional analyses have provided us with keystone microbes and functions. Mucosal biopsy amplicon sequencing indicated importance of *Akkermansia* in remission and hallmarks of the tumor-related IBD-microbiome, such as *Fusobacterium*. In general, strongest signals associated with FMT efficacy occur after primary transplantation. Our running hypothesis is that additional triggers at the mucosal interface surround this response to the primary transplantation. These mucosal triggers could be induced by beneficial microbes and their metabolites, which are required to reach an alternative immunological stable state and, in the meantime, blocked by pathobionts in other patients.

We have identified several mediators involved in these triggers. In our IBD&FMT `transcriptomeverse" (organoid and biopsy-based) we highlight overlooked response groups of genes that are associated with healing and remission. The relation of specific cytokines to the regulation of innate immunity, increase of eosinophils and differential regulation of cell death and apoptosis during early remission, are our key observations. This reprogramming of mucosal immunity has been confirmed by a unique characterization of UC by spatial transcriptomics. Finally, we employ *in vitro* investigations studying bacterial mediators for susceptibility to remission. For *Akkermansia* and *Fusobacterium* we have isolated outer-membrane vesicles (OMVs) and studied their transcriptional effect on IBD-organoids. Our wide understanding and approach, microbiome-related, pathological and *in vitro*, has indicated key processes at the host interface with which bacteria originating in FMT or the inflamed tissue may be involved.

Poster Session

P056

Dynamics of *Akkermansia* species in treatment of inflammatory bowel diseases by fecal microbiota transplantation

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Fecal microbiota transplantation (FMT) is a promising therapy for inflammatory bowel diseases (IBD), yet its mechanism of action remains unclear (1, 2). While *Akkermansia muciniphila* is emerging as a next-generation probiotic (3) and a biomarker of FMT for IBD success (1), recently discovered *Akkermansia* species have unknown roles in IBD (4, 5). This study investigated the prevalence and potential associations of novel *Akkermansia* species with FMT response in IBD patients. Longitudinal fecal samples of IBD patients (P) receiving FMT from donors (D) were analysed using 16S rRNA gene amplicon (P:44, D:24) and metagenomic shotgun sequencing (P:24, D:19).

Amplicon sequencing detected *A. muciniphila* in 45% of donors and 38% of patients, while species *A. biwaensis* and *A. massiliensis* were less prevalent: ≤ 13% of donors and ≤ 6% of patients. Metagenomics identified more *Akkermansia* species, *A. muciniphila* subgroups, and uncultivated strains (54-91% prevalences). Multiple *Akkermansia* spp. were observed in most donors, while that was the case only for 50% of patients pre-FMT, increasing to 75% of patients post-FMT. Non-responders post-FMT had significant differences in the relative abundance of *A. massiliensis* and uncultivated *Akkermansia* compared to donors (adjusted $p < 0.05$), while remission was linked to lower accumulation of other Verrucomicrobiota, suggesting a displacement by *Akkermansia* species.

These findings emphasize the complex dynamics of *Akkermansia* post-FMT and suggest that species other than *A. muciniphila* may be critical for treatment response. We show that 16S sequencing underestimates the prevalence of *Akkermansia* species compared to metagenomic sequencing and that donors and patients often harbor more than one *Akkermansia* species. Further work will focus on clarifying their functional roles, and to assess their potential as biomarkers and therapeutics.

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Poster Session

P057

Polyphasic analysis of the effect of roseoflavin, a structural riboflavin analogue, on the human skin microbiota

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In soil, *Streptomyces davaonensis* and few related species produce roseoflavin (RoF), a riboflavin (RF) analogue, to inhibit the growth of competitors [1; 2]. We hypothesized that RoF could serve as a microbiome modulator against potential skin pathogens, considering that most bacteria on human skin are gram-positive, which often depend on exogenic RF [3].

In the cultivation-based arm of our study, more than 250 isolates were obtained from the skin of 20 healthy human volunteers and identified via MALDI-TOF-MS. Securely identified species were tested for susceptibility to RoF in a RF-free medium supplemented with RF and/or RoF, respectively. For cultivation-independent studies, we obtained skin swabs from the forehead, elbow, and forearm of 15 healthy volunteers, which were pooled per site and incubated with RoF (100 and 500 µM) for 24 hours in the RF-free medium. Incubations with water and DSMO served as controls. After DNA and RNA extraction, 16S rRNA (cDNA) and 16S rRNA gene sequencing was used to investigate shifts in the community composition of the "present" (DNA-based) or "active" (cDNA-based) skin microbiota.

In the cultivation-based experiments, 21 gram-positive and 4 gram-negative species were tested. Out of those, 11 species (8 gram-positives, 3 gram-negatives) showed growth inhibition by RoF. 16S rRNA (gene) sequencing revealed significantly lower alpha diversity in all samples after 24 hours of incubation, albeit also for the control samples. Statistical analyses are still ongoing, to discriminate the effects of incubation time and applied substances/actives (water, DMSO, RoF) for each sampling site in detail.

The cultivation-based experiments showed inhibition effects of RoF largely for gram-positive species, thereby matching our previous results obtained for bacterial isolates from the human oral cavity [4]. Cultivation-independent data also revealed a shift in relative abundance of gram-positive and gram-negative taxa during the incubation phase, which appears to be largely caused by the incubation conditions and less by RoF. However, statistical analyses are still ongoing.

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[3] Gutiérrez-Preciado et al. (2015), PloS one, 10(5), e0126124.

[4] Schwendenmann et al. (2025). Folia Microbiol., in revision

Poster Session

P058

Regulation of *Methanobrevibacter smithii* cell surface genes in response to bacterial interactions in the gut microbiome

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Introduction

Methanobrevibacter smithii is a dominant archaeal species in the human gut microbiome, playing pivotal roles in maintaining gut homeostasis through hydrogen consumption and methane production. While these methanogens are essential for syntrophic relationships with fermentative bacteria, their impact on host health remains underexplored. This study investigates how *Methanobrevibacter smithii* adapts to bacterial co-cultures and how its cell surface modifications potentially influence both microbial interactions and host colonization.

Methods

We co-cultured *Methanobrevibacter smithii* with two key gut bacterial partners, *Christensenella minuta* and *Bacteroides thetaiotaomicron*. Following co-incubation until the stationary phase, total RNA was extracted, and transcriptomic sequencing (RNA-Seq) was performed. Differential gene expression analysis was conducted using R, focusing on identifying changes in cell surface-associated genes in response to bacterial interactions.

Results

Using transcriptomic analyses from *Methanobrevibacter smithii* grown in co-culture with *Christensenella minuta*, we revealed shifts in gene expression related to cell surface proteins. Out of 1,684 genes, we identified 334 upregulated and 352 downregulated genes. Among these, 9% (152 genes) were linked to cell surface functions, including 86 upregulated and 66 downregulated genes linked to cell surface functions such as membrane remodeling, envelope biogenesis, signal transduction, vesicular transport, secretion, and structural adaptations.

Conclusion

By examining these transcriptional changes, this study aims to elucidate how *Methanobrevibacter smithii* modifies its cell surface properties in response to bacterial interactions. These findings will contribute to the broader understanding of methanogen ecology in the gut microbiome and may help inform future research on human gut microbial dynamics and therapeutic applications.

Poster Session

P059

Reducing plastic, improving gut health? Challenges of microplastic fasting

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In this pilot trial, we investigated the presence of microplastics in the gut of healthy individuals and examined the effects of reducing plastic exposure in daily life. Sixty volunteers participated in the study by providing two stool samples: one before and one after a week of actively reducing plastic intake. Participants received plastic-free alternatives like glass bottles, plastic-free dental care products and wooden cooking utensils. Additionally, they were instructed to refrain from purchasing plastic-packaged products. Participants recorded their dietary habits and plastic exposure in detailed questionnaires, to follow their lifestyle changes and possible plastic sources.

Of the initial cohort, fifty-five volunteers successfully completed the study, providing both stool samples, which were frozen for future microplastic and microbiome analysis. Participants rated their success in following the intervention on average with a success rate of 76%. Avoiding plastic proved to be challenging, particularly regarding packaged foods: 40% of participants successfully avoided plastic packaging entirely, while 56% managed partial avoidance. Notable difficulties included limited plastic-free options for dairy and meat products, as well as challenges associated with dining out.

Despite these difficulties, the trial significantly raised awareness about personal plastic consumption. The majority (92.7%) of participants reported a lasting commitment to reducing plastic use beyond the study period. Many participants expressed an intention to incorporate more fresh products into their diets, avoid fast food, and replace plastic cooking tools with sustainable alternatives, including cutting boards and cooking spoons, following a more environmentally conscious approach. The strong engagement suggests short-term interventions can foster long-term behavioural changes.

Initial results of the microbiome analysis show that the microbial composition has not changed significantly because of the reduction in plastic intake. Neither alpha nor beta diversity have changed significantly. As plastic is part of our gut from birth, the question remains whether an extended fasting period could help to combat possible long-term effects. Future research will focus on analysing the collected faecal samples to quantify the effect of microplastic fasting on the abundance of microplastic particles to further explore the relationship between plastic exposure and gut health.

Poster Session

P060

Plastic in the Gut – investigating microplastic impacts on microbial health

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Microplastic particles (MPs), defined as plastic fragments smaller than 5mm, are widespread pollutants accumulating in ecosystems and the human food web. While research has explored their ecological consequences, their potential impact on the human gut microbiome remains largely unknown. This study used ex-vivo bioreactor experiments to assess the effects of different plastic types and particles on the gut microbiome. Bioreactors inoculated with healthy donor stool were maintained for five days with daily feeding. The cultures were exposed to MPs, including polystyrene (PS), polypropylene (PP), low-density polyethylene (LDPE), poly(methyl methacrylate) (PMMA), and polyethylene terephthalate (PET), at concentrations reflecting both realistic human exposure and higher doses to evaluate dose-dependent effects.

MPs did not significantly affect viable or total bacterial cell counts in the cultures. However, MPs led to a significant pH decrease, suggesting shifts in bacterial metabolism or composition. To further investigate these changes, 16S rRNA sequencing and metabolomics analysis were performed. These analyses revealed differences in microbiome composition between plastic types, with specific bacterial strains exhibiting increased or decreased abundance over time. Additionally, changes in the metabolome partially correlated with pH alterations, highlighting both shared and plastic-type-specific metabolic responses.

These findings suggest that MPs can influence gut microbial activity and metabolic processes. The differential effects observed between plastic types underscore the need for further research to clarify the mechanisms behind these interactions and their potential implications for human health. Interestingly, we found several similarities between MP-induced microbiome changes and those linked to certain diseases, though some contradictory results were also observed.

The bioreactor model enabled a direct observation of the effects of microplastic, providing a controlled environment to assess microplastic-microbiome interactions. However, in living organisms, additional factors such as diet, immune response, and individual microbiome variations play a role in determining long-term consequences. Exposure duration may be a key factor influencing the extent and nature of microbiome alterations, highlighting the need for further research on chronic microplastic exposure and its potential health risks.

Poster Session

P061

Investigating bacterial gene essentiality in context of the native intestinal microbiome

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For decades forward genetics has been used to identify new drug targets, conditionally essential genes or virulence factors in bacteria to help us define pathogenic traits. Using random mutagenesis these approaches cover whole genomes and allow for comprehensive genetic screens. However, several drawbacks hinder crucial discoveries especially in the context of natural host microbe interactions. Genome wide random mutagenesis relies on a substantial number of diverse mutants to identify essential genes. This limits our abilities to study events or compartments with tight bottlenecks or low colonization.

To address this problem, we have developed a system that allows for easy targeted gene knockout in bacteria via a CRISPR-Cas system called MULTICast. This system can not only be used in a genome wide approach to identify changes in abundance via standard sequencing techniques but also allows for fast easy and reliable targeted gene knockouts. MULTICast is based on a programmable transposon of *Vibrio cholerae* called Tn6677. It originated from a type I-F CRISPR-Cas system that allows specific integration of the transposon at a target site determined by a CRISPR guide which in this case is located inside the transposon.

This guide can be easily tracked through sequencing analysis and enables the investigation of relative abundances of multiple guides in a pool. In preliminary studies, we successfully generated a knock-out pool of 50 *Vibrio cholerae* genes simultaneously. MULTICast reaches a saturated library with significantly fewer bacterial cells and can therefore be used to analyze small compartments of the intestine at various timepoints. This novel spatial-temporal approach to gene essentiality enables us to investigate infection processes in a more granular and holistic manner compared to previous methods. Furthermore, MULTICast has the potential to shed light on how probiotic or commensal bacteria flourish or fail to colonize diverse compartments of the intestine. In fact, our preliminary data suggest that several genes exhibit patterns of essentiality during infection, both temporally and spatially. Currently we are utilizing this system in a genome-wide approach, involving pools of several hundred genes in a diverse group of organisms. This enables us to understand patterns of essential genes during gastrointestinal infection or colonization and potentially lead to the development of new treatment approaches.

Poster Session

P062

Patient-Donor signature in predicting clinical response of Faecal Microbiota Transplantation (FMT)

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In recent years, the incorporation of Fecal Microbiota Transplantation (FMT) into clinical practice has brought a novel therapeutic option for patients suffering from severe active ulcerative colitis (UC). Our latest findings suggest that microbial biomarkers detected just after the FMT could serve as a helpful predictor of the FMT treatment response.

The aim of this study is to construct a robust machine learning (ML) model capable of predicting the FMT response at early stage. Several techniques for classification and regression will be employed to predict post-FMT bacterial populations and abundance. Furthermore, by utilizing MICOM [1], we will be able to evaluate important prognostic metabolites and model the metabolic capacity of the predicted community.

Using this strategy, we can computationally combine patients with several FMT donors in order to find patient-donor pairs with the best possible metabolically competent microbial community. These computational modeling techniques have the potential to improve FMT strategies for UC patients by expanding our knowledge and prognostication abilities.

Poster Session

P063

Systematically understanding human gut microbiome compositional and functional differences between healthy and diseased populations

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The microbiome is a key driving factor of health and disease connecting all life ecosystems from soil to plants to humans. In the last decades, human environment and lifestyle factors have notably changed resulting in a loss of microbiome diversity which has been linked to disease. Probiotics are effective in alleviating various disease phenotypes; however, they often lack therapeutic specificity and are limited to a few traditional strains. Edible plant-associated microbiomes have been shown to shape the human microbiome and constitute a new probiotic source potentially restoring the loss of diversity. Probiotic development could benefit from advances in metagenome technology and meta-analysis methodology as it allows us to systematically identify microbiome alterations in diseased cohorts. Recent meta-analysis research identified microbiome diversity and composition alterations across diseases. However, most studies did not consider microbiome functionality, included few diseases and samples, and did not consider non-western populations. Functional analysis is key for better understanding the microbiome"s potential in shaping health and disease and ultimately developing new therapeutic approaches. As such, the objectives of this project are: 1) to systematically study composition and functionality alterations across diseases from a wide range of human gut metagenomes, and 2) to search the identified bioindicator taxa and functional features in edible plant-associated microbiomes. We collected around 5000 publicly available human gut metagenomes including multiple immune and metabolic diseases from diverse studies, population groups, and geographic locations. We performed alpha and beta diversity measurements between cases and controls at the taxonomy and functional levels and identified microbial species and KOs associated with the disease. Available metadata was included in the analysis to control for confounding variables. Overall, we found significant differences in alpha and beta diversity between cases and controls for some diseases with Crohn´s disease and Non-alcoholic fatty liver disease showing the strongest effects. Furthermore, several species and KOs were significantly associated with disease in most cohorts and many features overlapped multiple disorders. Overall, this work provides a systematic analysis of microbiome composition and functional features linked to disease that may be useful to identify potential targets for probiotic research.

Poster Session

P064

Characterization of the Subgingival Microbiome in Titanium and Superlatex Dental Implants: A Split-Mouth Study

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Background and aim of the study:

The oral microbiome is the second most complex microbial ecosystem in the human body. Its composition varies depending on the anatomical niche, and the dysbiosis of the subgingival microbiome has been strongly associated with the onset of periodontitis and peri-implantitis. While dental implants are a reliable solution for tooth replacement, they introduce surfaces that are prone to bacterial colonisation and biofilm formation. The type of material used in abutments may influence microbial adhesion and community composition. Titanium remains the gold standard in implantology, but superlatex alloys have emerged as potential alternatives due to their promising mechanical and antimicrobial properties. This study aims to characterize and compare the microbial communities adhering to titanium and superlatex implant abutments.

Material and Methods:

An observational, prospective, single-center study was conducted in ten subjects with dental implants, using a split-mouth design to minimize interindividual variability of subgingival microbiome. Each subject received both titanium and superlatex abutments in different implant sites. After abutment removal, adhered microbial community was collected and analyzed via nucleic acid extraction and real-time PCR microarray, targeting 93 oral microorganisms, including several periodontal pathogens. Whole genome sequencing (WGS) analysis was also used to obtain comprehensive taxonomic and functional profiling of the subgingival microbiome.

Results and Conclusions:

Microarray results, though evidencing interindividual variability in the subgingival microbiome, showed remarkable differences in the composition of the microbial community adhered to titanium or superlatex in all enroleld patients. Periodontal pathogens (e.g. *Porphyromonas endodontalis, Tannerella forsythia,* and *Treponema denticola*) were prevalent on titanium surfaces, whereas non-pathogenic subgingival microbes appeared prevalent on superlatex. WGS data are currently being analyzed to provide additional taxonomic and functional insights. These preliminary results suggest that superlatex abutments may be less prone to colonisation by periodontal pathogens compared to titanium, potentially offering microbiological advantages in peri-implant health.

50-word abstract summary:

This study compares subgingival microbial colonization on titanium and superlatex dental implant abutments. Real-time PCR microarray revealed higher levels of periodontal pathogens on titanium surfaces, compared to superlatex. WGS analysis is in progress to obtain further important details on microbial diversity and functional meaning of the observed differences.

Poster Session

P065

Role of small RNAs in plant-associated bacteria, *Azospirillum brasilense* under abiotic stress conditions

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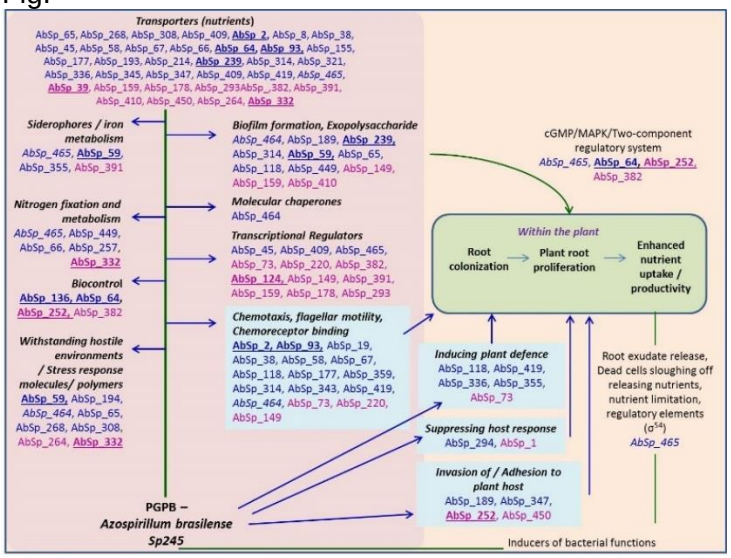
Background: The serendipitous discovery of bacterial regulatory RNAs have opened up a whole new field of small RNAs (sRNAs). Most sRNAs base pair to the mRNAs affecting its translation and/or stability. Different studies have been carried out to discover sRNAs in plant associated bacteria and play essential roles under abiotic stress conditions like iron limitation, oxidative and salt stress, heat and cold shock, and accumulation of glucose-phosphate. We have undertaken a study to explore the presence of sRNAs in *Azospirillum brasilense* Sp245 and the differential expression of the identified sRNAs in abiotic stress.

Results and Conclusion: In our study, expression-based sRNA identification (RNA-seq) revealed a list of ~468 sRNA candidate genes that were differentially expressed in nitrogen starvation versus non-starved (control) conditions. Following a meticulously designed pipeline for data analysis, we uncovered a list of 53 potentially novel sRNAs, of which 16 were randomly selected and validated for differential expression, which largely was found to be in congruence with the RNA-seq data. We selected two candidates, sSp_p4 and sSp_p6, for functional characterization. Plant microbe interactions are usually a part of a multigenic response involving a multitude of sRNAs and their associated mRNA targets. Target gene expression analysis of sSp_p4 confirmed that it influenced gene regulation and microbial traits such as poly-hydroxybutyrate synthesis, indole acetic acid production, and biofilm formation. sSp_p4 was overexpressed, knocked-down and complemented to characterize its physiological functions, and importance in plant growth-promoting traits. Its expression in WT and mutant strains studied in different nutrient conditions revealed variable regulation of different targets. sSp_p6 expression was found to be modulated in carbon and nitrogen stress while forming an interactive network with the target genes, *vnfG* (encoding vanadium-dinitrogenase) and σ-54 thereby establishing an essential role of this sRNA in biological nitrogen fixation by the strain. This study established the *A. brasilense* Sp245 sRNAs, sSp_p4 and sSp_p6 as the potential candidates for improving abiotic stress enduring capability in this strain. Since this bacterial strain is plant-growth promoting in nature, the modulation of the identified sRNAs will help improve the PGP potential of the strain by its improved stress response and eventually contribute to sustainable agriculture.

Fig. 1


Transporters (nutrients)
AbSp_65, AbSp_268, AbSp_308, AbSp_409, AbSp_2, AbSp_8, AbSp_38, AbSp_45, AbSp_58, AbSp_67, AbSp_66, AbSp_64, AbSp_93, AbSp_155, AbSp_177, AbSp_193, AbSp_214, AbSp_239, AbSp_314, AbSp_321, AbSp_336, AbSp_345, AbSp_347, AbSp_409, AbSp_419, AbSp_465, AbSp_39, AbSp_159, AbSp_178, AbSp_293AbSp_382, AbSp_391, AbSp_410, AbSp_450, AbSp_264, AbSp_332
Siderophores / iron metabolism
AbSp_465, AbSp_59, AbSp_355, AbSp_391
Nitrogen fixation and metabolism
AbSp_465, AbSp_449, AbSp_66, AbSp_257, AbSp_332
Biocontrol
AbSp_136, AbSp_64, AbSp_252, AbSp_382
Withstanding hostile environments / Stress response molecules/ polymers
AbSp_59, AbSp_194, AbSp_464, AbSp_65, AbSp_268, AbSp_308, AbSp_264, AbSp_332
Biofilm formation, Exopolysaccharide
AbSp_464, AbSp_189, AbSp_239, AbSp_314, AbSp_59, AbSp_65, AbSp_118, AbSp_449, AbSp_149, AbSp_159, AbSp_410
Molecular chaperones
AbSp_464
Transcriptional Regulators
AbSp_45, AbSp_409, AbSp_465, AbSp_73, AbSp_220, AbSp_382, AbSp_124, AbSp_149, AbSp_391, AbSp_159, AbSp_178, AbSp_293
Chemotaxis, flagellar motility, Chemoreceptor binding
AbSp_2, AbSp_93, AbSp_19, AbSp_38, AbSp_58, AbSp_67, AbSp_118, AbSp_177, AbSp_359, AbSp_314, AbSp_343, AbSp_419, AbSp_464, AbSp_73, AbSp_220, AbSp_149
PGPB – Azospirillum brasilense Sp245
cGMP/MAPK/Two-component regulatory system
AbSp_465, AbSp_64, AbSp_252, AbSp_382
Within the plant
Root colonization
Plant root proliferation
Enhanced nutrient uptake / productivity
Inducing plant defence
AbSp_118, AbSp_419, AbSp_336, AbSp_355, AbSp_73
Suppressing host response
AbSp_294, AbSp_1
Invasion of / Adhesion to plant host
AbSp_189, AbSp_347, AbSp_252, AbSp_450
Root exudate release, Dead cells sloughing off releasing nutrients, nutrient limitation, regulatory elements (σ^{54})
AbSp_465
Inducers of bacterial functions

Poster Session

P066

Uncovering the effect of wheat domestication and breeding on its rhizosphere microbiome

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Wheat is an important staple crop, accounting for ~20% of global caloric consumption. Producing enough wheat to meet growing demand while mitigating environmental damage is a major global challenge. A potential environment-friendly approach involves recruiting beneficial microbes to the rhizosphere, which serves as a hub for plant-soil microbe interactions and thus regulates plant health and productivity. However, previous studies suggest that domestication, modern breeding, and modern agricultural practices may have affected the delicate interactions between plants and soil microbes. In this project, we explore whether genetic changes that resulted from domestication of wheat, the development of modern cultivars and intensive fertilization have reduced beneficial relationships between wheat plants and rhizosphere microbes that can be restored to increase productivity. We characterized the rhizosphere microbiomes of wild, heirloom, and modern cultivars of *aestivum* and *durum* wheat in two soil types with and without fertilizers using shotgun metagenomics analysis. We found that the rhizosphere microbial composition differed between the genetic groups in both soil types, and fertilizers had the greatest impact on the microbiome in soil naturally containing lower levels of nutrients. Moreover, microbial diversity was lower in the rhizosphere of modern wheat grown in unfertilized soil compared to landraces relatives, suggesting difficulty of modern wheat plants to maintain the level of microbial diversity in the rhizosphere when nutrients are in scarcity. In addition, this methodology enabled the identification of specific microbial taxa with significantly different abundances between wheat groups, highlighting them as potential candidates for restoration in modern cultivars. Notably, bacterial taxa such as *Massilia* and *Diaminobutyricimonas*, recognized for their roles in promoting plant health, exhibited substantial differences across wheat types. Further investigation for the understanding of the interplay between wheat genetics, soil microbes, and environmental conditions could inform strategies for reintroducing ancient beneficial microbes to modern cultivars to optimize sustainable wheat production.

Poster Session

P067

Nitrogen-cycling plant microbiomes and their influence on crop nutrition

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Nitrogen (N) is crucial for forming proteins, nucleic acids, and chlorophyll, essential for photosynthesis. Its availability in soil directly impacts plant metabolism, growth, and productivity. In agriculture, N limitation is often countered with synthetic fertilizers, yet their excessive use contributes to eutrophication and climate change, necessitating more sustainable solutions. While microorganisms play key roles in the global N cycle, the influence of plant-associated microbiomes on N uptake remains poorly understood.

In this project, we investigate how soil microbiomes influence wheat N nutrition. In a preliminary experiment, microbiomes extracted from 34 donor soils were used to inoculate wheat plants under low-N conditions in a greenhouse trial. Based on plant phenotypic traits, 14 soils with contrasting effects on growth were selected for a main experiment, where plants were inoculated with the donor soils. This experiment included high-N and low-N treatments, as well as non-inoculated controls. A ^{15}N tracer experiment was included to assess microbial contributions to plant N uptake. 16S rRNA gene and ITS amplicon sequencing, along with ^{15}N analysis, will further characterize these soils and guide the selection of a subset for metagenomic analysis.

Currently, microbiomes from the five best-performing soils are being used in a reduced natural microbial community (NatCom) experiment, where wheat is grown in short cycles to reduce the diversity within microbial communities and potentially enrich beneficial traits. These NatComs are engineered by recurrently inoculating plants with rhizosphere microbiomes to shape their composition. In addition to molecular analyses, isolation focusing on N_2-fixing microorganisms was performed to provide further insights into potential microbial contributions to plant N uptake.

By integrating microbiology, plant physiology, and bioinformatics, this project aims to better understand N transformations in the rhizosphere and plant-microbe interactions while also developing microbial solutions for improving crop N nutrition and reducing reliance on industrial fertilizers.

Poster Session

P068

Application of plant growth promoting bacteria from arid regions in tomato cultivation

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The Hungarian agricultural environment is transforming via climate change. Drought is a major challenge for plant growth especially in intensive agricultural areas. Bacteria that stimulate plant growth are often isolated from extreme environments such as arid regions and these bacteria are able to increase the survival of other types of plants during the dry period, not just those from which they have been originated.

The objective of this study is to investigate whether the isolated bacteria from arid open and closed sand steppes with plant growth-stimulating properties can improve the growth of tomato under normal and drought stress conditions.

A total of 149 strains were isolated from the sandy grasslands and the selected nonpathogenic 48 strains were screened for plant growth promoting (PGP) traits, such as: osmotic stress tolerance, indole-3-acetic acid, exopolysaccharide, siderophore, and 1-aminocyclopropane-1-carboxylate deaminase production and phosphate solubilization. The strains with the best properties were tested in a phytotron system on tomato seedlings in short-term experiments.Based on the phytotron results, the long-term effects of two strains (*Kocuria* sp. FSP120 and *Brevibacillus* sp. FSP5 strains) were investigated in greenhouse experiments, where two different irrigation (100% and deficit) settings were introduced.

According to the results, the FSP120 strain had a positive effect on the average height of the plants, the number of leaves, the number of flower buds, flowers and fruits. In the 100% irrigated area, the marketable yield average of the FSP120 stand was 16% higher compared to the control group, while an 11% increase was observed in the case of deficit irrigation. As a result of the bacterial inoculation, we also observed an increase in the carotenoid content of the fruits.

Our experiment proved that the FSP120 bacterial strain isolated from an arid area was able to promote tomato growth under real conditions and also caused a positive change in fruit quality.

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Poster Session

P069

Identification of microbial keystone taxa for SynCom applications for sustainable agriculture

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Developing synthetic communities (SynComs) holds the potential for improving environmental and human health in the near future. In agriculture, for instance, it could compensate for biodiversity losses and subsequent functional traits in soils. However, due to the enormous diversity of soil microbiomes, with one gram of soil harboring more than 50000 species[1], establishing representative SynComs can be challenging. Moreover, most microbes remain uncultured due to their unknown specific isolation conditions or need for a minimal microbial consortium for successful establishment.

Applying ecological concepts to microbiome research, such as the keystone taxa concept[2,3], might help to prioritize microorganisms for targeted isolation and integration into representative SynComs. Implementing ecological concepts is one of the aims of the EU-funded MICROBE (Microbiome Biobanking (RI) Enabler) project, which focuses on novel approaches for isolating and cultivating microbiota from different environments. Here, we provide an eight-point definition of the characteristics of microbial keystone taxa. Among other aspects, we define a microbial keystone taxon as a microbial strain that has a high impact on the ecosystem independently of its abundance, plays critical functions in the ecosystem and organismal health, contributes to the stability of the microbial communities, and has highly dynamic genome containing key genetic material that can be passed to other community members. Furthermore, we describe an operational approach to detect "keystone taxa candidates" from complex *omics* data and to define isolation strategies. Our approach is based on microbial co-occurrence networks to define the keystone taxa from metabarcoding data and metagenomic data to map the functional potential of the identified candidates.

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Poster Session

P070

Synergistic effects of palm shell mulching and biogenic selenium nanoparticles from *Streptomyces euryhalinus* in improving water productivity and production in mango trees in sandy soils in the United Arab Emirates

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Mitigating water scarcity in agriculture necessitates new techniques to improve water use efficiency (WUE). This study examines the impact of four irrigation levels (100%, 75%, 50%, and 25% of crop evapotranspiration [ETc]) in conjunction with palm shell mulching (M), biogenic selenium nanoparticles (BSeNPs) from *Streptomyces euryhalinus*, or their combination (M + BSeNPs) on water use efficiency (WUE), evapotranspiration, physicochemical properties, yield, and net profit of mango trees utilizing a deep drip irrigation system. Irrigation needs during the growth phase varied from 81.36 to 825 mm/day, decreasing to 18.36 to 3.4 mm/day at 25% ETc. The M and M + BSeNPs treatments augmented fruit weight by 35-59%, but BSeNPs improved yield by 26% at the minimum irrigation rate treatment (IR 25%). Integrated M + BSeNPs treatments enhanced carbohydrate, protein, and moisture levels, while markedly decreasing ETc and improving WUE. The yield response factor stayed beneath the FAO (2002) criterion, indicating resilience to water stress. Energy savings from M + BSeNPs treatments varied between 39.24% and 66.58%. The implementation of IR at 25%, in conjunction with M and BSeNPs, enhanced marketable yield, with relative gains of 42-58% compared to the control group. The decrease in irrigation expenses augmented net benefits by 31%. In summary, the implementation of palm shell mulching and BSeNPs with reduced irrigation (IR 25%) offers a sustainable approach to boost water use efficiency, increase yield, and optimize economic returns in mango trees in sandy soils in the United Arab Emirates.

Poster Session

P071

Enhancement of the grey mangrove (*Avicennia marina*) growth by rhizosphere-competent, polyamine-producing actinobacteria in the United Arab Emirates

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Actinobacteria residing in the rhizosphere can enhance plant growth and development. Thirty-nine actinobacterial isolates derived from the rhizosphere of the grey mangrove (*Avicennia marina*) in the United Arab Emirates were assessed for their capacity to synthesize polyamines (PAs): putrescine (Put), spermidine (Spd), and spermine (Spm), as well as their competence as rhizosphere isolates. Consequently, the prolific PAs-producing isolates, *Micromonospora* UAE1 and *Streptomyces* sp. UAE1, were chosen. In greenhouse conditions, the application of either isolate significantly (P<0.05) enhanced the weight and length of shoot and root tissues, photosynthetic pigments, compared to control plants, indicating that *Micromonospora* UAE1 and *Streptomyces* sp. UAE1 can stimulate the growth of *A. marina*. The infestation of soil with these isolates led to marked increases in endogenous polyamines (Put, spd, and Spm) and various plant growth regulators (PGRs), such as auxins (indole-acetic acid, indole-pyruvic acid, gibberellic acid) and cytokinins (isopentenyl adenine, isopentenyl adenosine, and zeatin), alongside a corresponding decrease in abscisic acid in the roots and shoots of *A. marina*. Both *Micromonospora* UAE1 and *Streptomyces* sp. UAE1 were, however, unable to generate measurable quantities of PGRs *in vitro*. Plant growth promotion (PGP) was more significant in the presence of *Streptomyces* sp. UAE1 than *Micromonospora* UAE1; consequently, this relative superiority in performance demonstrated the advantage imparted to *Streptomyces* sp. UAE1 as rhizosphere-competent compared to *Micromonospora* UAE1, which was not. Our findings underscore the necessity of incorporating rhizosphere competence in the selection of PGP candidates. This study is the inaugural report on the synthesis of PAs by marine actinobacteria and illustrates the capacity of PA-producing actinobacteria to enhance the growth of halophytic plants (e.g., *A. marina*) by elevating the endogenous concentrations of PAs and other plant growth regulators (PGRs).

Poster Session

P072

A phosphate-solubilizing, rhizosphere-competent isolate of *Actinoplanes philippinensis* promotes cucumber development in calcareous soil in the United Arab Emirates

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This study aimed to assess the capacity of non-streptomycete actinomycetes (NSA) to solubilize insoluble phosphates in soil and enhance plant growth in the United Arab Emirates (UAE). Thirty-five strains were identified utilizing *Streptomyces* phage and dry heat methods from calcareous soil lacking in accessible phosphorus in the UAE. Nine of these NSA isolates solubilized powdered rock phosphate (PRP) in both solid and liquid mediums. Five isolates were first chosen based on their rhizosphere efficacy. An isolate of *Actinoplanes philippinensis* resulted in a notable decrease in pH in a liquid medium supplemented with PRP, solubilized substantial quantities of phosphorus, and formed both acid and alkaline phosphatases, along with other organic acids. This isolate of *A. philippinensis* was selected from five isolates due to its remarkable rhizosphere competence and its significant capacity to colonize cucucmber roots to a depth of 14 cm. In the greenhouse, the introduction of *A. philippinensis* to soil enriched with either single super-phosphate (SP) or PRP markedly enhanced the growth of roots and shoots in cucumber plants compared to those cultivated in non-inoculated soil supplemented with SP or PRP. The substantial increases in the concentration of accessible phosphorus in the soil, as well as the levels of nitrogen, phosphorus, potassium, sulfur, magnesium, iron, and zinc in the roots, and nitrogen, phosphorus, potassium, sulfur, magnesium, and iron in the shoots of inoculated plants were also apparent. The stimulation of plant development by *A. philippinensis* was most significant with SP as a soil amendment in comparison to PRP. In contrast, a non-phosphate-solubilizing, non-rhizosphere-competent NSA isolate of *Actinoplanes missouriensis* could not enhance accessible soil phosphorus, nutrient concentrations in roots and shoots, nor facilitate plant growth. The evaluated plant growth regulators do not seem to contribute to the observed stimulation of plant growth. *A. philippinensis* and *A. missouriensis* did not generate measurable quantities of indole-acetic acid, indole-pyruvic acid, gibberellic acid, isopentenyl adenine, isopentenyl adenosine, or zeatin *in vitro*. This work is the inaugural published report illustrating the capacity of phosphate-solubilizing NSA to enhance plant growth. This is the inaugural published report of rhizosphere-competent NSA that can solubilize SP or PRP in soils.

Poster Session

P073

The influence of wheat microbiomes on drought stress resilience

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Wheat is the most grown crop plant by area worldwide and a widely valued part of western human diet. However, drought periods, that are projected to increase due to climate change, pose a major agricultural risk to crop yields. One potential strategy to increase drought stress resilience of wheat is the application of plant-growth promoting bacteria. In this study, we aimed to identify drought stress suppressing microbiomes through plant phenotype monitoring. Furthermore, bacteria were isolated from the rhizosphere of drought stressed wheat to decipher the plant-microbe interactions during drought

We cultivated wheat plants in 20 distinct agricultural soils collected across Lower Austria in greenhouse trails. To simulate drought stress, a water withholding regime was applied. The five best and worst performing soils in elevating drought stress resilience were selected based on dry weight, stomatal conductance, as well as water and chlorophyll content. To control for potential abiotic factors, living microbial cells were extracted using a 0.2% pyrophosphate solution from each of the selected soils and used as an inoculant for a subsequent experiment. Inoculated wheat plants were grown in steamed artificial soil under drought stress and controlled conditions to assess the effect of soil microbiomes and stress on the plant phenotype and wheat-associated microbiomes. Drought stress had an impact on plant growth, whereas different soil microbiomes had only minor effects. Treatments with three different soils were selected based on their effect on plant traits. Root and rhizosphere microbiomes of stressed and unstressed plants were subjected to 16S rRNA gene amplicon sequencing as well as isolation campaigns. As *Flavobacterium* strains were prominently isolated, a comparative genomics analysis has been initiated. We will present results on how drought stress affects wheat growth when grown in identical soils but containing distinct microbiota.

Poster Session

P074

Unravelling the microbiome of Atlantic Nori (*Porphyra*) at early development stages – a polyphasic approach to microbiome-based aquaculture solutions

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Algal-microbiome interactions are crucial for the health and development of the host, but our current knowledge of microbiome-based strategies for sustainable aquaculture practices is still limited. We employed a polyphasic approach using cultivation-dependent and -independent methods to determine the composition and function of microbial communities associated with organic certified Atlantic Nori (*Porphyra dioica* and *P. umbilicalis*) cultivated under a land-based integrated multitrophic aquaculture system. Cultivation of *Porphyra*-associated bacteria and 16S rRNA gene and shotgun metagenome sequencing were performed to shed light on the microbiomes of algal samples at three early life stages and their culturing water, harvested from indoor photobioreactors. Analysis of 16S rRNA gene amplicons revealed that the phyla *Pseudomonadota* and *Bacteroidota* were dominant in algal tissue. Genera such as *Blastopirellula*, *Algoriphagus*, *Hyphomonas* and *Marinobacter* were enriched in algal samples, with their relative abundances varying across life stages. Notably, the genera *Ensifer* and *Paraglaciecola* were consistently found in both species. Culturable bacteria from Nori (n=134) affiliated mostly with the families *Roseobacteraceae*, *Flavobacteriaceae* and *Alteromonadaceae*, with several presenting potential novel species. Shifts in taxonomic composition across life stages were recorded among culturable bacteria, including numerous producers of the algal growth-promoting hormone auxin (e.g. *Alteromonas*, *Aliivibrio* and *Yoonia-Loktanella*). Shotgun metagenomics allowed for the characterization of eukaryotic and viral communities, showing an enrichment of *Bacillariophyta* (diatoms) in algae tissue, in contrast with *Ascomycota* fungi enrichment in culturing water. *Uroviricota* (mainly bacteriophages) was the major virus phylum detected, while *Nucleocytoviricota* (large DNA viruses) was depleted in algae tissue. Seventy-eight medium and high-quality bacterial metagenome-assembled genomes (MAGs), mainly from the families *Alteromonadaceae* and *Roseobacteraceae*, were recovered, with 22% of possibly new, undescribed bacteria. Functional analysis of MAGs is expected to illuminate the roles of bacterial symbionts of Atlantic Nori. This study reveals complex, phylogenetically diverse microbiomes with algal morphogenesis-inducing capabilities during early *Porphyra* development, highlighting the potential of microbiome-based interventions to support the sustainable growth of algae in aquaculture.

Poster Session

P076

Microbes-4-Climate – microbial services addressing climate change risks for biodiversity and for agricultural and forestry ecosystems

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Microbes-4-Climate (M4C) is a project that aims to deepen the comprehension of the complex relationships among microorganisms, plants, and soil within the framework of Climate Change. By offering access to advanced Research Infrastructures (including those of MIRRI-ERIC, ELIXIR, AnaEE-ERIC, LifeWatch-ERIC and EMPHASIS), training, and assistance, the project seeks to encourage research tackling the multifaceted challenges presented by Climate Change to terrestrial biodiversity and ecosystems. M4C offers researchers involved in the above topics the ability to use free of charge the services and resources of cutting-edge Research Infrastructures (RI) spread across multiple countries via a TransNational Access (TNA) program. This project aims to break down geographical barriers, foster collaboration, and accelerate scientific progress by providing researchers with unparalleled support and access to resources and expertise. Details concerning the M4C TNA can be seen here: https://microbes4climate.eu/tna/

Poster Session

P077

Two novel approaches to transition from single strain bioinoculants to consortia

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Sinlge strain bioinoculants can suffer from reduced efficacy and inconsistent performance under field conditions. The success and stability of these agicultural bioinoculants is heavily shaped by interactions with the resident microbiome. Consortia-based bioinoculants can persist and function better in the presence of a competive resident microbiome, due to community emergent properties.

Typically, the design of a bioinoculant consortia, is either a top-down approach driven by -omics, or a bottom-up approach driven by synthetic biology, both of which pose challenges. Here we present two novel approaches for the design of bioinoculant consortia that are based on colocalization instead. The bead method [1] and the bait method [2] can be used to design microbial consortia whose members synergistically reinforce each othes as a community emergent property.

Our innovations enabled the high-throughput isolation of 1000+ consortia directly from environmental niches like the rhizosphere, using the bead and bait methods. More than 100 promising consortia were cultivated and characterized, leading to a catalogue of microbial strains and functions. Lead consortia outperformed the corresponding single strains in plant protection and growth promotion in greenhouse trials with potato and tomato plants.
In conclusion, these methods offer high-throughput discovery of consortia-based agricultural bioinoculants. Harnessing these social interactions within the plant microbiome for the design of bioinoculants holds the potential to revolutionize microbe-assisted crop production.

1. Taparia, T., Agergaard, A, J., Sørensen, J, S. (2024). A method for co-isolating two or more bacterial strains co-located on a micro-scale surface. European Patent Office, 24184732.6

2. Taparia, T., Puro, S, M., Sørensen, J. S. (2024). A method for obtaining one or more supporting bacteria for a bacterial composition. European Patent Office, 24184776.3

Poster Session

P078

Rooted in resilience – the sunflower microbiome as a source of growth-promoting and drought-resistant microbes

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The seed microbiome has the potential to support germination and early plant development, and can help plants to cope with environmental stresses such as drought. Despite these beneficial properties, the seed microbiome remains underexplored compared to the root and soil microbial communities. This study aims to characterise the bacterial and fungal communities present in sunflower (*Helianthus annuus*) seeds for two main purposes: (1) to identify plant growth-promoting and drought-resistant microorganisms for later isolation studies and functional assays, and (2) to compare them with the root and soil microbiome to explore microbial selection and potential horizontal transfer. We conducted a comprehensive metabarcoding analysis of the bacterial and fungal communities present in sunflower seeds (inner seed and outer shell), roots (rhizosphere and endosphere), and surrounding soil, utilising 16S rRNA and ITS2 metabarcoding protocols. Samples from five sunflowers were collected in each of four Flemish sunflower fields. DNA was extracted from all compartments and sequenced on an Illumina NextSeq 2×300 bp platform. Preliminary results show that bacterial diversity follows a decreasing gradient from the soil to the rhizosphere, endosphere, and ultimately the seed. Additionally, inner seeds exhibited a higher alpha diversity than outer shells. Beta diversity analysis revealed a distinct bacterial community composition, where soil and rhizosphere samples clustered separately from seed samples, which grouped more closely with endospheric samples. We will further investigate the impact of field location and sunflower cultivar on the microbiome composition, and identify key microbial taxa consistently present across multiple sunflower compartments and agricultural fields, which indicates the presence of a core microbiome. This research provides the foundation for developing microbial-based strategies to improve germination and drought resilience in sunflower cultivation.

Poster Session

P079

N_2O consumption potential in long-term tillage and fertilization experiment correlates with *nosZII* community proportion instead of microbial community structure and diversity

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Question:

Reduced tillage intensity is known to increase soil organic carbon in the topsoil, however conflicting results are reported regarding the impact on N_2O emissions. In our seasonal study, we observed a positive trade-off between increased soil C content and reduced N_2O emissions in organically fertilized no-till corresponding to a reduced (*nirK*+*nirS*)/(*nosZI*+*nosZII*) ratio, driven by higher *nosZII* abundance (Govednik et al., 2024). This study aimed to confirm this pattern and evaluate its implications for N_2O consumption potential by analysing microbial community composition and potential denitrification activity (PDA).

Methods

Long-term legacy of two different tillage systems [no-till (NT) and conventional ploughing (CT)] combined with three different fertilization strategies [mineral fertilization (MIN), compost (ORG) and unfertilized control (CON)] was studied. PDA was performed with and without C-mix addition in presence and absence of acetylene. Functional gene abundances were quantified using qPCR and microbial community structure was examined by 16S amplicon sequencing and WGS.

Results

The highest N_2O consumption potential was observed in NT-ORG, (Fig 1), confirming the seasonal pattern. Addition of C-mix to the assay significantly increased N_2O consumption in CT. Gradient in soil physical and chemical properties reflected the tillage driven separation of microbial communities, which however did not correlate with N_2O consumption potential (Fig 2). Among denitrifier genes, *nosZII* exhibited negative correlation with PDA rates and $N_2O/(N_2O+N_2)$ ratio (Fig 2).

Conclusions

Tillage and fertilization influenced microbial communities by shifting N functional guilds linked to N_2O consumption potential. However, while both practices affected microbial diversity, these changes did not directly translate to functional responses. Our findings suggest that overall community structure and diversity did not drive N_2O consumption potential. Instead, the negative correlation between *nosZII* gene and the $N_2O/(N_2O+N_2)$ ratio highlights the need to examine functional subsets to understand microbial processes like denitrification.

Govednik et al. 2024. STOTEN, 928: 172054, https://doi.org/10.1016/j.scitotenv.2024.172054

Fig 1: $N_2O/(N_2O+N_2)$ ratio as calculated from PDA rates in the absence and presence of acetylene, respectively.

Fig 2: Results of abiotic and biotic soil parameters partitioning (top) and their correlation with PDA rates and ratios (bottom).

Fig. 1

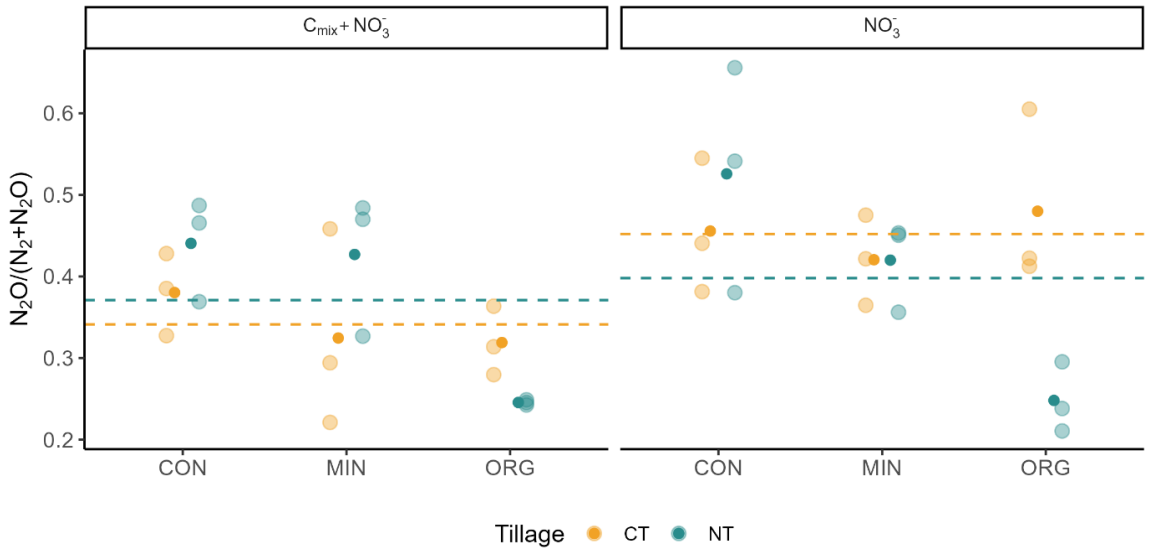
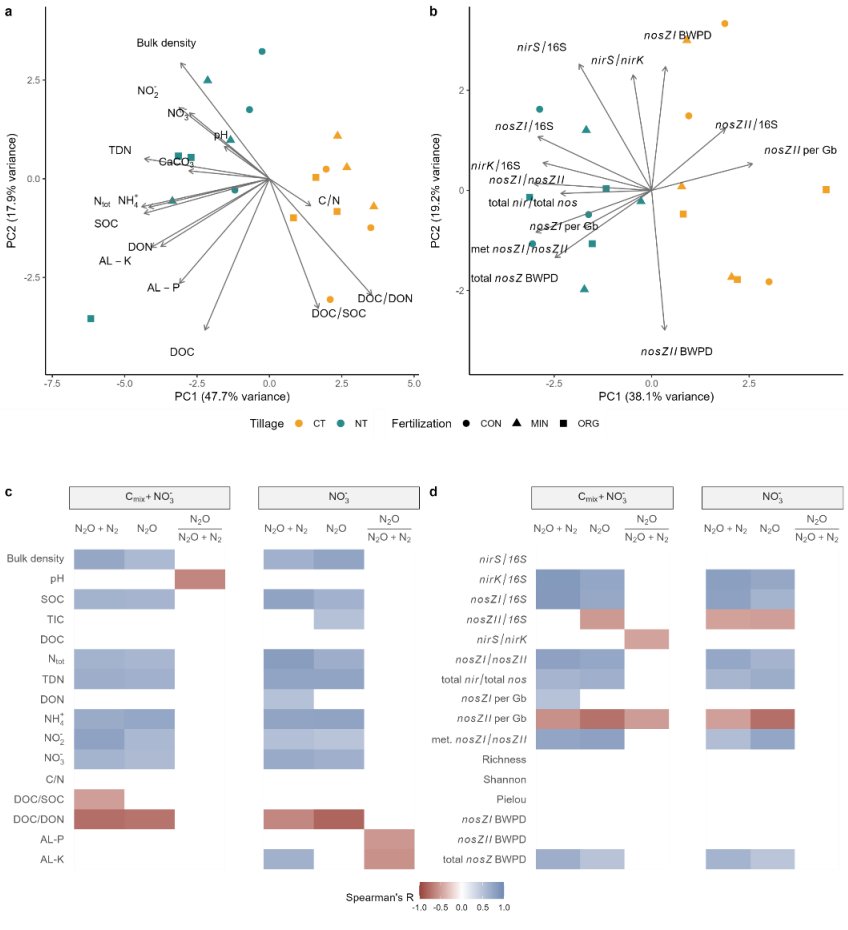


Fig. 2



Poster Session

P080

Effects of artificial humic substances on soil and plant health in horticulture

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The maintenance of plant health is key to horticultural farming and strongly affected by the plant and soil microbiome. Soil edaphic properties and agricultural interventions using soil amendments shape microbial diversity and its associated functions. In the context of circular bioeconomy and the SPIN-FERT Project (Grant agreement ID: 101157265, DOI: https://doi.org/10.3030/101157265), artificial humic substances are of special interest for improving soil and plant health. The beneficial properties of soil organic matter on plant health and disease resistance are well established, however whether this applies to artificial humic substances remains unknown. Furthermore, it is not clear how the microbiome adapts following humic substance amendment and how such adaptations can promote plant-beneficial functions.

To understand the influence of horticultural interventions on the soil and plant microbiome by manipulating microbial diversity and organic matter content we formulated five substrates to grow tomato seedlings. The substrates are modified using soil transplants and are treated with artificial humic substances and *Rhizoctonia solani* AG 4*,* a fungal plant pathogen. Disease incidence and plant growth are assessed. The soil, rhizosphere and phyllosphere are sampled for amplicon sequencing to observe microbiome shifts after treatment. Additionally, the soil metabolome is analyzed to determine changes in the soil microbiome"s functional potential.

We hypothesize that artificial humic substances increase soil microbial diversity and disease resistance in tomato seedlings. By altering the microbiome composition, the plant can have a wider selection of microorganisms to recruit into its own microbiome while the fungal pathogen is met with a more diverse battery of potential antagonists. Our findings may contribute to more efficient manipulation of the microbial aspects of agriculture to promote and improve healthy produce.

Poster Session

P081

The microbial communities associated with winter wheat are shaped by agricultural management practices

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The extensive use of chemical pesticides and fertilizers has detrimental impacts on both agricultural and natural ecosystems. These practices can disrupt the microbial communities associated with soil and plants, which are critical for sustaining healthy symbiotic interactions within the plant holobiont. The application of living microorganisms in the form of biopesticides, biostimulants, and biofertilizers represents a promising and sustainable alternative. However, it is essential to adopt a holistic approach that encompasses a thorough understanding of how microbial products influence the indigenous plant and soil microbiome. Here we analyzed the impact of different management practices on the microbiome of winter wheat, by collecting samples from a large-scale, long-term field trial in Grubno, Poland. The different practices included i) a treatment with two commercially available microbial products (Valibiotics AG), ii) a hybrid treatment that combined the microbial products with a reduction in chemical fungicides, iii) a conventional control group treated only with chemical fungicides, and iv) an untreated control group. Quantitative real-time PCR and high-throughput sequencing targeting the bacterial 16S rRNA gene and fungal ITS region was performed to analyze the effect of the management practice within the leaf, root, seed and rhizosphere microbiomes. Sample type was the major factor influencing the bacterial and fungal community structure of winter wheat. Differences between the sampling timepoints were more pronounced within fungal communities compared to bacteria. Changes in the bacterial and fungal community structure of wheat leaves, roots, and the rhizosphere were observed as a result of the different management practices. While the management practice had a significant influence on the bacterial leaf communities at the first sampling timepoint, no effect was observed at the second sampling timepoint. Interestingly, no significant change was observed in the seed microbiome. Furthermore, a significantly higher fungal diversity was observed in wheat leaves treated with only the biological products compared to leaves where pesticides were applied. This study demonstrates that agricultural management practices are drivers of the microbiome of winter wheat, emphasizing the critical role of the plant microbiome in promoting sustainable agriculture.

Poster Session

P082

What differentiates perennial from annual cereals? – A microbiome perspective

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Modern agricultural practices are often unsustainable, negatively impacting the surrounding environment and contributing to a decline in biodiversity. To mitigate these issues, implementing perennial grain cropping may serve as a viable alternative to traditional annual wheat cultivation. Perennial plants offer numerous ecosystem services, such as preventing soil erosion and reducing nutrient runoff, due to their extensive root systems. However, there is limited knowledge regarding the microbial communities associated with perennial cultivation systems. Our study is the first to examine the microbial structure within the endophytic compartments of perennial wheatgrass (*Thinopyrum intermedium* L.) compared to its annual counterpart, winter wheat (*Triticum aestivum* L.). We designed a comprehensive sampling strategy across three distinct sites in Europe over two consecutive years, followed by high-throughput sequencing of the 16S rRNA gene fragment. Our findings reveal that while aboveground microbial diversity remained unchanged, perennial wheatgrass harbored a greater diversity of bacteria in its belowground compartments. The diversity of root bacteria was influenced by various soil chemical parameters, including the carbon:nitrogen ratio, as well as soil microbial parameters such as soil respiration and dehydrogenase activity. Consistent findings across sampling sites indicate stable mechanisms in the assembly of microbiota associated with perennial grain cropping, highlighting their potential role in promoting biodiversity within sustainable agricultural systems.

Poster Session

P083

Shifts in rhizobacterial communities of seedlings and plants in flowering stage of two varieties of yellow lupin (*Lupinus luteus*) treated with short-term heat shocks

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Agriculture is currently one of the most affected industries by climate change worldwide. Despite that cold and warm waves periodically affect the crop yields, their effect on associated plant microbiomes has been less explored. In this context, varieties of lupins are cultivated as an attractive source of protein for human and animal consumption, however, studies on the effect of climatic stressors on lupin crops are limited, especially stress by heat shock. In this study, we investigated the response of rhizobacterial community in seedlings and plants in flowering stage (anthesis) of two varieties of yellow lupin (*Lupinus luteus*) exposed to short-term heat shocks. Seeds of two varieties of *L. luteus* (Core 78 and Core 195) were germinated, grown under chamber conditions, and exposed to short-term heat shocks (2 h at 39°C during 3 consecutive days). Shifts in rhizobacterial community were investigated by 16S rRNA gene amplicon sequencing. Our results showed a lower richness (OTUs) and diversity (Shannon and Pielou"s Evenness indexes) in plants in flowering stage treated with heat shock in both lupin varieties compared with seedlings and controls without heat shock treatment. Principal coordinate analysis (PCoA) also revealed that heat shocks triggered changes in the rhizobacterial community in both lupin varieties. Coincidently, Proteobacteria, Actinobacteriota, Acidobacteriota and Planctomycetota were the most abundant phyla observed, independently of phenological stages and heat shock treatments. In both lupin varieties, the co-occurrence network analysis revealed ≥50% of positive connectivity within the rhizobacterial community. Despite that differences in clustering between plants with and without heat shocks were revealed, the Xanthobacteraceae family was suggested as keystone taxa independently of heat shock treatments. Additionally, the application of heat shocks also resulted in a lower growth (e.g., height and weight of stems and seeds) of both lupin varieties at flowering stage plants compared with those without heat shock treatment. Thus, our study evidence a potential impact of hot waves not only on the rhizobacterial community but also on the fitness of lupin plants, particularly in those under flowering stage.
Funding: Projects FONDECYT #1221228 and #1240602 (ANID, Chile), Doctoral Scholarship #21211278 (ANID, Chile) and Strengthening of Regional Center project code R20F0003 (ANID, Chile).

Poster Session

P084

Characterization of cooperative bacterial consortia based on in situ cultivation approach from rhizosphere of lupine plants

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Artificial microbial consortia based on plant growth-promoting (PGP) microbes have been widely commercialized and used as biofertilizers to increase plant nutrient availability. However, the functional complementary between microbial members of the consortia is a critical aspect that has not been considered in their formulation. Here, we develop a cooperative microbial consortium based on their PGP complementarity by in situ cultivation using a "microwell chamber" (MWC) and "fungal highway column system" (FHW) from the rhizosphere of lupine cultivars. Bacterial strains were isolated and characterized by exometabolic interaction and their complementarity of PGP traits. Eleven isolates were described as both drought-tolerant, auxin producers (2–8 µg mL−1) and 1-aminocyclopropane-1-carboxylate (ACC)-degrading bacteria (4–44 nmol αKB mg protein−1 h−1). Co-culture assays revealed that consortium formulated by *Bacillus* sp. 31+ *Burkholderia* sp. 34 grew synergistically and enhanced their growth by 55% and 10% compared with single cultures. Cooperative interactions between consortia members were observed in PGP traits, increasing ACC deaminase activity from 35 to 87 nmol αKB mg protein−1 h−1 (2 times) and IAA production increasing to 38 µg mL−1 from 0.24 by *Bacillus* sp. 31 and 3 µg mL−1 by *Burkholderia* sp. 34. Bidirectional cross-feeding assays showed that both, *Burkholderia* sp. 34 and *Bacillus* sp. 31 enhanced the growth when their supernatants were used. This consortium was tested for their impact on the germination of drought stress (5% PEG and 7.5% PEG) lupine seeds. The germination experiments demonstrated that seeds inoculated with cooperative bacterial consortium containing *Bacillus* sp. 31+ *Burkholderia* sp. 34 showed higher total length (39%) than uninoculated plants under 5% of PEG. Although the underlying mechanisms remain elusive, our data suggest that the application of cooperative consortia based on established natural bacteria-bacteria interactions, mediated via targeted cross-feeding, can be an effective strategy for the development of effective PGP consortia for lupine crops.

Funding: Projects FONDECYT #1221228 and #1240602 (ANID, Chile), Doctoral Scholarship #21211278 and #21210306 (ANID, Chile) and Strengthening of Regional Center project code R20F0003 (ANID, Chile).

Poster Session

P085

Humic acid amendment modulates microbiome restoration by soil transplants

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The functionality of the soil microbiome is closely associated with microbial diversity and community structure. Soil transplant and humic acid has been used individually used to improve the soil microbial diversity. However, the impact of soil transplantation and humic acid and their combination on soil bacterial diversity across varying conditions has not been thoroughly examined. This study aims to explore the potential application of soil transplantation and humic acid for the restoration of depleted soil microbiomes. Employing a comprehensive methodology that incorporates both culture-dependent growth assays and culture-independent techniques, such as quantitative PCR (qPCR) and amplicon sequencing, the following research questions were formulated: 1) Does soil transplantation enhance soil bacterial diversity? 2) How does this treatment interact with the addition of humic acid? 3) Is there a consistent response observed across different soil types? Three distinct soil types were analyzed to evaluate whether the soil and its microbiome respond differently to the treatments, and to understand how the restoration of the soil microbiome is influenced by the native microbiome and various soil characteristics. The incorporation of humic acid resulted in significant alterations in bacterial community diversity and composition. Notably, humic acid appears to inhibit the rapid colonization of copiotrophic bacteria, thereby allowing slower-growing bacteria to establish, reproduce, and ultimately contribute to an increase in overall bacterial diversity. This study offers valuable insights into the restoration of soil microbiomes through the application of soil inocula.

Poster Session

P086

Minor changes in the structure of bacterial Rhamnolipids cause major differences in plant responses

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Rhamnolipids (RLs) are secondary metabolites produced by certain species of different bacterial genera. Their core structure comprises a hydrophilic moiety consisting of one (mono-RLs) or two (di-RLs) rhamnose molecules and a hydrophobic mono- or di-lipidic moiety. While the majority of RL-producing bacteria is able to produce a complex of mono- and di-RLs, variation in the fatty acid chain(s) is mainly determined by the bacterial species.

Playing a role in quorum sensing, we hypothesized that the structural range of RLs enables a certain level of specificity in the transfer of information, and that organisms that naturally co-occur with RL-producers are able to perceive and react specifically in response to structural changes. Therefore, we analyzed the impact of mono- and di-RLs on plant protection and growth as well as the impact of variation in the lipidic moiety. We found a distinct structure - response relation with partly opposing effects on the vitality of *Arabidopsis thaliana*. A mixture of mono-RL congeners induced prolonged stress in plants, and in consequence, inhibited plant growth. In contrast, plants benefited from a treatment with a mixture of di-RL congeners showing a significantly lower infection rate with the plant-parasitic nematode *Heterodera schachtii* as well as a neutral to even positive influence on plant growth. Our investigations demonstrate that RLs were not directly lethal for the plant pathogen indicating that RLs stimulate molecular plant responses. Observed differences in the plant reactive oxygen species profile and infection pattern upon treatment with individual RL congeners further confirmed, that this stimulation highly depends on the specific RL structure.

We show that structural variation in quorum sensing involved bacterial RLs resulted in a respectively altered response profile of *A. thaliana*, an organism that is not part of the quorum sensing community. This may indicate that bacterial communication is either intended to address a broader audience or that co-occuring organisms learned to convert and adopt information originating from outside of their own taxa.

Poster Session

P087

Microbial teamwork: Functional benefits of compatible *Bacillus* mixtures for sustainable agriculture

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In response to the growing demand for sustainable agricultural practices, plant growth-promoting bacteria (PGPB) have emerged as promising tools for enhancing crop productivity. This study assessed the PGP potential of *Bacillus* strains isolated from diverse environmental sources and examined the influence of compatibility-based strain selection on cucumber growth. We observed substantial variation in PGP traits among *Bacillus* isolates, with strain origin playing a significant role - highlighting the impact of environmental context on microbial functional capacity. A total of 269 bacterial isolates including *Bacillus* and unidentified isolates from different environmental sources (rhizosphere, animal faeces) were biochemically tested for *in vitro* PGP activities, of which 46 strains were selected for biofertilizer formulation. These strains were further classified into "compatible" and "incompatible" groups according to their swarming behaviour and phylogenetic relationships. Biofertilizer mixtures enhanced cucumber performance overall, but compatibility-based mixtures led to more pronounced effects, including earlier flowering, increased plant mass, greater root branching, and a higher root mass fraction. Notably, while both compatible and incompatible groups exhibited comparable cumulative PGP scores, the compatible mixtures produced superior growth outcomes—indicating that functional synergy, rather than additive trait presence, drives biofertilizer efficacy. These findings suggest that prioritizing strain compatibility in biofertilizer design can optimize plant growth responses and support more targeted and sustainable microbial interventions in agriculture.

Poster Session

P088

Management of mango dieback disease caused by *Lasiodiplodia theobromae* in the United Arab Emirates through the application of endophytic actinobacteria

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Mango plantations in the United Arab Emirates (UAE) face a significant challenge with dieback caused by the fungus *Lasiodiplodia theobromae*. A total of 36 actinobacterial isolates were found in the roots of mango trees in the UAE. Of these, 12 were streptomycetes actinobacteria (NSA) and 24 were streptomycetes actinobacteria (SA). Antifungal metabolites, extracellular cell-wall-degrading enzymes (CWDEs), or a mixture of the two are thought to be responsible for the antagonistic effect of eleven isolates (8 SA and 4 NSA) against *L. theobromae*. All isolates were tested *in vivo* for their capacity to lessen the severity of lesions on fruits infected with *L. theobromae* using a "novel" mango fruit bioassay. For the purpose of testing mango seedlings for this pathogen, three isolates were chosen: two belong to *Streptomyces* spp. and one belong to *Actinoplanes* spp. These isolates showed the strongest inhibitory effect *in vitro*. Our research showed that *Streptomyces* UAE1 had antibiosis, CWDE production (especially chitinase), and siderophores as its antifungal properties. On the other hand, *Streptomyces* UAE2 and *Actinoplanes* UAE1 were identified as having antibiotic and CWDE production as their respective connections. Before being inoculated with the pathogen, mango seedlings that were pre-inoculated in greenhouse trials with the most promising actinobacterial isolates showed significant disease resistance. Applying specific biocontrol agents (BCAs) significantly reduced mango dieback disease severity indices compared to just the pathogen, proving their efficacy in managing the disease. When compared to the other two biocontrol agent treatments, the number of defoliated leaves and conidia counts of *L. theobromae* were significantly reduced in mango seedlings treated with *Streptomyces* UAE1. The reduction was threefold and sixfold, respectively. *Streptomyces* UAE1's synergistic antifungal properties, acting through various pathways, prevented *L. theobromae's* in planta invasion. To our knowledge, this is the first study to use endophytic microbial antagonists as a biological control agent against *L. theobromae* in mango seedlings. The capacity of endophytic actinobacteria, whether native to the UAE or found elsewhere, to inhibit mango dieback disease has never been reported before.

Poster Session

P089

The microbiome of sugar beets displaying a healthy phenotype in SBR-infested fields

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Sugar Beet (Beta vulgaris) is the main source of sugar production in temperate climates. In recent years, the leafhopper-transmitted bacterial disease "Syndrome Basses Richesses" (SBR) caused major economic losses. We observed sugar beet displaying a healthy phenotype in otherwise highly SBR-infested fields. This research aimed to compare the microbiomes of these disease-tolerant sugar beets to neighbouring symptomatic beets. Bacterial and fungal communities of sugar beet rhizosphere, endosphere, and phyllosphere were analysed using 16SrRNA and ITS amplicon sequencing. In addition, pathogen presence was tested using pathogen- specific PCR protocols. Fungal community composition differs between symptomatic and non-symptomatic beets in all plant compartments, while bacterial community composition always differs in phyllosphere, differences in endosphere and rhizosphere are field-dependent. Non-symptomatic beet microbiomes partially contained the SBR- causing pathogens (Candidatus Arsenophonus phytopathogenicus, Candidatus Phytoplasma solani). Our results indicate that the healthy phenotype in SBR-infested sugar beet fields incorporates pathogen tolerance and suppression of symptoms, and is at least partially based on differences in the sugar beet microbiome. Future breeding efforts and research on microbial isolates from non-symptomatic sugar beets and their role in SBR prevention can contribute to sustainable solutions for sugar beet production in SBR-affected regions.

Poster Session

P090

Investigating bacterial interactions in lung infections by 3D bioprinting and air-liquid interface model

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Chronic lung infections often comprise polymicrobial communities shaped by genetic and ecological interactions. Within such infection communities, *Pseudomonas aeruginosa* (*Pa*) shows intra-clonal diversification, driven by genome-wide mutations and micro-niche-specific selective pressures. Pa employs its Type VI secretion system (T6SS) against *Staphylococcus aureus* (Sa), which influences its microbial dominance during infection progression. However, the spatial and genetic dynamics underlying these interactions are still not understood.

Consequently, we have developed a lung infection model using Air-Liquid Interface (ALI) epithelial cultures, closely mimicking human airways. Additionally, we use 3D bioprinting to precisely position bacterial cells onto ALI cultures at defined microscale distances, providing controlled conditions for investigations of bacterial interactions in connection with colonization of airway epithelial tissues. This approach allows us to study bacterial complementation, competition, T6SS-mediated antagonism and *Pa* dynamic phenotypes at different infection sites.

We have successfully optimized 3D bioprinting for *Pa* strains on ALI cultures, ensuring bioink biocompatibility with both *Pa* and BCi-NS1.1 (Bronchial cell line-nonsmoker) airway epithelial cells. We obtained uniform and reproducible bacterial droplets at micrometre precision. We have performed preliminary investigations of the interactions between Pa/Pa Type VI secretion mutants (PAO1 Δ*H1, PAO1* Δ*H2)* and *Escherichia coli,* which have demonstrated increasing antagonistic behaviour at decreasing spatial distances, underlying the role of micro-scale positioning in interspecies interactions. The ongoing work is focusing on *Pa-Sa* interactions to study the genetic and ecological factors conditioning their coexistence/competition in lung infections.

By associating an in vitro physiologically relevant lung infection model with 3D bioprinting precise spatial control, we strive to further understand bacterial genetic and ecological interactions which shape lung infections, with the aim of developing an innovative approach which can provide improved understanding of infection dynamics and possible advancement of more targeted antimicrobial strategies.

Poster Session

P091

Bacterial composition of *Polygenis* fleas associated with wild echimyid rodents in the brazilian pantanal wetland

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Fleas present an important role as vectors of various zoonotic bacterial agents, especially *Bartonella* spp., that can infect domestic and wild animals, as well as humans. Understanding flea microbiomes is essential to assess their potential role in disease transmission and ecological interactions. This study aimed to explore the bacterial composition and diversity in *Polygenis* fleas. For this purpose, fleas were collected from wild rodents in December 2018 and February 2019 in the Nhecolândia region, Pantanal, MS, central-western Brazil. A total of 21 flea specimens, identified as *Polygenis* (*Polygenis*) *bohlsi bohlsi*, were collected from 12 *Thrichomys fosteri*. To assess bacterial diversity, fleas were individually processed, cleaned and disinfected, and DNA was extracted using the QIAGEN® DNeasy Blood & Tissue Kit, including homogenization and extraction controls. The DNA was used to construct libraries targeting the V3-V4 region of the 16S rRNA gene and sequenced on the Illumina NovaSeq 6000 platform. Raw sequences were processed, and the DADA2 pipeline was used to infer ASVs. Taxonomic assignments were performed using the Greengenes 2 database. Microbiome analyses were conducted using the *Phyloseq* and *Microeco* packages. High-throughput sequencing of 16 fleas (12 females, 4 males) generated a mean of ~74K (±40K) paired-end reads per library. After quality control, ~32K (±19K) sequences were retained, identifying 1,989 bacterial ASVs. After decontamination and rarefaction, 1,815 ASVs remained. Microbial diversity varied by sex. Of the total ASVs, 72.4% (1,296 ASVs) were exclusive to male fleas, 22.4% (400 ASVs) to females. Alpha diversity was significantly different for Faith"s Phylogenetic Distance and Core Dominance Abundance (Mann–Whitney U, $p \leq 0.05$). Beta diversity, measured by the Bray-Curtis dissimilarity distance, also showed significant sex-based differences ($p \leq 0.05$). Pseudomonadota was the predominant phylum, with a mean abundance of 80.9% (±27.9). Within this phylum, *Bartonella* was exclusively detected in males (36.9% ±44.3), while *Wolbachia* was predominant in females (82.3% ±23.8) and showed lower abundance in males (2.9% ±3.0). This study provides insights into the microbiome of *Polygenis* fleas, highlighting the predominance of Pseudomonadota and the distribution of *Bartonella* and *Wolbachia*. Future studies should investigate the effect of *Wolbachia* abundance on the vectorial compacity of *Polygenis* fleas in transmitting *Bartonella* spp. to rodents.

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Keywords: *Bartonella*; *Wolbachia*; pathogens, symbionts.

Poster Session

P092

The effect of microbiome in non-symbiotic coral on the larvae settlement

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Corals play a crucial role for sustainable marine ecosystems and global carbon cycle. Thus coral restoration is important in ocean engineering. The function of coral microbiome is more critical in non-symbiotic, azooxanthellate corals and the complex of community leads to healthy coral life and also the pattern of abundance and diversity shapes coral resilience and adaptation to environmental change. The high latitude corals are facing a decline or a distribution change by increased seawater temperature like as coral reefs around tropical area encountering a tremendous decline and bleaching event. In this study, we investigated the microbiome composition in azooxanthellate coral and compared it between corals under reproduction period and non-reproduction period and also habitat seawater and non-habitat seawater with seasonal variation. In addition, we examined the effect of microbiome on the coral larvae settlement. The majority of OTUs significantly shifted in corals under reproduction period and in coral habitat seawater indicated distinction in the relative abundance of bacteria compartment/site-wise. Richness and diversity were higher, and more taxa were enriched in the corals under reproduction period and coral habitat seawater in summer. Flavobacteria and alphaproteobacteria dominated corals under reproduction period and coral habitat seawater in summer. Flavobacteriaceae and Oceanospirillaceae showed the most dramatic difference between corals in reproduction period and non-reproduction period. Flavobacteriaceae and rhodobacteriaceae showed the biggest composition difference between coral habitat seawater and non-habitat seawater. In the larvae settlement experiment, coral larvae settled on the microbiome coated surface 70% higher than non-coated surface and the normal polyp development were enhanced in group on the microbiome coated surface. We suggest that coral restoration through their microbiome could be a self-sustaining tool in worldwide coral decline. This work was supported by Marine Biotics project (20210469) funded by Ministry of Ocean and Fisheries, Korea.

Poster Session

P093

Mycolicibacterium sp. PO1's pyrene degradation was modulated by *Dermacoccus nishinomiyaensis* in a density-dependent manner

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The pyrene-degrading bacterium *Mycolicibacterium* sp. PO1 was isolated from mangrove sediments, and its pyrene-degrading capacity was enhanced by interacting with strains from the same source [Wanapaisan *et al.*, 2018, *J. Hazard. Mater.*, **342**, 561-570]. However, traditional enrichment methods significantly reduced the opportunity to obtain interacting strains due to competition, limiting our understanding of the actual behavior of microbial communities. In this study, we used fluorescence-based, single-cell technology to screen environmental bacteria capable of modulating the pyrene-degrading ability of PO1 and investigated the underlying mechanisms.

Pyrene was crystallized at the bottom of 96-well plates, and a minimal liquid mineral medium was added to these "pyrene micro crystal plates". PO1 and an environmental bacterium extracted from wheat cultivation soil were single-cell inoculated using flow cytometry to prepare 756 two-strain consortia. The cultures were statically incubated at 30°C for up to 18 days, during which residual pyrene was quantified using fluorescence microscopy (Figure).

We obtained 11 and 19 two-strain consortia containing environmental bacteria capable of enhancing or suppressing the degradation ability of PO1, respectively. 16S rRNA gene sequencing revealed that *Dermacoccus nishinomiyaensis* was the most abundant strain, comprising both pyrene-degradation-enhancing and -suppressing partners. We isolated *D. nishinomiyaensis* strains DO1 (suppressing) and DO2 (enhancing) for detailed analyses. When PO1_mS (PO1 containing the mScarlet gene in its chromosome) was co-cultured with DO1 or DO2 at 10^3 CFU/mL, DO1 suppressed PO1's pyrene degradation, while DO2 enhanced it. DO2 formed larger bacterial clumps than DO1, and PO1 appeared to be attracted to DO2, which could facilitate pyrene degradation. When the initial cell concentration was increased to 10^7 CFU/mL, both DO1 and DO2 inhibited PO1's pyrene degradation and biofilm formation. The antagonism assay suggested that DO1 and DO2 released growth-inhibitory compounds only when co-cultured with PO1. A genome comparison between DO1 and DO2 revealed nine SNPs and four insertion/deletions. Notably, two transcriptional regulators appeared to be nonfunctional in DO2, which may contribute to the differing effects on PO1's pyrene degradation. DO1 and DO2 altered PO1's pyrene degradation in a density-dependent manner, suggesting the involvement of quorum sensing, which should be further studied.

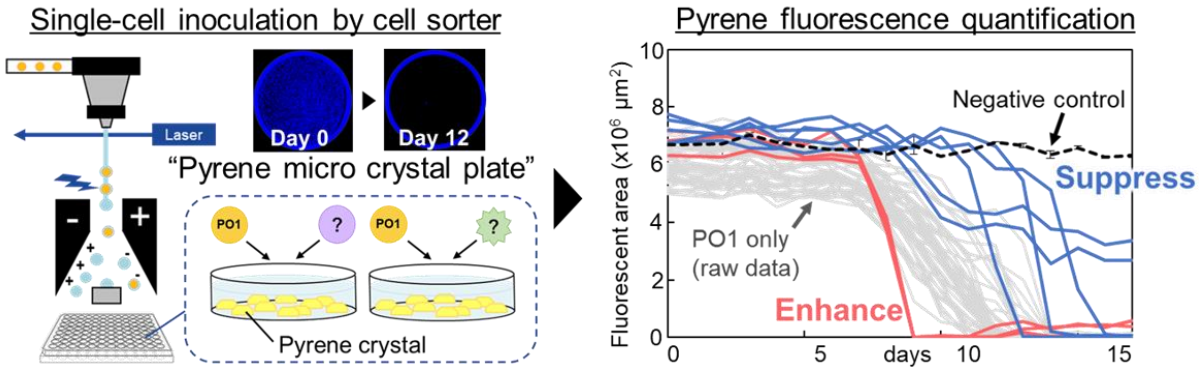

Figure. Screening overview

Poster Session

P094

Characterization, antibacterial, and cytotoxic activities of silver nanoparticles using the whole biofilm layer as a macromolecule in biosynthesis

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Recently, multi-drug resistant (MDR) bacteria are responsible for a large number of infectious diseases that can be life-threatening. Globally, new approaches are targeted to solve this essential issue. This study aims to discover novel antibiotic alternatives by using the whole components of the biofilm layer as a macromolecule to synthesize silver nanoparticles (AgNPs) as a promising agent against MDR. In particular, the biosynthesized biofilm-AgNPs were characterized using UV-Vis spectroscopy, electron microscopes, Energy Dispersive X-ray (EDX), zeta sizer, and potential while their effect on bacterial strains, and normal cell lines was identified. Accordingly, biofilm-AgNPs have a lavender-colored solution, spherical shape, with a size range of 20–60 nm. Notably, they have inhibitory effects when used on various bacterial strains with concentrations ranging between 12.5 and 25 µg/mL. In addition, they have an effective synergistic effect when combined with phage ZCSE9 to inhibit and kill Salmonella enterica with a concentration of 3.1 µg/mL. In conclusion, this work presents a novel biosynthesis preparation of AgNPs using biofilm for antibacterial purposes to reduce the possible toxicity by reducing the MICs using phage ZCSE9.

Fig. 1

Poster Session

P095

Screening and identification of novel chitin deacetylases for the sustainable valorisation of chitin

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Chitin is one of the most abundant organic molecules after lignocellulose, with an annual global production estimated between 10^{10} and 10^{11} tons. However, its direct applications are largely limited to low-value products (e.g., animal feed, agricultural fertilizers) due to the high crystallinity and rigid structure. In contrast, derivatives such as chitosan, *N*-acetylglucosamine, and chitooligosaccharides, which result from chitin's deacetylation and/or hydrolysis, have gained attention for their water solubility and various beneficial bioactive properties. As a result, there is growing interest in discovering novel chitinases and chitin deacetylases (CDAs) that could enable the targeted and environmentally friendly production of these compounds, as opposed to the conventional chemical methods. To this end, both in silico and functional screening approaches are being employed to identify new CDAs from bacterial strains sourced from diverse ecological environments, ranging from European soils to the extreme conditions of the Antarctic Ocean. So far, three putative CDAs have been identified from a soil-derived *Streptomyces sp.* MEL8 strain, a marine Antarctic *Acinetobacter sp.* C33 strain, and from the type species *Nonomuraea angiospora* DSM 43173T. Biochemical characterization of these enzymes has revealed promising properties, including their temperature activity profiles and tolerance to various salts, solvents, and detergents. These findings are expected to support the development of sustainable bioprocesses for chitin valorization, with applications spanning multiple industrial sectors, such as polymer functionalization, enzyme immobilization, biocatalysis, as well as packaging, biomedical, pharmaceutical, cosmetic, and food industries.

Poster Session

P096

In-Silico characterization of Sirtuins in acetic acid bacteria reveals a novel phylogenetically distinctive group

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Question

Sirtuins are NAD^+-dependent deacylases involved in metabolism, stress response, and longevity regulation across all domains of life. While their functions in eukaryotes and some bacteria are well documented, their presence and role in acetic acid bacteria (AAB) remain largely unexplored. This study aimed to characterize sirtuin homologs in AAB using *in silico* approaches to assess their evolutionary relationships and potential functions (Jugović & Trček, 2025).

Methods

Publicly available AAB genomes were screened for sirtuin homologs, and phylogenetic classification was performed using the Neighbor-Joining method. Protein structure predictions were generated using AlphaFold, and conserved motifs were identified with sequence alignment and motif discovery tools. A Python script was made using sequence alignment and similarity scoring.

Results

Our findings reveal that sirtuins are present in 21% of AAB genomes, with *Acetobacter* and *Novacetimonas* uniquely harboring three distinct types. Two of these types, SIR2 and SIR2_2, form a previously unrecognized phylogenetic clade distinct from known sirtuin classes, called SirAAB, which is subdivided into SirAAB-L and SirAAB-S (Figure 1). Structural modeling confirmed the presence of conserved NAD^+-binding sites, supporting their function as deacylases. To address the detection of SIR2_2 variants, a Python script was developed.

Figure 1. Unrooted phylogenetic tree represents the classification of AAB sirtuins. Sirtuins labeled from SIRT1 to SIRT7, Class U sirtuin, SirTM and *Escherichia coli* sirtuin are reference sequences. The newly identified SirAAB class is subdivided into SirAAB-L and SirAAB-S, based on phylogenetic clustering. Bootstrap values indicate the confidence level of each branch (green = high confidence, red = low confidence). The scale bar represents 10% of estimated sequence differences.

Conclusions

These results highlight the evolutionary diversity of bacterial sirtuins and suggest their potential role in metabolic regulation and stress adaptation in AAB. The revelation of this novel sirtuin group may open avenues for biotechnological applications, including enhancing bacterial stress tolerance and optimizing industrial bioprocesses.

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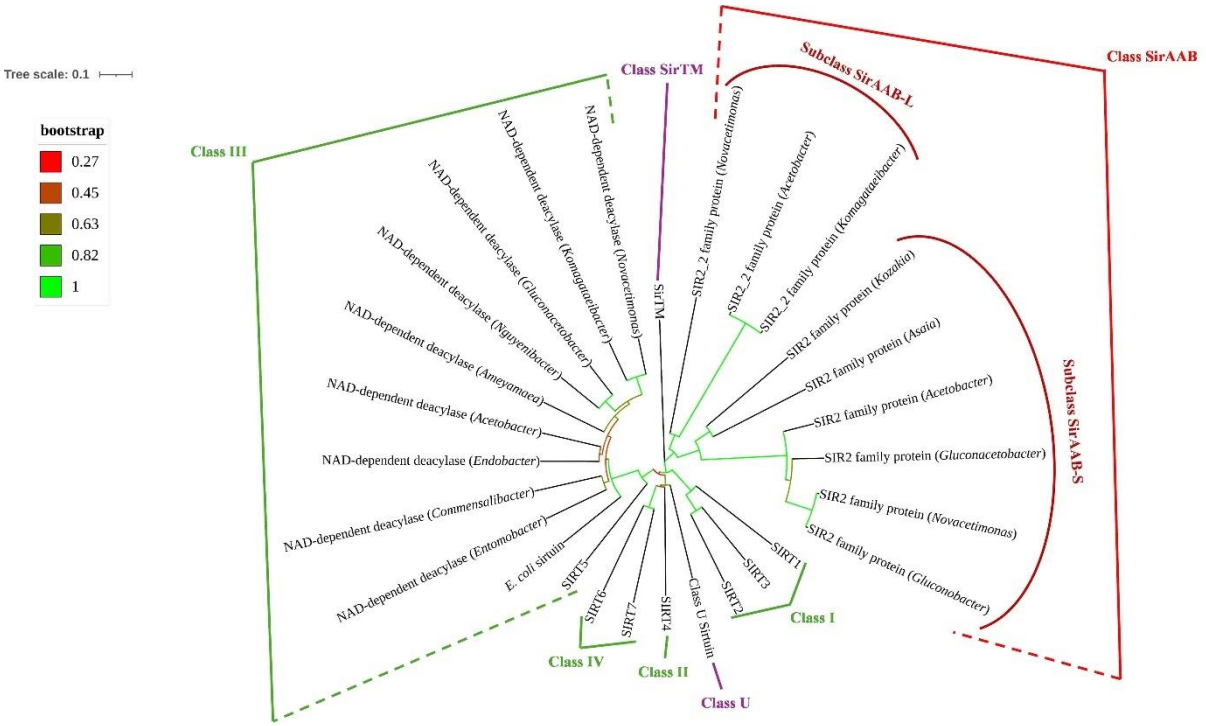
Fig. 1



Poster Session

P097

Bacterial diversity of marine biofilm communities in terra nova bay (Antarctica) by culture-dependent and -independent approaches

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The Antarctic continent is known as the coldest, driest, windiest and most inaccessible continent on the Earth [1]. Antarctic microorganisms display unique physiological features to survive in such harsh constantly shifting conditions, including the formation of complex biofilm structures [2]. Despite microbial diversity of the Southern Ocean waters being previously described as comparable to that of tropical and temperate oceans, Antarctic marine biofilms, which are considered hot spots of microbial diversity, are still poorly investigated [3]. In our work, we applied both culture-independent and -dependent approaches to investigate bacterial diversity of marine biofilm communities colonising polyvinyl chloride panels submerged in Terra Nova Bay (Ross Sea, Antarctica). Panels were deployed in two sites subjected to a different degree of anthropogenic impact (Road Bay [RB] impacted site and Punta Stocchino [PTS] control site). Biofilm samples were collected after 3 or 12 months to evaluate both short- and long-term microbial colonisation. Taxonomic composition of the microbial community was studied by 16S rRNA gene amplicon sequencing. *Proteobacteria* was the predominant phylum, followed by *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia,* and *Firmicutes*. Impacted RB biofilms were found to contain a relevant fraction of potentially pathogenic bacterial genera, accounting for 27.49% of the whole community. In parallel, a total of 86 psychrotolerant bacterial strains were isolated from the biofilm samples using culture-dependent techniques designed to enrich in *Actinobacteria*. These strains were assigned to three different phyla: *Actinobacteria* (54.65%), *Firmicutes* (32.56%) and *Proteobacteria* (12.79%). 2.73% of genera identified by metabarcoding were recovered also through cultivation, while, remarkably, 11 additional genera were uniquely yielded by cultivation. Moreover, functional screening of the isolates revealed their hydrolytic and oxidative enzyme activity patterns, giving new insights into the metabolic and biotechnological potential of microbial biofilm communities in Terra Nova Bay seawater.

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Poster Session

P098

Eco-evolutionary dynamics of host–microbiome interactions in a natural population of closely related mouse subspecies and their hybrids

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Closely related host species share similar symbionts, but the effects of host genetic admixture and environmental conditions on these communities remain largely unknown. We investigated the influence of host genetic admixture and environmental factors on the intestinal prokaryotic and eukaryotic communities (fungi, parasites) of two house mouse subspecies (*Mus musculus domesticus* and *M. m. musculus*) and their hybrids in two settings: (i) wild-caught mice from the European hybrid zone and (ii) wild-derived inbred mice in a controlled laboratory environment before and during a community perturbation (infection). In wild-caught mice, environmental factors strongly predicted the overall microbiome composition. Subspecies' genetic distance significantly influenced the overall microbiome composition, and each component (bacteria, parasites and fungi). While hybridization had a weak effect, it significantly impacted fungal composition. We observed similar patterns in wild-derived mice, where genetic distances and hybridization influenced microbiome composition, with fungi being more stable to infection-induced perturbations than other microbiome components. Subspecies' genetic distance has a stronger and consistent effect across microbiome components than differences in expected heterozygosity among hybrids, suggesting that host divergence and host filtering play a key role in microbiome divergence, influenced by environmental factors. Our findings offer new insights into the eco-evolutionary processes shaping host–microbiome interactions.

Poster Session

P100

Artificial humic substances for soil remediation and carbon sequestration

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Soils are complex systems shaped by minerals, organic matter, and organisms, forming the foundation of life on land and food production. Yet, agriculture often disrupts soil processes, releasing carbon as CO_2, causing erosion, and polluting waterways. Humic substances (HS) are a pivotal component of soil organic matter, naturally formed during the decomposition of plant and animal biomass. Although their assembly is slow, they enhance nutrient availability, soil structure and microbial activity. Hydrothermal humification (HTH), defined as the processing of waste plant biomass with heat, alkali and autogenous pressure, can produce Artificial Humic Substances (AHS) in under 2 hours. HTH is tailored to produce linear disordered polymers, rich in oxygen-containing groups, along with other low-molecular-weight intermediates [1]. The presence of base ensures a complete retro-aldol addition reaction of sugars from cellulose and hemicellulose by keeping pH above 7 and preventing dehydration and fast uncontrolled condensation of intermediates. The resultant AHS have been shown to have a chemical structure and behavior similar to natural HS extracted from soil, peat, or leonardite [1-3]. AHS have a positive effect on plant growth and trigger C accumulation in soil via photosynthetic bacteria activity [3-5]. Our vision is to harness AHS to target microbial communities and metabolic processes. We aim to boost Carbon Use Efficiency (CUE), enhance carbon fixation, and optimize photosynthesis. This approach will unlock AHS's potential as a climate solution, sequestering C in soils.

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Poster Session

P101

How microbes affect one another's mutation rates in mixed communities

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The mutations that are fundamental for adaptation occur at different rates, dependent on ecological factors. For example, our previous work shows that high-density bacterial populations have lower spontaneous mutation rates, a phenomenon dubbed density-associated mutation rate plasticity (DAMP) (Krašovec *et al*. 2017, https://doi.org/cb9s).

This negative relationship between mutation rate and population density arises from the collective ability of microbial populations to control hydrogen peroxide (Green *et al*. 2024, https://doi.org/m8zt). Thus, *Escherichia coli*"s reduction in mutation rate in denser populations is lost in peroxide degradation-deficient cells and restored by the presence of wild-type cells in a mixed population.

This raises the question of whether similar effects of one organism on another"s mutation rate occur in mixed-species communities.

We have tested communities of *E. coli* cocultured with *Enterococcus faecalis* or *Pseudomonas aeruginosa* using high-throughput fluctuation assays. We find that average mutation rates and levels of DAMP are affected by coculture. In other words, rates of antimicrobial resistance in species 1 do depend on the presence/absence of a species 2.

Advancing understanding of mutation rates in mixed communities is critical for understanding the interactions of ecology and evolution and may help identify mechanisms that promote or hamper emergence of important traits such as antimicrobial resistance.

Poster Session

P102

Gut microbiota of wild and domestic animals in Slovenia – insights into diversity, taxonomic profiles and the effects of domestication

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Understanding animal gut microbiota is essential from a One Health perspective, as it is closely linked to environmental microbial networks and the spread of zoonotic diseases. By understanding how diet, taxonomy, domestication, and habitat shape these communities, we gain valuable insights for conservation strategies and agricultural practices, as well as public health management.

Here, we present a microbial profiling study of 715 fecal samples from over 50 animal species, including both domesticated and wild animals, collected across Slovenia. The samples were subjected to 16S rRNA gene (V3-V4 hypervariable region) sequencing and analysis of zero-radius operational taxonomic units (ZOTU). We examined taxonomic composition, diversity patterns, and variability both within and between species.

Our results reveal significant differences in alpha diversity linked to diet, with herbivores displaying higher diversity than carnivores. Beta diversity analyses show that microbiomes cluster primarily by host taxonomy, though environmental factors also contribute to variation. In particular, microbiota of wild and domestic relatives (e.g., wild boar vs. pig) exhibits distinct taxonomic and functional profiles. A core microbiome analysis of animal classes (Mammalia and Aves) highlights taxa with potential ecological and functional importance, providing targets for future culturing and omics studies.

The patterns observed in this study provide a framework for understanding how animal microbiomes are shaped by their environments and evolutionary history. These insights could guide future research into developing targeted interventions for animal conservation, agricultural sustainability and mitigating public health risks associated with zoonotic diseases. Furthermore, the microbiota library can serve as a foundation for developing Microbial Source Tracking methods to identify sources of fecal contamination in Slovenia and surrounding regions.

Poster Session

P103

Harnessing indigenous greek microalgae for sustainable biofuel and lutein production

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The energy crisis and climate change from fossil fuel use have driven the search for renewable alternatives. Photosynthetic biomass cultivated for bioenergy production has the potential to yield substantial amounts of lipids, which can be readily converted into a liquid fuel feedstock known as biocrude, which is compatible with the existing petroleum refinery technologies. However, cultivating biomass solely for biofuel production displays economic restraints due to high costs. A recent technological shift emphasizes a biorefinery approach, utilizing biomass not only for biodiesel but also for extracting high-value products, aligning with the principles of a circular economy. In this study, we exploited the Greek algal biodiversity by isolating and genetically characterizing by sequencing 18S rRNA gene and ITS region of indigenous microalgae strains from the coastal area of Aspropyrgos and Koumoundouros lake, in Attiki region, Greece. Physicochemical growth study was performed in 4 different conditions of NaCl (35 mM, 75 mM, 100 mM and 250 mM) and Na_2Co_3 (80 mM, 100 mM, 160 mM, 200 mM and 250 nm) in respect to optimization of biomass production. Concentrations effects of NaCl and Na_2CO_3 on the fatty acid methyl esters (FAME) and lutein profile of two selected strains was analyzed by gas chromatography and high-performance liquid chromatography. Our results indicating that under specific conditions applied, both strains shift to consistent higher lipid and lutein production, a fact that is very important for further industrial exploitation.

Poster Session

P104

Gut microbiome as a conservation tool – a study on the endemic small mammal, balkan snow vole (*Dinaromys bogdanovi*)

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Animals held in under human care face unique challenges, including changes in diet and husbandry practices all of which can impact their microbiomes differently than in wilderness. In this study, we investigated the gut microbiome of the Balkan snow vole, a charismatic rodent inhabiting the Balkan mountains. This small mammal, also known as Martino's vole (*Dinaromys bogdanovi* Martino, 1922; Arvicolinae; Cricetidae; Rodentia), is believed to be the sole surviving member of an ancient lineage of Tertiary origin, having evolved independently from other extant voles and lemmings since the late Miocene. It is classified as a paleoendemic species and categorized as Vulnerable (VU) by the IUCN, while in Croatia, it is listed as Data Deficient (DD) and is strictly protected by national regulations as an endemic species of the Dinaric karst.

Zagreb Zoo in Croatia has been actively involved in the *Dinaromys* research and has housed an *ex situ* population since 2011, originating from nine individuals collected from Mt. Dinara. In addition to various studies on the species" diet and behavior conducted at the zoo, we collected faecal samples from 18 adult individuals in captivity and compared their microbiome taxonomic composition with that of 29 wild adult individuals sampled from Pelješac, Croatia, in March and April 2023.

The analysis of 16S rRNA gene profiles obtained via amplicon sequencing revealed a substantially higher bacterial diversity in wild individuals compared to those kept under human care. A large portion of the shared taxa in both wild and captive animals belonged to Lachnospiraceae and Muribaculaceae, which are known components of the core mammalian microbiota. However, all captive animals exhibited a significantly higher proportion of Lactobacillaceae and Ruminococcaceae compared to wild animals. Conversely, wild animals showed a significantly higher abundance of ASVs belonging to Oscillospiraceae, Eggerthellaceae, Christensenellaceae, and the Clostridiales vadinBB60 group than captive ones.

Our study represents an important first step in establishing a microbiome dataset for this endemic species. Understanding its gut microbiome is crucial, as microbiome management strategies—such as microbial engineering or stewardship (e.g., prebiotics, probiotics, transplants)—can help mitigate the effects of captivity and support overall health. By contributing to this knowledge, we aim to enhance conservation efforts for this unique and vulnerable species.

Poster Session

P105

Metatranscriptomic investigation of functional genes associated with biofouling in an anaerobic membrane bioreactor (AnMBR) equipped with a rotary disk and floating media

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Introduction

Anaerobic membrane bioreactors (AnMBRs) offer an energy-efficient approach for wastewater treatment by integrating anaerobic digestion with membrane filtration. Despite their advantages, membrane biofouling remains a critical challenge, primarily due to the accumulation of extracellular polymeric substances (EPS) and microbial biofilm formation, which are influenced by quorum sensing (QS) mechanisms. This study aims to investigate key functional genes associated with biofouling in an AnMBR equipped with a rotary disk and floating media, utilizing metagenomic and metatranscriptomic analyses.

Methods

A lab-scale AnMBR treating synthetic wastewater was operated with two chemical oxygen demand (COD) conditions (350 and 700 mg/L). Samples were collected from both media-attached and suspended microbial communities for DNA and RNA sequencing. Functional gene annotation was performed using KEGG and QSDB databases to identify genes related to QS, EPS biosynthesis, and amino acid metabolism. Taxonomic assignments were determined using DIAMOND and MEGAN software to assess microbial diversity and gene expression levels.

Results

The metatranscriptomic analysis revealed that QS-related genes, particularly those involved in acyl-homoserine lactones (AHLs), were highly expressed in suspended microbial communities, correlating with biofilm formation. EPS biosynthesis genes associated with galactose and fructose-6-phosphate metabolism showed increased expression in suspended samples, suggesting their significant role in biofouling. Amino acid biosynthesis genes, contributing to protein-based biofilms, were also more actively transcribed in suspended bacteria. The microbial analysis identified Bacteroides, Syntrophus, and Geobacter as key contributors to biofouling, demonstrating their active involvement in QS and EPS production.

Conclusions

This study provides insights into the microbial mechanisms driving biofouling in AnMBRs, highlighting the role of QS and EPS biosynthesis genes in biofilm development. The findings suggest that controlling the activity of suspended microbial populations could mitigate biofouling, improving AnMBR performance and membrane longevity. Further research on biofouling control strategies targeting microbial interactions and gene expression is recommended.

Poster Session

P106

Old Story, New Approach – development of a 16S-23S sequencing approach for highly accurate pathogen identification from clinical and environmental samples

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Rapid and reliable bacterial identification from clinical and environmental samples is essential for optimizing diagnostics and antimicrobial therapy. While amplicon-based Sanger and NGS sequencing of the 16S rRNA gene is a common molecular method, its species discrimination is limited due to high sequence homology. Sequencing of the 16S-ITS-23S rRNA region has been proposed for more accurate pathogen identification but is still rarely used, mainly due to the lack of curated databases and primers. To overcome this obstacle, we leveraged the recently published GROND database (Genome-derived Ribosomal Operon Database) containing quality-checked 16S-ITS-23S rRNA operon sequences for all genomes in GTDB and Refseq.

MAFFT multiple sequence alignments were created using GROND-curated 16S-23S regions representing all species in GTDB and validated with RefSeq data. The resulting alignment contained 10,487 species in GTDB and bases present in more than 95 % of all sequences at a given position were considered conserved and formed the basis for identifying highly conserved regions. Candidate primers were then validated by alignment to all 103,991 genomes in GTDB 207.

Two bacterial PCR primer sets, generating 1 kb and 1.5 kb fragments, were designed from those conserved regions. Fragmenting the 4.3 kb 16S-ITS-23S rRNA region into multiple, slightly overlapping segments provides a more reliable approach by enhancing PCR efficiency with shorter amplicons and reducing the risk of complete fragment loss. PCRs were successfully tested on 31 isolates spanning seven bacterial classes. Initial species assignment was verified through Sanger sequencing and subsequent assembly, followed by BLAST analysis to determine the isolate identity at least to the species level using the GROND databases. In some cases, strain-level identification was possible via the NCBI nucleotide collection database. While minor discrepancies exist between databases, taxonomic resolution improves when analyzing the 16S-ITS-23S region rather than only the 16S rRNA region.

The development and evaluation of a multiplex PCR of both primer sets is planned, followed by NGS techniques. We further developed a universal primer set for bacteria and archaea, and first tests have shown promising results when applied to the aforementioned 31 bacterial isolates. It will soon be tested on archaea. Consequently, among other potential applications, metagenomic approaches for diverse microbiomes could be explored.

Poster Session

P107

Correlation between glucose deprivation and biofilm formation of ESBL – producing *Escherichia coli,* recovered from lower respiratory tract

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The World Health Organization (WHO) has classified extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-E), as critical pathogens for public health due to their increasing antimicrobial resistance profile. Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC), particularly pandemic *E. coli* sequence type 131 (ST131), are associated with high mortality due to ineffective antimicrobial treatment. In addition, treatment is often complicated by the formation of biofilms. The aim of our study was to determine whether biofilm formation depends on different culture media, with an emphasis on glucose concentration, and whether it is related to the genotype of clinical ESBL-EC strains, isolated from the lower respiratory tract between 2018 and 2022.

The ability to form biofilms was monitored by cultivation of the 116 ESBL-EC isolates in a microtiter plate (Calgary Biofilm Device). After 24 and 48 hours, biofilm formation was quantified spectrophotometrically in two different media with/without biocides. In addition, all isolates were classified into phylogenetic and clonal groups and sequence types and analyzed for the presence of selected virulence-associated genes (VAGs) and antimicrobial resistance genes using the PCR method.

Strong biofilm formation was observed in 18.1 % of ESBL-EC isolates in Luria-Bertani medium (LB) and in 51.7 % in minimal medium supplemented with 0.02 % glucose (MG-glc) after 24 hours of incubation. Using Spearman correlation coefficient, a statistically significant positive correlation was found between strong biofilm formation in MG-glc after 24 hours of incubation, sequence group ST131, phylogenetic group B2, antimicrobial resistance gene *bla*CTX-M-9, biocide resistance gene *emrE* and VAGs *afa*/*dra*, *fyuA*, *iha*, *iroN*, *irp2*, *kpsMT*II, *sat* and *usp*. Further, biofilm production was enhanced when MG-glc was supplemented with selected biocides at a 1/100 MIC concentration.

The results of our study indicate that very low glucose concentration triggers biofilm formation of extraintestinal ESBL-EC isolates of sequence type ST131 and that biofilm formation may also be related to the presence of some VAGs. These results could explain the survival and thus the spread of the isolates of the ST131 clonal group by biofilm formation on surfaces deficient in basic nutrients such as glucose and on medical devices treated with biocides.

Poster Session

P108

Isolation and draft genome sequence of *Paenibacillus sp.* JAB-Hum

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The genus *Paenibacillus*, a group of gram-positive bacteria differentiated from *Bacillus* due to morphological differences, includes a variety of biologically active compounds that have potential applications in fields such as medicine, agriculture, and livestock. These bacteria play an important role in the health and economy of society. In this study, we focused on *Paenibacillus sp. JAB-Hum*, a strain isolated from soil rich in organic matter, which was characterized by its potential to produce phenolic compounds. Notably, this strain is capable of producing shikimic acid at concentrations of approximately 18 g/L after 20 hours of fermentation in Horikoshi medium. Shikimic acid, essential for the biosynthesis of aromatic compounds, is traditionally produced by chemical synthesis. However, microbial fermentation, particularly using microorganisms like *Paenibacillus*, has emerged as a more efficient and sustainable alternative. To further explore its potential, we sequenced the draft genome of *Paenibacillus sp. JAB-Hum*. This genomic analysis aims to identify novel enzymes suitable for industrial applications. Our findings revealed a complete set of genes involved in shikimic acid synthesis, along with a significant collection of genes associated with plant growth promotion. 16S rRNA gene sequence analysis and Type Strain Genome Server (TYGS) analysis confirmed that *JAB-Hum* belongs to the genus *Paenibacillus*. Genome sequencing using a MiSeq sequencer yielded 19,037,241 reads, and the assembled genome sequence of *Paenibacillus sp. JAB-Hum* contained 38 scaffolds, with a total length of 6,451,526 bp and a GC content of 46.69%. In total, 5,672 predicted coding sequences were identified within the genome. Average nucleotide identity (ANI) analysis showed that *JAB-Hum* is most closely related to *Paenibacillus suaedae* chi10 (97.04%), and digital DNA-DNA hybridization (dDDH) analysis revealed a value of 74.3%. These findings highlight *Paenibacillus sp. JAB-Hum* as a promising candidate for shikimic acid production, likely due to the presence of key enzymes involved in the metabolic pathway responsible for its synthesis.

Keywords: *Paenibacillus sp.*, Shikimic acid, Draft genome sequence, Microbial fermentation, Plant growth promotion

Poster Session

P109

Enzyme immobilization of a novel arabinose isomerase – a strategy for enhanced tagatose production

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The rising global prevalence of diabetes and obesity has increased the demand for healthier sugar alternatives. D-Tagatose, a low-calorie natural sweetener, has gained attention as a safe substitute. Current production methods include chemical conversion with calcium hydroxide and enzymatic bioconversion, with enzymes offering advantages such as fewer by-products, milder reaction conditions, and lower production costs. In this study, we characterized a novel L-arabinose isomerase (AraR) from the rumen metagenome, with potential applications in low-calorie dairy products. AraR was heterologously expressed in Escherichia coli BL21 (DE3) using the pET28a vector and purified via affinity chromatography followed by gel filtration. Its enzymatic activity, assessed by the cysteine-carbazole-sulfuric acid colorimetric method, confirmed efficient conversion of D-galactose to D-tagatose. AraR exhibited thermostability, with optimal activity at 40–50 °C and functioning effectively under slightly acidic conditions (pH 6.0–6.5). Unlike many isomerases, it does not require metal ions (Mn^{2+} or Co^{2+}) for catalysis, simplifying industrial applications. Enzyme immobilization further enhanced its properties, increasing the optimal temperature by 10 °C (to 50–60 °C) and improving thermal stability at 40 and 50 °C. Additionally, immobilization extended AraR"s half-life, maintaining 100% activity for 28 days. These findings highlight AraR as a promising biocatalyst for large-scale rare sugar production, offering enhanced stability and efficiency for industrial applications.

Keywords: biotransformation; sweeteners; isomerization; tagatose

Poster Session

P110

Screening soil microorganisms for antimicrobial activity against *Escherichia coli*

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Multidrug-resistant bacteria, particularly those on the World Health Organization (WHO) list of priority bacterial pathogens (1), call for an urgent need of new antimicrobial agents. *Escherichia coli* is in the critical group of the WHO 2024 list, which shows how important the search for new antimicrobial agents against *E. coli* is. The aim of our study was to screen soil microorganisms for their antimicrobial activity against *E. coli*. Soil samples were collected from the tree rhizosphere in a biodiverse area of Maribor Island, Slovenia, and analysed for bacteria with antimicrobial activity against *E. coli*. Soil samples were collected in sterile polyethylene tubes and brought to the laboratory, where the samples were diluted to 10^{-3} and 10^{-4} in sterile saline solution and plated on nutrient agar (NA) and ISP2 agar and incubated at 25 °C for 5 days. After incubation, colonies with distinct inhibition zones were transferred to fresh NA plates and incubated again at 25 °C until visible growth occurred. Colonies were then used in agar overlay assays with and without chloroform on Mueller-Hinton agar performed in triplicate (2). Two uropathogenic *E. coli* strains DL92 and DL100 (3) were used to evaluate antimicrobial activity. Inhibition zones were measured after 16 hours of incubation. Of the 60 colonies with zones of inhibition initially identified on NA or ISP2 agar, 11 exhibited antimicrobial activity against DL92 and/or DL100 in an agar overlay assay; the inhibition zones measured as radial distance from the colony edge, ranged from 1 to 12 mm. Subsequent sequencing of the 16S rRNA gene revealed that seven isolates were *Streptomyces* sp. and one isolate was *Bacillus* sp. These results suggest that soil bacteria from natural environments may be a source of potential new antimicrobial compounds.

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Poster Session

P111

Impact of aminoglycoside antibiotic therapy on the intestinal microbiota of horses – molecular insights and gastrointestinal health implications

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Gastrointestinal disorders, one of the main causes of equine mortality, can be associated with changes in the gut microbiota, influenced by diet and the use of antimicrobials, especially aminoglycosides. These antibiotics, widely used, have a controversial impact on the gut microbiota, making it essential to understand their consequences in order to optimize treatments and minimize adverse effects. Thus, we assessed, through 16S rRNA V4 region sequencing, the changes in the fecal microbiota of horses treated with aminoglycosides. Eight horses (3 to 10 years old, 300-400 kg) were used, divided into two groups: GA (amikacin) and GG (gentamicin). The antibiotics were administered intravenously for 3 consecutive days every 24 hours (6.6 mg/kg for gentamicin and 20 mg/kg for amikacin). Fecal samples were collected at five time intervals, and the extracted and sequenced DNA was analyzed for gut microbiota composition using QIIME2 and MicrobiomeAnalyst. After quality control, sequencing resulted in 8,522,583 reads (mean 71,021), grouped into 7,055 ASVs, of which 66.3% were shared, while 18.5% were exclusive to GA and 15.2% to GG. The observed richness of ASVs did not show a difference ($p>0.05$). However, species richness and evenness, represented by the Shannon index, showed a significant difference. Beta diversity revealed that microbiota composition differed ($p<0.05$) between groups and over time. The most abundant phyla were Firmicutes (43%), Bacteroidota (36%), and Verrucomicrobiota (10%), with a similar distribution between the groups. The abundance of short-chain fatty acid-producing families Lachnospiraceae, Prevotellaceae, and Anaerovoracaceae, with log2FC close to zero, suggests a gut microbiota with a shared core between groups. In contrast, *Desulforispora, Lactobacillus*, and Prevotellaceae-Ga6A1-group were more abundant in GA, while Lachnospiraceae probable genus 10, *Lactococcus*, *Clostridium methylpentosum* group, Lachnospiraceae_NK4B4, and *Streptococcus* were enriched in GG. The administration of aminoglycosides did not significantly alter the richness of the equine gut microbiota but influenced its composition over time and between groups. These variations may impact the abundance of specific taxa, highlighting the importance of evaluating the effects of these antibiotics to optimize therapeutic strategies and minimize risks to gut health.

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Poster Session

P112

Structural prediction elucidates the DNA-binding mechanism of Pnd, a nucleoid-associated protein encoded on the catabolic plasmid pCAR1

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Nucleoid-associated proteins (NAPs) bind to DNA, organize bacterial nucleoids, and regulate DNA folding and transcription. The carbazole-degradative plasmid pCAR1, which belongs to the incompatibility (Inc) P-7 group, possesses three genes encoding NAPs: MvaT homolog Pmr, NdpA homolog Pnd, and HU homolog Phu. Our previous study demonstrated that the stability and transferability of pCAR1 in *Pseudomonas putida* KT2440(pCAR1) decreased significantly when *pmr* and *pnd*, or *pmr* and *phu* were disrupted simultaneously. Furthermore, disruption of *pnd* resulted in the decreased transcription of 174 genes [Suzuki-Minakuchi *et al.*, *Appl. Environ. Microbiol.*, 81: 2869-2880 (2015)]. These results suggested that Pnd binds to DNA and affects the transcription of genes on the chromosome and pCAR1. In this study, we investigated the DNA binding mechanism of Pnd.

Chromatin immunoprecipitation sequencing (ChIP-seq) was conducted to identify the DNA regions where Pnd binds in *P. putida* KT2440(pCAR1). However, the difference between the input control and the ChIP was small, and no significant binding sites were detected. The electrophoretic mobility shift assay (EMSA) demonstrated that the C-terminal-His-tagged Pnd, which was heterologously expressed in *Escherichia coli*, has DNA-binding ability, but no sequence specificity was observed. The structure of Pnd was predicted to form dimers using the AlphaFold version 2.2.0 (Fig. 1A). The dimeric model has a large cavity with many positively charged amino acid residues (Fig. 1B). These positive residues of Pnd formed hydrogen bonds with the backbone phosphate of the DNA double helix during a 10-ns molecular dynamics simulation (Fig. 2). Nine positively charged residues per monomer, located within 5 Å of the DNA, were substituted with alanine. The DNA-binding ability of the resultant protein, Pnd-9Ala, was assessed by EMSA. Pnd-9Ala exhibited a decreased DNA-binding ability compared to native Pnd. This result supports the structural model in which the Pnd dimer traps DNA in a cavity.

Given that the sequence specificity of Pnd was not observed in this study, NAPs other than Pnd encoded on pCAR1 may define the binding region of Pnd in the cell. Further studies are necessary to investigate the interactions between Pnd and other NAPs encoded on pCAR1, Pmr and Phu, and on chromosome in *P. putida* KT2440(pCAR1). This will clarify the intracellular functions of Pnd.

Fig. 1

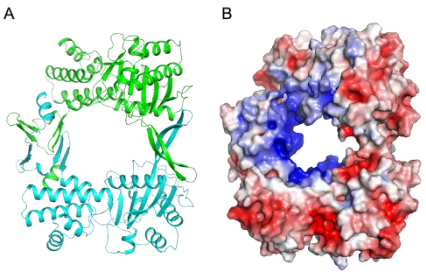

Fig. 1 Structure prediction of Pnd.
(A) Pnd dimer model predicted by AlphaFold2. Each monomer is represented in green and cyan. (B) Electrostatic surface potential of the Pnd dimer model. Red and blue indicate negative and positive charges, respectively.

Fig. 2

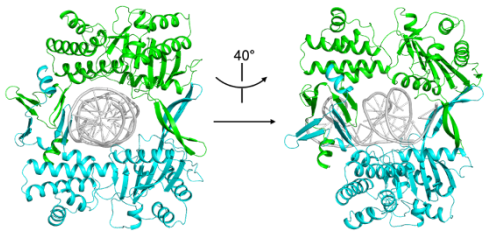

Fig. 2 DNA-binding model of Pnd.
DNA-binding model of Pnd predicted by molecular dynamics simulation. Each monomer is represented in green and cyan, and the DNA (PDB: 6Q1V) is represented in gray.

Poster Session

P113

Microbial connectivity and adaptive strategies driving biogeochemical cycling in ice-covered lakes of the McMurdo dry valleys, Antarctica

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The McMurdo Dry Valleys (MDV), located in Southern Victoria Land, Antarctica, adjacent to McMurdo Sound, represent one of the most extreme desert environments on Earth. These valleys are characterized by exceptionally low temperatures, humidity, and annual precipitation, as well as diurnal freeze-thaw cycles, desiccating winds, high sublimation and evaporation rates, and intense solar radiation. The MDV lack higher life forms and exhibit lower microbial biomass compared to temperate soils. The valleys also contain several enclosed drainage basins, such as Lakes Fryxell, Hoare, Joyce, and Bonney, which were formed by glacial movement. In this study, we assembled metagenome-assembled genomes (MAGs) from water samples collected from Lake Fryxell to investigate the roles of microbial communities in biogeochemical cycling within this ice-covered lake. Additionally, we compared these findings to previous metagenomic analyses from Lake Bonney to examine microbial adaptation and evolution in extreme environments, focusing on community structure and connectivity between lakes in the Dry Valleys. Our study provides novel insights into biogeochemical cycling in extreme environments and highlights the effective adaptation strategies and ecological roles of microorganisms in these ice-covered lakes.

[Supported by grants from KOPRI (PE25130)]

Poster Session

P114

Functional interchangeability of replication initiation proteins and *oriV* in PromA plasmids

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PromA is a plasmid group first proposed in 2009(1). These plasmids exhibit a broad host range and have been found in variety of environments. However, many of them lack accessory genes, including antimicrobial resistance gene or heavy metal resistance gene, making their ecological roles in the environment unclear. In our prior study, we obtained plasmids belonging to distinct subgroups of PromA(2). Here, we compared their features with a particular focus on their replication mechanisms.

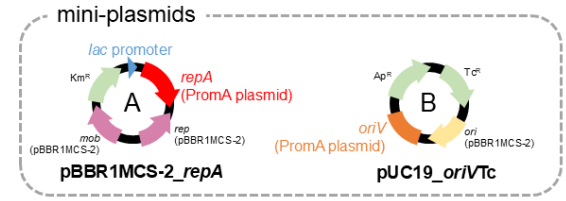
In PromA plasmids, replication is presumed to be initiated by RepA (a replication initiation protein) binding to iterons, which were repeat sequences within *oriV* (the origin of vegetative replication). Previously, we identified both RepA and *oriV* by using mini-plasmids(3). A comparative analysis of RepA and *oriV* across distinct PromA subgroups revealed that iterons were highly conserved (more than 85%). In contract, the *repA* gene and RepA protein exhibited lower sequence identity (*repA* gene: less than 70%, RepA protein: less than 80%). To investigate the functional interchangeability of RepA proteins and *oriV* among PromA subgroups, we conducted transformation experiments. A pUC19 vector carrying *oriV* was introduced into *Pseudomonas putida* harboring a mini-plasmid expressing different RepA proteins. These experiments were performed with 25 combinations of RepA and *oriV* derived from pKIFA124 (PromAα), pMH0613-68 (PromAβ-1), pYK0414-12 (PromAβ-2), pSN1104-11 (PromAγ), pSN0729-62 (PromAδ) (see figure). All combinations achieved successful replication, indicating that different RepA proteins could recognize and bind to *oriV* across distinct PromA subgroups. These findings suggested that RepA proteins and *oriV* of PromA plasmids were functionally interchangeable across distinct subgroups and that the iteron-binding region in RepA proteins might be conserved. Currently, we are investigating whether the RepA proteins of pMRAD02 (PromAε) could function with the *oriV* from other PromA plasmids.

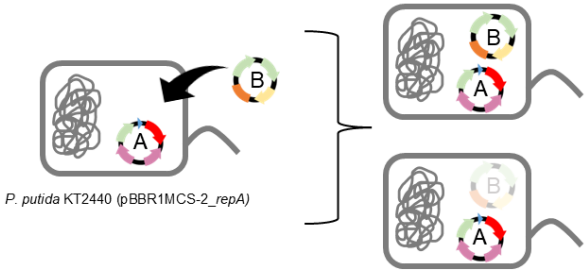
1) van der Auwera et al., 2009, *Antonie van Leeuwenhoek*, 96:193

2) Tokuda et al., 2023, *Microb Genom*, 9: 001043

3) Hayakawa et al., 2022, *Appl Environ Microbiol*, 88: e01114

Fig. 1





repA	*oriV*
pKIFA124 (α)	pKIFA124 (α)
	pMH0613-68 (β-1)
	pYK0414-12 (β-2)
	pSN1104-11 (γ)
	pSN0729-62 (δ)
pMH0613-68 (β-1)	pKIFA124 (α)
	pMH0613-68 (β-1)
	pYK0414-12 (β-2)
	pSN1104-11 (γ)
	pSN0729-62 (δ)
⋮	⋮

25 combinations

Poster Session

P115

Characterization of mobilizable IncQ2 plasmids – host range and antibiotic resistance gene distribution

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Horizontal gene transfer of antibiotic resistance genes (ARGs) via plasmids plays a crucial role in the emergence and dissemination of antimicrobial-resistant bacteria. Therefore, understanding the dynamics of antimicrobial resistance plasmids and ARGs is of great epidemiological significance. In our previous study[1)], an IncQ2 (a subgroup of mobilizable IncQ[2)]) plasmid, pKHI42, carrying multiple ARGs was obtained from a wastewater treatment plant in Germany. While IncQ1 plasmids, a well-studied subgroup of IncQ plasmids, are known for their broad host range, the host range of IncQ2 remains unclear. In this study, we aimed to characterize the IncQ2 plasmid, determine the ARGs it carries and identify its hosts.

To investigate the distribution of ARGs among IncQ2 plasmids, we analyzed the public plasmid database PLSDB[3)]. Of the 84 IncQ2 plasmids in PLSDB, 74 (88%) carried ARGs, while among 724 IncQ1 plasmids, 680 (94%) carried ARGs. The most common ARGs in IncQ2 were *qnrS2* (32/84), *tetA* or *tetC* (18/84), and *bla* genes (25/84). In contrast, IncQ1 plasmids exhibited a different distribution, with *qnrS2* (4/724), *tet* genes (456/724), and *bla* genes (531/724).

Interestingly, IncQ1 was isolated from a limited range of hosts, mainly *Enterobacterales*, whereas IncQ2 plasmids were found in more diverse set of hosts, including aquatic bacteria (*Aeromonadales*, *Acidithiobacillales*), suggesting distinct host adaptability. Notably, 15 of the 84 IncQ2 isolates were from some Gram-negative species of ESKAPE pathogens, which includes clinically significant antibiotic-resistant pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.). This suggests that IncQ2 plasmids could serve as crucial vehicles for ARGs, in both environmental and clinical ecosystems. Currently we are analyzing the host range of pKHI42, which was shown to be mobilized by a self-transmissible plasmid pKHI41[1)].

1) Hauschild et al., submitted for publication.

2) Loftie-Eaton and Rawlings, 2012, *Plasmid* 67, 15–34, doi: 10.1016/j.plasmid.2011.10.001.

3) Molano et al., 2025, Nucleic Acid Res., 53 (D1): D189-D169.

Poster Session

P117

Assessing the influence of agro-industrial by-products on the hemolymph and gut microbiota of *Tenebrio molitor*

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Edible insects are considered a potential resource for addressing food security issues due to their sustainability, high nutritional content, and ability to convert agro-industrial by-products, aligning with a circular economy perspective. The rearing substrate not only influences insect"s performance, but also plays a crucial role in shaping the insect-associated microbiota, which in turn affects the host's physiology and immune system. Particularly, the gut microbiota of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), one of the most extensively farmed insect species, has received little attention when raised on different agri-food by-products; as well as the hemolymph, a key mediator of nutritional and immunological homeostasis in insects, remains largely unexplored. Therefore, this research aimed to investigate the impact of different agro-industrial by-products composed by wheat bran or wheat bran + tomato peels or wheat bran + brewer"s spent grain on the microbial communities within the hemolymph and gut microbiota of *T. molitor* larvae. The analysis was conducted using high throughput 16S rRNA gene sequencing, while the abundance of bacterial communities was measured through qPCR targeting the 16S rRNA gene. Moreover, several chemical and biochemical parameters of larval hemolymph, including pH, and sugars were evaluated. Results highlighted that insects" performance varied depending on the by-products, characterized by different nutrient profiles. Microbiological analysis revealed that the larval hemolymph and gut microbiota varied depending on the agro-industrial by-products. This research offers valuable insights into the characterization of the hemolymph and gut microbiome in *T. molitor* under different rearing conditions, helping to bridge the knowledge gap, particularly in hemolymph microbiome studies. The use of agro-industrial by-products could potentially provide a cost-effective substrate for larvae to feed on, promoting a circular economy by reducing waste. Future studies will be focused to elucidate the dynamics of the hemolymph and gut microbiota, key elements to enhance insect health in mass rearing environment.

Poster Session

P118

Energy conservation during oxygen-independent denitrification and N_2O production in *Fusarium oxysporum* is primarily linked to substrate level phosphorylation

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Nitrous oxide is a potent greenhouse gas produced via denitrification in soils. Fungal denitrification has long been recognized as a potential source of N_2O emissions from soils. While many fungi produce significant amounts of N_2O, denitrification as the primary driver of this production has been questioned. Therefore, we investigated the role of denitrification for N_2O production and energy conservation in the model fungal denitrifier *Fusarium oxysporum*. Nitrite-supplemented *F. oxysporum* was incubated under hypoxic and oxic conditions for 24 h. Incubations in the absence of nitrite served as controls. N_2O production was similar in oxic and hypoxic treatments, but nitrite depletion was more prominent in oxic conditions. Ammonium concentrations increased in both treatments compared to their nitrite-free controls. Nitrogen balancing indicated that approximately half of the nitrite was converted to ammonium and less than 1 % were converted to N_2O in both treatments. Oxic incubations showed an increased glucose consumption and higher CO_2 production when supplemented with nitrite. Based on the ammonium found in the oxic incubations, at least 7.5 % of the electrons released during glycolysis were directed to the reduction of nitrite to ammonium. Pathway enrichment analysis indicated an up-regulation of glycolysis associated gene expression compared to the nitrite-free control. Expression of genes central to denitrification, including fungal *nirK* and *p450nor*, was significantly increased in both treatments relative to nitrite-free controls. These results suggest that (i) the expression of *nirK* and *p450nor* is oxygen-insensitive and primarily depends on nitrite, (ii) nitrite reduction to ammonium rather than denitrification is the primary electron sink under eutrophic conditions, and (iii) nitrite reduction to ammonium enhances glycolysis associated substrate level phosphorylation via regeneration of NAD+.

Poster Session

P119

Expanding the diversity of culturable coral-associated bacteria through aerobic, microaerophilic and anaerobic conditions – a quest for novel marine drug producers

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Benthic marine invertebrates, such as corals, play pivotal roles in ocean ecosystems and rely on microbial symbionts to function and thrive. Coral-associated bacteria are rich chemical reservoirs for innovative biotechnological applications. However, most cultivation attempts use nutrient-rich media and aerobic conditions, not corresponding to the habitat provided by the coral host, wherefore many coral symbionts remain uncultured.

This study aimed to (i) expand the cultivable diversity of coral-associated bacteria, (ii) compare the cultured fraction with the total bacterial community diversity, and (iii) evaluate the isolates" secondary metabolite biosynthesis potential and bioactivities. Bacteria of eight octocoral (*Leptogorgia sarmentosa*) specimens were cultured under aerobic, anaerobic and microaerophilic conditions, on diluted marine agar, and minimal medium supplemented with coral extract. Coral fragments and "culture plate washes" were 16S rRNA gene amplicon sequenced for bacterial diversity analyses.

Amplicon sequencing revealed intra-species variability in microbiome composition and dominance of the families *Endozoicomonadaceae, Mycoplasmataceae* and *Spongiibacteraceae*. This contrasts with the culture-dependent approach where the dominating families were *Roseobacteraceae*, *Vibrionaceae* and *Shewanellaceae*, the latter two showing less than 1% relative abundance in the *in-situ* samples. Of 1317 ASVs identified in the entire dataset, 233 (17.7%) belonged to the cultured fraction, and of these cultured ASVs, 27 (11.6%) derived from microaerophilc and anaerobic conditions. Our culture collection comprises 328 strains of 38 described genera plus 37 isolates not classifiable at genus-level. Notably, 13 genera have not been previously cultured from corals, including *Desulfotalea*, *Halodesulfovibrio* and *Propinigenium*, obtained only under anaerobic conditions. Of 196 screened isolates, 45 encode for polyketide synthase genes. Antibacterial assays highlighted *Bacillus safensis* LS_113 for its potent antibiotic activity against human pathogens, including methicillin-resistant *Staphylococcus aureus* and enteropathogenic *Escherichia coli*.

Our work demonstrates that alternative culturing conditions with varying oxygen regimes broaden the diversity of cultivable coral-associated bacteria. It provides access to taxonomic novelty and strains with promising secondary metabolite coding potential, underscoring their value as bioresources for blue biotechnology innovation.

Poster Session

P120

Unveiling the pangenomic trends in the phylum Patescibacteria through circularisation of metagenome-assembled genomes

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Patescibacteria forms a distinct monophyletic clade of diverse, largely uncultivated bacteria. These tiny bacteria (100-200nm in width & < 1 Mb genome size) are difficult to cultivate, have reduced genomes, lack many essential pathways, and appear to have symbiotic or parasitic relationships with others to compensate for these deficiencies. Obtaining circularised genome sequences, rather than only genome bins, is crucial for studying Patescibacteria eco-evolution in the context of gene loss and their symbiotic relationships to ensure full knowledge of the gene content of each genome. Circularised genomes also serve as reference scaffolds for future assemblies. In this study, we attempted to circularise Patescibacteria genomes reconstructed from 29790 metagenomes and analyse the pangenome of this phyla.

We performed circularisation using JORG, which refines and circularises metagenome-assembled genomes (MAGs) from Illumina metagenomes through iterative assembly (SPAdes & MIRA), binning (MetaBAT2), and read mapping (Mirabait). We screened 6000, 18485, and 5305 metagenomes from terrestrial, human, and marine data. Using MuDoGeR V1, we recovered 2971 (out of 51918) terrestrial, 608 (out of 459523) human, and 1483 (out of 126106) marine MAGs of Patescibacteria. We started with MAGs with 1 - 10 contigs and an average coverage of 25x - 250x. Based on our benchmarks, 76 (out of 2971) terrestrial, 53 (out of 608) Human and 52 (out of 1483) marine passed the required criteria for circularisation. We observed that memory usage varied from 800MB to 15GB and time from 125 to 1440 min for bins with coverage 25x - 250x. We created a Singularity container to install the tool and a script to filter the genomes with a high likelihood of circularisation. We have circularised 16 terrestrial genomes and 26 marine genomes and elongated 15 terrestrial MAGs and one marine MAG affiliated with Patescibacteria. We are currently circularising MAGs from human data.

A preliminary pangenome analysis of Patescibacteria reveals a loss of genes associated with secondary metabolism, suggesting that as their genomes shrink, their dependence on other species increases. Our study will shed light on the eco-evolutionary dynamics of Patescibacteria and advance our understanding of genome reduction in complex microbial communities. Further, our optimisation of JORG makes it ideal for refining viruses and small bacterial and archaeal genomes in Illumina platforms.

Poster Session

P121

Unveiling the microbial diversity of Etoliko lagoon – a unique extreme environment

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Etoliko Lagoon, a landlocked lagoon in Western Greece, represents an extraordinary ecosystem for novel microbial discoveries, particularly putative new phyla. This study explored the microbial diversity within the lagoon's anoxic sediments, characterized by extreme conditions including anoxia, high sulfate concentrations, and a permanent thermocline, using both next-generation sequencing and culture-dependent approaches.

Next-generation sequencing analysis revealed an exceptionally diverse microbial community with significant taxonomic novelty. Among the **205** unassigned bacterial OTUs identified by Illumina MiSeq, **5 were classified as putative new phyla**, potentially representing previously undocumented branches of the bacterial tree of life. Additionally, **10** putative new families, **9** putative new classes, **10** putative new orders, and **5** putative new genera were detected, highlighting the lagoon's remarkable taxonomic uniqueness.

Complementary Oxford Nanopore MinION analysis generated **1100 ASVs** with **94** unassigned taxa, while the eukaryotic community analysis identified **334 OTUs**, with **178** remaining unclassified. The dual sequencing approach provided comprehensive insights, with Illumina offering high accuracy for dominant taxa assessment and Nanopore providing enhanced taxonomic resolution through its long-read capability.

Culture-dependent techniques successfully isolated viable anaerobic bacteria from the sediment, including *Clostridium sulfidigenes*, *Paraclostridium benzoelyticum*, and *Terrisporobacter petrolearius*, whose genomes were subsequently sequenced.

The discovery of **putative new phyla** establishes Etoliko Lagoon as a significant reservoir of novel microorganisms. Further investigation of these uncharacterized organisms promises valuable insights into microbial evolution and adaptations to extreme environments. Understanding this unique microbial diversity is essential for comprehending the lagoon's ecological significance, as these communities drive biogeochemical cycles, nutrient cycling, and organic matter decomposition. This research advances our understanding of life's limits on Earth and the potential for microbial adaptation to harsh conditions.

Poster Session

P122

NFDI4Microbiota – empowering microbiota research with integrated data and digital solutions

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Over recent years, advances in omics data generation have opened up significant opportunities for researchers working with "big data." As a result, the challenges in the field have evolved, shifting from data collection to the reuse, interpretation, and analysis of vast, heterogeneous datasets. The NFDI4Microbiota consortium was formed to meet these new demands by offering data access, analytical services, training, and infrastructure to support the broader microbiological community.

NFDI4Microbiota aims to serve as a central hub established to assist both the German and international microbiological research communities. It comprises ten partner institutions backed by five professional societies and over 50 participating organizations. The consortium aims to accelerate the digital transformation within the microbiological community by providing solutions for data management through cutting-edge computational methods. It also seeks to streamline the entire research workflow, from initial data generation to the final stages of publication, including data submission. The consortium offers specialized training programs, infrastructure, and advanced computational tools to achieve this streamlining process.

The wide array of tools, methodologies, and resources offered by NFDI4Microbiota is designed to facilitate the transformation of new and existing data into meaningful scientific knowledge. Training opportunities are available in open science, research data management, databases, programming, data science, and data analysis. Furthermore, implementing a cloud-based infrastructure provides access to analytical tools, a knowledge base, and systems for analyzing, integrating, and storing microbiological data.

By creating this centralized resource, NFDI4Microbiota aims to become the essential connection for microbiota research in Germany and beyond. It offers researchers the infrastructure, expertise, and easy access to tools and training that enhance their work while supporting Open Science principles and ensuring that data remains Findable, Accessible, Interoperable, and Reusable (FAIR) in the future.

Poster Session

P123

Effects of submaximal training on the intestinal microbiota of horses kept on pasture

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Physical activity modulates human gut microbial metabolism and may induce changes in the intestinal microbiota of horses undergoing physical conditioning, with metabolic and endocrine repercussions. In this context, the metabolic effects of submaximal training have been studied in horses; however, the impact of physical conditioning on the gut microbiota remains poorly understood. This study investigated the influence of submaximal training on the composition of the intestinal microbiota of pasture-fed horses at three time points: before training (Submax), after seven weeks of exercise (Week7), and after 30 days of recovery (Trev30). The composition and diversity of the gut microbiota were analyzed by sequencing the V4 region of the 16S rRNA gene using the Illumina platform and bioinformatics tools. Beta diversity analysis revealed variations in species composition between groups ($p = 0.001$) without a significant impact on alpha diversity ($p > 0.05$). The abundance of the phylum *Proteobacteria* increased at seven weeks (Week7), followed by a reduction after 30 days (Trev30), suggesting an adaptive response to training. The forage-based diet helped maintain gut microbiota stability, preventing major changes in bacterial composition and promoting a balanced environment throughout the study. Fecal pH showed a slight decrease after seven weeks of exercise, with no statistically significant differences between groups ($p \geq 0.05$), reflecting metabolic adjustments. It stabilized after the recovery period at Trev30, suggesting a post-training microbial readjustment. Submaximal training temporarily altered the intestinal microbiota but did not induce permanent changes in the microbial community.

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Poster Session

P124

How the environment selects – a functional approach to microbial community analysis

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How do human-mediated activities influence the dispersal and selection of microbial communities in marine and soil environments? Can functional-level analyses, independent of taxonomic classification, reveal patterns shaped by environmental factors?

The Baas Becking tenet stating that "everything is everywhere, but the environment selects" suggests that to understand what the environment selects, we need to study the microbial functional features that facilitate that selection, not just taxonomy. Our study introduces a taxonomically independent approach to comparing microbial metagenomic samples through what we call the Functional Distance (FD) metric. Our method implements a beta diversity metric that quantifies gene ortholog similarity, providing a direct measure of functional composition between metagenomic samples without relying on species classification. By focusing solely on functions, it enables a clearer understanding of how environmental pressures shape microbial gene pools.

Unlike methods reliant on specific taxonomic frameworks, our approach allows comparisons based on genes with known functions, enabling the comparison of any given pair of metagenomic samples in terms of their functional gene content. This better reflects microbial gene flow, as microbes from different species can have similar functional capabilities. Our tool helps to better understand what the environment selects at the functional level, rather than being limited by taxonomic biases. We developed, tested, and will make this metric available for broader use in metagenomic research. We applied the FD metric to marine metagenomic datasets to assess the impact of environmental factors and uncovered distinct spatial patterns in gene distribution, including spatial autocorrelation and similarity gradients in polar regions and along the equator. Our results show that functional similarity is shaped either by dispersal or by local effects. Some genes show high sensitivity to environmental gradients, exhibiting greater diversity and even distribution, while others are more constrained, dominated by a few variants. By going beyond taxonomy, the Functional Distance metric provides a more accurate representation of microbial gene flow. Our findings emphasize the necessity of studying microbial dynamics through functional traits rather than taxonomic classifications, paving the way for a deeper understanding of microbial ecology.

Poster Session

P126

LipBK – a microbial lipase with potential for environmental and biotechnological applications

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Microorganisms play a fundamental role in maintaining environmental balance, actively participating in nutrient cycling and the degradation of organic compounds. Microbial lipases, in particular, have broad biotechnological applications due to their ability to catalyze lipid hydrolysis, influencing both natural and industrial processes. In this study, we characterized LipBK, a lipase produced by *Burkholderia* sp. (strain D15), investigating its biochemical properties and potential for sustainable applications. LipBK exhibited optimal activity at pH 4.5 and temperatures between 40 and 60°C, demonstrating stability under challenging conditions. Enzymatic assays using olive oil as a substrate confirmed its efficiency in releasing long-chain fatty acids, both in whole-cell form and as a purified enzyme (specific activity of 0.297 U/mg). Its ability to function across a wide range of temperatures and pH values reinforces its potential for applications involving lipid degradation and the conversion of organic waste into value-added products. The search for efficient and environmentally responsible biocatalysts has driven the study of new microbial enzymes. LipBK"s stability and functionality highlight its potential in innovative approaches such as the bioremediation of contaminated environments, the treatment of oily effluents, and the development of more sustainable industrial processes. Advancing the understanding of enzymes like LipBK contributes to the development of microbial-based strategies, promoting more efficient solutions to environmental and biotechnological challenges.

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Poster Session

P127

Effects of incremental exercise test and maximum lactate steady state on the gut microbiota of pasture-raised horses

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Exercise plays a key role in shaping equine gut microbiota, yet its short-term effects remain underexplored. This study aimed to evaluate how acute exercise influences microbial diversity and composition in horses subjected to the Incremental Exercise Test (IET) and Maximum Lactate Steady State (MLSS). Ten horses were assessed, and fecal samples were collected at baseline (T0), 24h (T24), and 48h (T48) post-exercise. Microbial DNA was extracted, and the V4 region of the 16S rRNA gene was sequenced.Firmicutes and Bacteroidota were dominant across all conditions. In IET, microbial richness increased at T24 and declined at T48, while beta diversity remained unchanged ($p > 0.05$). However, specific bacterial families, including **Acidaminococcaceae** and **Anaerovoracaceae**, transiently increased post-exercise, suggesting metabolic adaptation. In MLSS, microbial composition was stable, but in MLSSLACEX, where lactate exceeded 1mM, beta diversity significantly shifted ($p = 0.007$), with increased **Euryarchaeota** and **Fibrobacterota**, indicative of metabolic stress. Alpha diversity was significantly higher at MLSS-T24 ($p = 0.0021$), possibly reflecting microbial adaptation to sustained aerobic effort.Fecal pH significantly decreased at T48 in IET ($p < 0.05$), indicating increased bacterial fermentation, while remaining stable in MLSS. The results highlight that acute exercise induces transient but intensity-dependent shifts in gut microbiota, with high-intensity sessions (MLSSLACEX) triggering greater microbial disruption than steady-state exercise (MLSS). These findings suggest that training strategies considering microbial resilience could optimize equine performance while preserving gut homeostasis.

Górniak W, Cholewińska P, Szeligowska N, Wołoszyńska M, Soroko M, Czyż K. Effect of intense exercise on the level of bacteroidetes and firmicutes phyla in the digestive system of thoroughbred racehorses. *Animals* (2021) 11:1–9. doi: 10.3390/ANI11020290

Fig1: Relative abundance of bacterial phyla across different exercise protocols showing microbial composition shifts in response to exercise intensity.Fig2:PCoA of beta diversity comparing gut microbiota between MLSS and MLSSLACEX.

Fig. 1

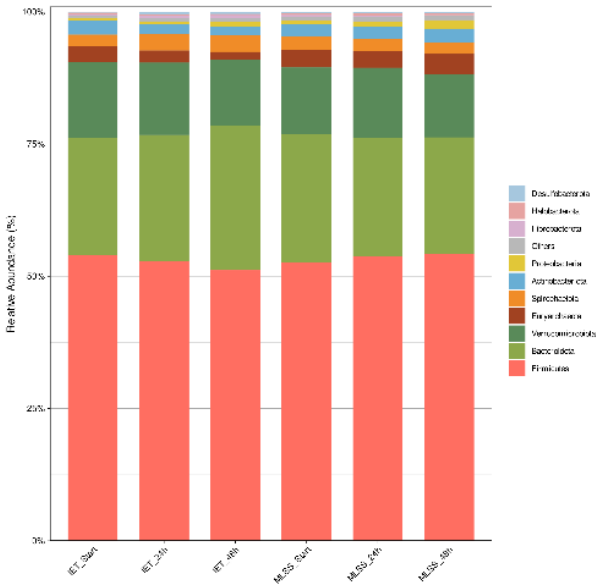
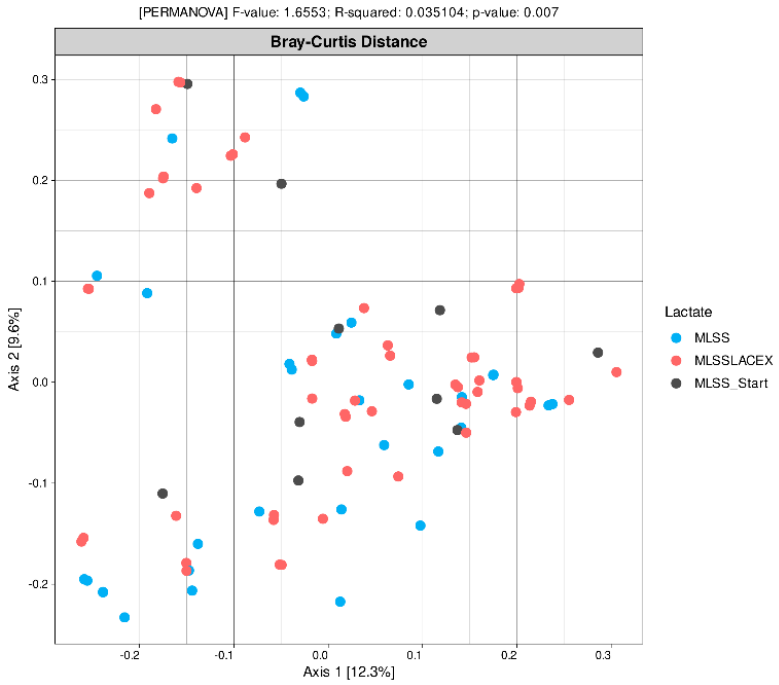

100%
75%
50%
25%
0%
Relative Abundance (%)
IET_Start
IET_24h
IET_48h
MLSS_Start
MLSS_24h
MLSS_48h

Fig. 2


[PERMANOVA] F-value: 1.6553; R-squared: 0.035104; p-value: 0.007
Bray-Curtis Distance
Axis 2 [9.6%]
Axis 1 [12.3%]
0.3
0.2
0.1
0.0
-0.1
-0.2
Lactate
MLSS
MLSSLACEX
MLSS_Start

Poster Session

P128

Assessment of bacterial fauna on three cockroach species collected in houses in Cape Coast, Ghana

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Question: Do different species of cockroaches in houses harbour different bacteria?

Methods: A hundred households were randomly selected in Cape Coast, Ghana. Four cockroach traps were set per each house and monitored for up to 48 hours and removed. The traps were set bi-weekly for three months. In each house, a trap was placed in the bedroom, living room (hall), kitchen and bathroom/toilet. When a trap collected a cockroach, the trap was removed and put in a ziplock bag and transported to the laboratory. The cockroaches were singly placed in a sterile tube containing 2 mL of saline water in the laboratory. The tube was shaken thoroughly for two minutes. The liquid was used for bacteria culture.

Results: Three species of cockroaches, namely American, Brown-banded, and German cockroaches, were collected after six weeks of survey in 100 houses. Seven species of bacteria, *Bacillus* spp, *Enterococcus faecalis*, *Acinetobacter* spp, *Enterobacter cloacae*, *Staphylococcus* spp, *Pseudomonas* spp, and *Staphylococcus aureus,* were isolated from the cockroaches. *Enterococcus faecalis* was the most prevalent bacteria across the three cockroach species, with the highest presence in American cockroach (20%), followed by German (16%) and Brown Banded (12%) cockroaches. *Acinetobacter spp.* was present only on German cockroaches (4%). *Bacillus* spp. and *Staphylococcus* spp. were seen on American and German cockroaches but not in Brown Banded cockroaches. *Pseudomonas spp.* was present in both German (4%) and Brown Banded (4%) cockroaches. *Enterobacter cloacae* was isolated only from Americana cockroaches (4%).

Bacterial diversity was highest in the bedroom and kitchen, with the kitchen containing potentially harmful bacteria like *Enterococcus faecalis, Staphylococcus aureus*, and *Bacillus spp*. *Acinetobacter* species, *Enterobacter cloacae*, and *Pseudomonas* species were exclusively found in the bedroom, whereas *Staphylococcus* species, including *Staphylococcus aureus*, were only identified in the kitchen

Conclusion: The findings throw more light on the bacterial species present on different cockroaches in houses, which may help in developing better sanitation strategies, pest control methods, and awareness campaigns to minimise health risks.

Poster Session

P129

Evaluation of *Bacillus* spp. for Biocontrol of mycotoxigenic fungi in microgreens

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Microgreens are edible seedlings of vegetables, herbs, or grains with the first two fully developed cotyledon leaves, usually harvested 7-20 days after germination. In recent years there has been a growing interest in the cultivation of microgreens, as they have an enriched nutritional composition beneficial to humans (e.g. vitamins, minerals, antioxidants, macro- and micronutrients). It is known that beneficial bacteria belonging to the genus *Bacillus* can improve the growth of microgreens. However, it is increasingly being reported that microgreens become infested with filamentous fungi. There is a lack of detailed information in the scientific literature on this subject and, in particular, on whether fungal infections capable of producing mycotoxins may be unsafe for consumers. This study aimed to evaluate the efficacy of *Bacillus* spp. inoculation in mitigating the adverse effects of *Aspergillus* sp. infection and subsequent mycotoxin production in radish microgreens. Radish seeds were surface-disinfected and then inoculated with *Bacillus* spp. using a vacuum infiltration method (600 mmHg for 5 minutes). Seeds were sown in soil within separate micro-greenhouses; one group of plants was subsequently inoculated with Aspergillus sp., while the other served as a non-fungal contaminated control. Following the growth period, the dry weight, root length, and shoot length of the plants were measured. Mycotoxin levels were determined using LC-MS/MS analysis following extraction via a modified QuEChERS method. The results indicate that *Bacillus* inoculation conferred a slight growth advantage, with inoculated microgreens exhibiting up to a 10% increase in shoot and root length and dry weight compared to non-inoculated controls. However, *Aspergillus* infection impacted plant growth, reducing the dry weight of infected roots by up to two-fold and shoot dry weight by 12% compared to the non-infected control. No significant difference in chlorophyll content was observed between the control and *Aspergillus*-infected cotyledons. Importantly, mycotoxin levels in both control and *Bacillus*-inoculated plants remained below acceptable limits.

This work was supported by The National Science Centre of Poland within the OPUS-23 contract number 2022/45/B/NZ9/04254.

Poster Session

P130

Identifying the bacterial diversity within the white rot fungus *Fomitiporia mediterranea*, the molecular strategies governing the associations and the underlying consequences

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Question

The role of fungal endosymbiotic bacteria has been thoroughly characterized for certain *Mucoromycota*, but there are still few descriptions of the bacterial communities residing within the hyphae of *Basidiomycota* and the impact they have on their host. We are using the white rot fungus and grapevine pathogen *Fomitiporia mediterranea* as a model to investigate the nature and mechanics of its endosymbiotic association with bacteria.

Methods

The presence of endosymbionts within our fungal model was studied using fluorescence microscopy and transmission electron microscopy. Using a large collection of *F. mediterranea* isolates, we used amplicon sequencing and shotgun metagenomics to characterize the bacterial diversity present within the hyphae and to infer the influence of geographic and host origin of the fungus. The degree of inter-dependence between fungal host and symbiotic bacteria was further evaluated using cured fungal lines and pure cultures of isolated bacteria.

Results

The presence of endofungal bacteria is widespread in *F. mediterranea* isolates. Microscopy approaches have confirmed their endohyphal localization and demonstrated their activate state. The bacterial communities colonizing *F. mediterranea* are diverse, but often dominated by one major bacterial taxon. Complete removal of bacteria using antibiotics has proven difficult for some strains, while others can spontaneously lose their endosymbionts over the course of *in vitro* replication.

Conclusions

F. mediterranea is host to diverse bacterial taxa and their detailed functions remain to be explored, but are likely to involve both mutualistic and opportunistic colonizers. Reducing the bacterial load within *F. mediterranea* has a significant fitness effect on the host. Indirect control of the fungus via its bacterial communities could be an innovate solution against the disease it causes in grapevines.

Poster Session

P131

Endophytes and potato cryopreservation – a microbial perspective

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The potato ranks as the fourth most important food crop. Thus, it is essential to maintain the heterozygous genotypes *in vitro* to preserve its genetic diversity. *In vitro* culturing includes surface sterilization to remove external bacteria and fungi, followed by propagation under sterile, sugar-rich conditions. However, this approach is labor-intensive as the plants require periodic transfer to fresh growth medium. Therefore, cryopreservation approaches have been established and implemented in genebanks for long-term storage to preserve vegetatively propagated collections. For this, meristematic tissues, i.e. apical or lateral shoot tips, undergo tissue dissection, osmotic adaptation, cryoprotection, cryogenic treatment using liquid nitrogen to halt all biochemical activities and preserving genetic integrity, and controlled rewarming. However, rewarming can trigger the colonization of endophytes around the explant, which can compromise its ability to develop into a plantlet.

In this project, we analyzed 382 potato accessions for their regrowth potential post-cryopreservation. While 50% showed a good regrowth (≥60% of plants regrown), 39% recovered poorly (≤60% of plants regrown) and 11% failed to recover. To investigate microbial influences, an ITS and 16S rRNA gene amplicon survey was conducted on all these accessions. As anticipated, the microbial diversity was in general low due to sterile cultivation conditions. A total of 637 bacterial amplicon sequencing variants (ASVs) spanning 53 different orders were identified. While some ASVs might originate from exogenous sources, others belong to known plant-associated groups, including Bacillales. In addition, bacteria were isolated from 19 potato accessions resulting in 52 bacterial isolates, belonging to 19 different strains based on 16S rRNA gene sequence comparison. The isolates were characterized for their potential in producing IAA, siderophores and osmolytes, as well as in ACC deamination, and phosphate solubilization. This characterization guided the selection of strains isolated from both well and poorly recovering accessions to test their impact on *in vitro* plant performance and to analyze their genomes. In conclusion, this study provides first insights in the microbial communities associated with potato cryopreservation and their functional traits, representing a crucial step towards enhancing regrowth success and refining long-term preservation strategies in genebanks.

Poster Session

P132

Discovery of novel *Streptomyces* from Austrian soil and compost – bioactive potential and secondary metabolite profiling

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Filamentous actinobacteria of the genus *Streptomyces* produce a vast array of secondary metabolites and are the primary sources of clinically used antibacterial compounds. This study aimed to isolate and characterize newly *Streptomyces* strains, evaluate their antimicrobial and anticancer properties, and perform mass spectrometric analysis of their secondary metabolites. Additionally, the effects of stimulants, such as rare earth elements and N-acetylglucosamine, on antibiotic production—whether activation, enhancement, or inhibition—were investigated.

A total of 59 isolates were recovered from four soil and compost samples collected in Austria and identified as *Streptomyces* species through 16S rRNA sequence analysis. The antimicrobial activity of these isolates was assessed against multiple indicator strains, including Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli, Klebsiella pneumoniae*), and yeast (*Candida albicans*). Notably, 10% of the isolates exhibited inhibitory effects on at least one indicator strain.

The crude extracts from the culture supernatants of two bioactive isolates were tested for anticancer activity against human hepatoma (HepG2) and human breast cancer (MCF-7) cell lines. One isolate demonstrated strong cytotoxic activity against both cancer cell lines.

Multilocus sequence analysis (MLSA) of four genes (*16S rRNA, rpoB, atpD, recA*) and genome comparisons using average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) suggested that *"isolate 7"* may represent a novel *Streptomyces* species. Whole-genome sequencing data of *"isolate 7"* were analyzed using antiSMASH, revealing 25 predicted biosynthetic gene clusters (BGCs) responsible for secondary metabolite production. The most common types of predicted secondary metabolites were nonribosomal peptides (NRPs) and polyketides.

Untargeted liquid chromatography–ultraviolet–mass spectrometry (LC-UV-MS) analysis of bioactive crude extracts identified several relevant *m/z* values of putative metabolites, essential for further structural elucidation.

Our findings indicate that secondary metabolites from *Streptomyces* isolates exhibit promising bioactivity and hold potential for therapeutic applications, warranting further investigation.

Keywords: *Streptomyces*, secondary metabolites, antimicrobial activity, anticancer activity, genome sequencing, digital DNA-DNA hybridization (dDDH), mass spectrometry (LC-UV-MS)

Poster Session

P133

Hidden viral players – diversity and ecological roles of viruses in groundwater microbiomes

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The ocean contains 10^{10} virus-like particles (VLP) per liter, vastly outnumbering host cells. Viruses significantly affect nutrient cycling, reprogramming host cells, and promoting horizontal gene transfer. Pristine groundwater is a vital source of drinking water, yet we lack an understanding of the role of viruses in this aquatic ecosystem. We examine virus diversity, ecological importance, and functional interactions in a two-year study of seven wells within a pristine groundwater system. From ~1.3 terabases of metagenomics data, we identified >7 million virus contigs, resulting in 257,252 and 82,245 virus operational taxonomic units (vOTUs) of ≥5 kb or ≥10 kb, respectively, representing a ~22-fold increase compared to publicly available groundwater vOTUs (*n*=3,584, ≥10 kb). Taxonomically, 99% of the groundwater viruses were species-level unique, even when compared to a global ocean dataset. Approximately 81% could be taxonomically classified, with 99% belonging to the Caudoviricetes. Ecological analysis demonstratedsite- specific endemism in virus communities, evidenced by strong grouping based on the sampled groundwater wells (p <0.001). Our host prediction analysis found that 88% of viruses infect 78% of microbes from the same sample, including ecologicallyimportant groups like Patescibacteria and Proteobacteria. Additionally, about 5% of viruses may reprogram ~32% of host pathwaysthrough auxiliary metabolic genes (4,093 AMGs found), linked to ~29 host phylum. One example of AMGs is GAPDH (K00134), which breaks down glucose for energy and carbon utilization, nitrite reductase (K15876), and sulfate adenyltransferase (K00957) which help groundwater microbes to thrive in oxygen-depleted conditions. Overall, this research offers valuable insights into groundwater virus communities and the mitigation of human impacts on groundwater resources.

Poster Session

P134

Ecogenomic insights into the carbohydrate metabolism of the marine bacterial genus *Aquimarina* – an emerging opportunist relevant to aquaculture

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The marine bacterial genus *Aquimarina* (*Flavobacteriaceae*, *Bacteroidota*) is globally distributed, occurring in sediments, seawater, and diverse hosts, including macroalgae, sponges, corals, mussels, sea urchins, crustaceans, and fish. Some *Aquimarina* species act as opportunistic pathogens of economically relevant crustaceans and seaweed, likely due to their versatile carbohydrate-degrading capacities and secondary metabolism. Within *Flavobacteriaceae*, *Aquimarina* stands out indeed for its abundance and diversity of biosynthetic gene clusters (BGCs), with novel peptide antibiotics recently identified. Many species are also efficient degraders of polysaccharides such as chitin, cellulose, xylan, and carrageenan.

Through a meta-analysis of 139 publicly available *Aquimarina* genomes (68 cultured, 71 uncultured), we **(i)** refined the genus' phylogenomic framework and **(ii)** examined the distribution of its polysaccharide- and chitin-degrading enzymes across hosts and habitats. Average nucleotide identity comparisons identified 15 putative novel species (ANI<95%), 10 exclusively represented by metagenome-assembled genomes (MAGs) retrieved from seaweed microbiomes. Seaweed-associated strains (*N*=91) dominated the dataset, spanning most phylogenomic clades, except for the large "*A. longa-A. macrocephali-A. megaterium-A. atlantica-A. spinulae*" clade which lacked seaweed-associated representatives.

Aquimarina genomes commonly harbour GH18, GH19, and GH20-type chitinases, as well as lytic polysaccharide monooxygenases (LPMOs). A total of 601 endo-chitinases (cleaving chitin polymers) and 445 exo-chitinases (releasing monomers/dimers from chitin oligomers) were identified across the 139 genomes. A maximum of 12 and an average of 4 endo-chitinases, 11 and 3 exo-chitinases, and 3 and 1 LPMOs were observed among genomes. No direct correlation with taxonomy or habitat has been found so far, but a deeper phylogenetic inspection of chitinase genes for clade-specific differences is still ongoing.

Our findings reinforce *Aquimarina* as generalist degraders of complex carbohydrates, with broad substrate utilization likely contributing to their ecological versatility. Surprisingly, pathogenic *Aquimarina* strains infecting chitin-rich crustaceans do not possess more endo or exo-chitinases than non-pathogenic strains. Future work will focus on heterologous expression and kinetic characterization of selected *Aquimarina* chitinases, as none have been experimentally characterized to date.

Poster Session

P135

Diversity and function of the excreted and exposed proteins of human-associated archaea

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Methanogenic archaea are key players in the human gastrointestinal tract yet their role in health and disease in humans remains only scarcely characterized. Among them, *Methanobacteriales* are highly prevalent, but their surface architecture has not been further investigated since the 1980s. Their cell envelope consists of a lipid monolayer — distinct from bacteria and eukarya — and a thick pseudomurein layer, that varies between species and even strains and shows a high resistance to many bacterial antibiotics. Understanding these structural components is essential for elucidating their interactions with other gut microbes and the host mucosa. *Methanobrevibacter smithii* have in general a bold surface but can exhibit a dense layer of adhesins at one end; their functions and at which growth state they appear, however is unknown.

This study investigates the methanogenic cell surface, in particular exposed and excreted proteins to find species- and or strain specific patterns to shed light into the methanogen way to adhere to the mucosal surface and the effect of specific structures on their survival.

Initially, we analyzed 729 genomes of human-associated archaea, identifying shared genes based on predicted protein localization. We then focused on two dominant gastrointestinal species, *M. smithii* and recently identified *Candidatus Methanobrevibacter intestini*. Despite their close phylogenetic relationship, they show structural differences in their cell envelopes. Besides a genome-based approach, proteomic analysis of both species enabled the validation of predicted proteins and their relative abundance. In addition, scanning electron microscope imaging and transcriptomic profiling allowed us to examine structural changes across growth phases to identify proteins that are linked to intra-cellular communication and biofilm formation.

Preliminary findings indicate substantial gene sharing between *M. smithii* and *C. M. intestini* (1041 shared genes), whereas more distantly related methanogens exhibit a lower overlap (e.g., 562 genes). Notably, the distribution of membrane, cell wall, and extracellular proteins is strikingly similar between *M. smithii* and *C. M. intestini* but differs in other methanogens.

By elucidating the cell surface architecture, this study advances our understanding of their role in the human microbiome and their potential impact on the human host.

Poster Session

P136

Antibiotic-resistance gene diversity and mobilization in bacteria from salmon farms

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Florfenicol is a broad-spectrum antibiotic widely used as a veterinary drug for its therapeutic efficacy against bacterial diseases of farmed animals. In Chile, the 2nd largest salmon producer in the world, florfenicol (FFC) has been the drug of choice to treat outbreaks and over 300 tons of antibiotics are used each year to control bacterial diseases. The intensive use of FFC imposes a selection pressure on aquatic bacteria and can promote the occurrence and spread of antibiotic resistance, even at sub-inhibitory concentrations. In this work, we investigated the prevalence of florfenicol-related resistance genes in bacteria from an intensive aquaculture area in southern Chile, with focus on the potential dissemination of florfenicol resistance genes through horizontal gene transfer (HGT). For this, total DNA of the seawater bacterial fraction obtained from two salmon farms was purified and sequenced. Through a metagenomics analysis, antibiotic resistance gene (ARG) prediction was performed using the Resistance Gene Identifier (RGI) and CARD database. ARG composition, abundance and sequence were analyzed using assembled and raw read data. ARG"s drug classes from salmon farm bacteria showed similar abundance profiles to global ocean bacteria. The *floR* gene was the most abundant resistance gene showing a conserved sequence, although with variations from the reference gene. These differences were recovered by RGI prediction and by mapping reads using SNP base-calling, observing a total of 13 gene variants. The variants were analyzed by heterologous expression, revealing the coexistence of high- and low-resistance sequences in the environmental bacteria. Finally, the genomic context of *floR* was assessed for mobilization elements. Plasmid prediction was carried out using PlasmidHunter and PlasClass, and plasmid-specific databases (MobileOG-db, PLSDB, oriTDB). The contigs containing *floR* were identified as plasmid fragments carrying a LysR regulator and a putative ISVsa3 family transposase belonging to IS91-like elements. This study demonstrates that *floR* is present in marine bacterial communities with diverse nucleotide sequence and resistance phenotypes, and suggests it can be mobilized through HGT. Additionally, it highlights the importance of combining metagenomic and phenotypic approaches to study the genetic variability of antibiotic-resistant bacteria associated with salmon farms.

Poster Session

P137

Phototrophicity and genomic composition in Plant-Associated *Sphingomonas faeni* Strains

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Question: How do different phototrophic traits in *Sphingomonas faeni* correlate with genomic composition, and what are the evolutionary implications of these adaptations in Arctic and boreal environments?

Methods: We sequenced the genomes of 26 plant-associated *S. faeni* strains from Arctic and sub-Arctic regions, and utilized a reference genome from NCBI. Whole-genome alignment, phylogenetic analysis, and pan-genome analysis were conducted to explore genetic diversity and phototrophic capabilities. The presence of *xanthorhodopsin* (XR) and *aerobic anoxygenic phototrophy* (AAP) genes was identified, and structural predictions of XR were performed using SWISS-MODEL. Photoreceptor genes (BLUF, BphP, PYP) were identified and quantified.

Results: The strains exhibited three distinct phototrophic groups: 7 strains were AAP-positive, 17 contained XR genes, and 3 were non-phototrophic. AAP-positive strains, exclusively found in *Vaccinium myrtillus* and *V. vitis-idaea*, formed a distinct and tightly clustered phylogenetic group. XR and non-phototrophic strains exhibited clustering based on phototrophicity, with ecological niche serving as a secondary determinant of their phylogenetic organization. The pan-genome analysis identified 24,618 genes, with 1,293 core genes. AAP strains had 221 shared genes above 85% similarity threshold, including a second copy of *5-aminolevulinic acid* (5-ALA) in the photosynthetic gene cluster (PGC). XR-containing strains showed conserved but functionally variable carotenoid-binding regions. Phototrophic strains had a higher number of photoreceptors.

Conclusions: Phototrophicity in *S. faeni* correlates with overall genomic composition, with AAP-positive strains displaying higher genetic conservation and XR strains exhibiting greater genetic diversity. The presence of dual 5-ALA genes in AAP strains suggests an evolutionary adaptation for enhanced photosynthesis. The greater number of photoreceptors in phototrophic strains supports the hypothesis that light-sensing capabilities are enhanced in light-utilizing bacteria. These findings highlight the genomic flexibility of *S. faeni* in adapting to Arctic and boreal plant niches, offering insights into the evolutionary dynamics of microbial phototrophy.

Fig. 1

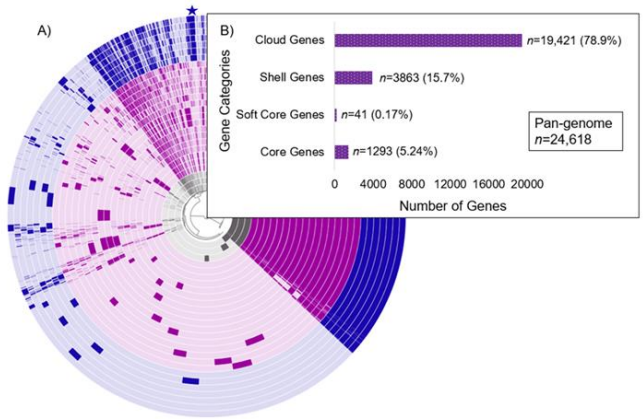
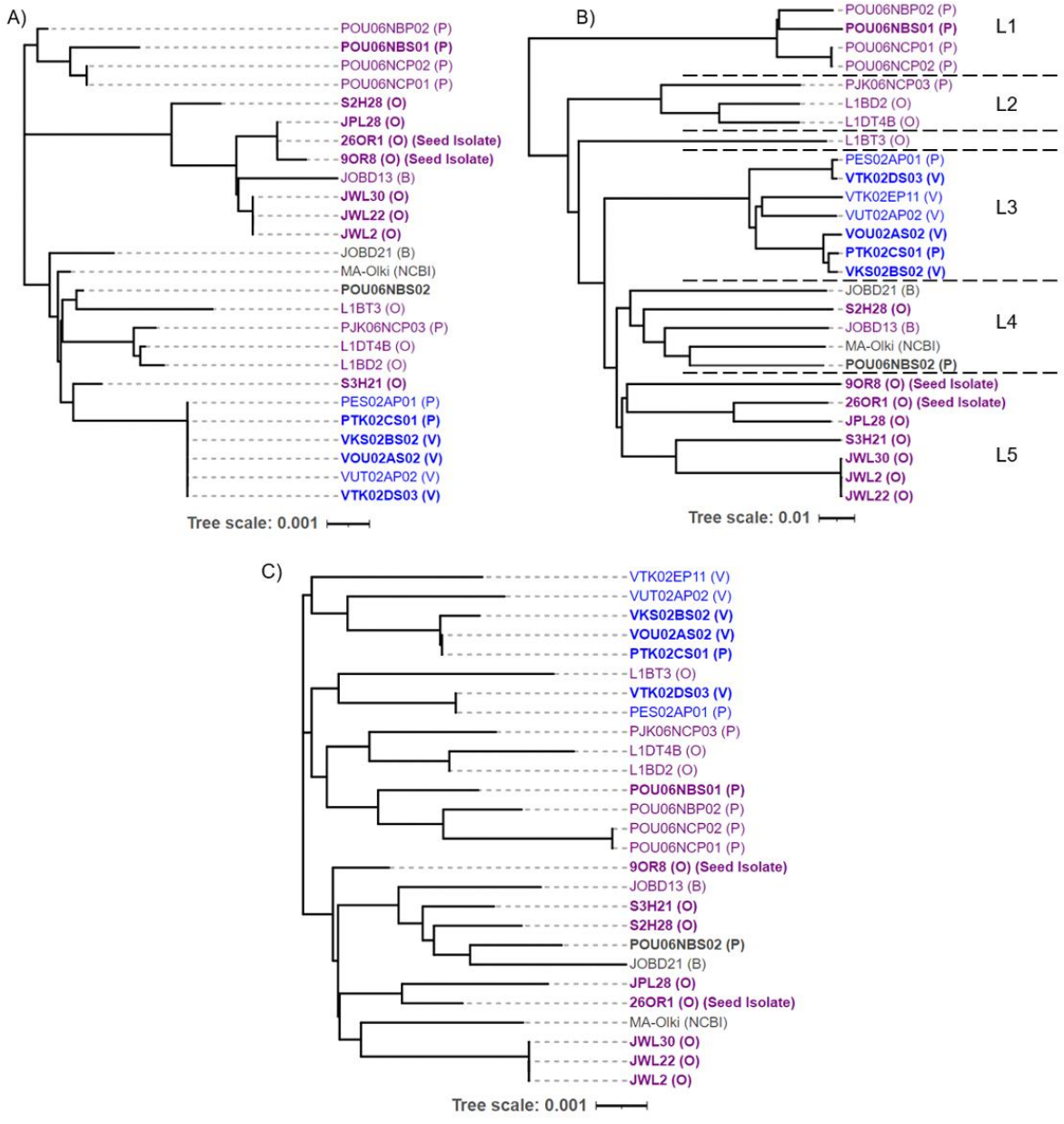

A)
B)
Gene Categories
Cloud Genes
n=19,421 (78.9%)
Shell Genes
n=3863 (15.7%)
Soft Core Genes
n=41 (0.17%)
Core Genes
n=1293 (5.24%)
Pan-genome
n=24,618
0 4000 8000 12000 16000 20000
Number of Genes

Fig. 2


A)
POU06NBP02 (P)
POU06NBS01 (P)
POU06NCP02 (P)
POU06NCP01 (P)
S2H28 (O)
JPL28 (O)
26OR1 (O) (Seed Isolate)
9OR8 (O) (Seed Isolate)
JOBD13 (B)
JWL30 (O)
JWL22 (O)
JWL2 (O)
JOBD21 (B)
MA-Olki (NCBI)
POU06NBS02
L1BT3 (O)
PJK06NCP03 (P)
L1DT4B (O)
L1BD2 (O)
S3H21 (O)
PES02AP01 (P)
PTK02CS01 (P)
VKS02BS02 (V)
VOU02AS02 (V)
VUT02AP02 (V)
VTK02DS03 (V)
Tree scale: 0.001
B)
POU06NBP02 (P)
POU06NBS01 (P)
POU06NCP01 (P)
POU06NCP02 (P)
PJK06NCP03 (P)
L1BD2 (O)
L1DT4B (O)
L1BT3 (O)
PES02AP01 (P)
VTK02DS03 (V)
VTK02EP11 (V)
VUT02AP02 (V)
VOU02AS02 (V)
PTK02CS01 (P)
VKS02BS02 (V)
JOBD21 (B)
S2H28 (O)
JOBD13 (B)
MA-Olki (NCBI)
POU06NBS02 (P)
9OR8 (O) (Seed Isolate)
26OR1 (O) (Seed Isolate)
JPL28 (O)
S3H21 (O)
JWL30 (O)
JWL2 (O)
JWL22 (O)
L1
L2
L3
L4
L5
Tree scale: 0.01
C)
VTK02EP11 (V)
VUT02AP02 (V)
VKS02BS02 (V)
VOU02AS02 (V)
PTK02CS01 (P)
L1BT3 (O)
VTK02DS03 (V)
PES02AP01 (P)
PJK06NCP03 (P)
L1DT4B (O)
L1BD2 (O)
POU06NBS01 (P)
POU06NBP02 (P)
POU06NCP02 (P)
POU06NCP01 (P)
9OR8 (O) (Seed Isolate)
JOBD13 (B)
S3H21 (O)
S2H28 (O)
POU06NBS02 (P)
JOBD21 (B)
JPL28 (O)
26OR1 (O) (Seed Isolate)
MA-Olki (NCBI)
JWL30 (O)
JWL22 (O)
JWL2 (O)
Tree scale: 0.001

Poster Session

P138

Rhizosphere bacteria from the Bolivian highlands improve growth and drought tolerance in quinoa (*Chenopodium quinoa* Willd.)

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Drought is one of the most destructive abiotic factors for agricultural production, causing considerable yield losses. Quinoa (*Chenopodium quinoa* Willd.) is cultivated worldwide in different environmental conditions due to its nutritional characteristics and ability to grow in harsh environments. This study aims to select drought stress tolerant rhizosphere bacteria from the Bolivian altiplano to evaluate their quinoa growth-promoting capacity, including *in vitro* germination, seedling growth under drought stress in greenhouse conditions and field studies.

Rhizosphere soil from the southern highlands of Bolivia was collected to isolate 164 drought-stress tolerant bacteria. From these, 28 strains were shown to produce indole acetic acid, and/or to possess nitrogen-fixing or phosphate solubilizing capacity under *in vitro* conditions. Furthermore, all strains were evaluated for improvement of *in vitro* quinoa seed germination. Based on these properties, nine bacterial strains were formulated in three different matrixes and evaluated for quinoa seedling growth promotion during drought stress in a three-month greenhouse experiment. Three strains were shown to significantly ($P < 0.05$) increase root length of the quinoa seedlings. One strain was selected and shown to significantly ($P < 0.05$) increase leaf number in a field trial under semi-arid conditions in the southern altiplano in Bolivia. DNA sequencing and phylogenetic analyses of the 16S locus putatively identified the three strains with growth-promoting potential under drought stress as members of the genera *Bacillus*, *Pseudomonas* and *Serratia*.

Microorganisms from the arid Bolivian altiplano constitute a potential biological source of bioinoculants to improve quinoa productivity and provide sustainable mitigation of climate change effects.

Key words: *Bacillus*, Drought tolerance, PGPR, *Pseudomonas*, Quinoa, *Serratia*.

Poster Session

P139

Competence for transformation shapes kin discrimnation and colony morphology phenotypes in *Bacillus subtilis* during experimental evolution

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Natural competence for transformation is hypothesized to contribute to genome repair and phenotypic stability through the uptake of extracellular DNA. To investigate the evolutionary consequences of impaired DNA uptake, we conducted experimental evolution with competent and competence-deficient (ΔcomGA) *Bacillus subtilis* strains PS-216. Focal strain was evolved on swarming agar over 20 cycles with repeated contact with isogenic or non-kin contact swarms.

We observed increase in frequency of strains with KD phenotype change in competence deficient evolved populations compared to evolved competent populations regardless of whether they encountered isogenic or nonkin contact swarms. This suggests that genetic competence for transformation may contribute to stability of KD phenotype, while the KD linked sociality has no effect on stability of KD phenotype. Additionally, we observed a higher prevalence of divergent colony morphotypes in non-competent lineages exposed to contact strains. Interestingly, this pattern was not observed in evolution experiments lacking repeated encounters with contact strain, implying that the influx of extracellular DNA from neighboring swarms which were not exposed to experimental evolution may negatively affect morphotype diversification through genome repair.

Although additional experiments are required to confirm these trends and rule out alternative explanations, our findings support the hypothesis that natural competence may help preserve phenotypic traits in *B. subtilis* populations. These results provide new insight into the role of horizontal gene transfer in phenotypic stability of bacterial populations.

Poster Session

P140

Distribution and Ecology of Prophage-Like Elements in *Bacillus subtilis* Across Global and Local Scales

P. Štefanič[1], E. Stare[1], V. Floccari[1], J. Kovac[2], R. Hertel[3], U. Nunez da Rocha[4], A. Kovacs[5], I. Mandić-Mulec[1], M. Lenz Strube[6], A. Dragos[1]

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[3]Georg-August-University of Göttingen, Göttingen, Germany
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While many bacteria allocate a significant portion of their genome to prophage elements, our understanding of their ecological roles remains incomplete. Similarly, the factors influencing the abundance and distribution of prophages within host species, particularly in non-pathogenic or beneficial bacteria, are still poorly understood. In this study, we examine prophage elements in *Bacillus subtilis*, a widely studied model organism of beneficial bacteria within the Firmicutes phylum, which is known for its high prevalence of prophages.By analyzing bacterial genomes from the NCBI database alongside isolates from a specific geographic region, we uncover local influences on prophage abundance and distribution. Additionally, we compare predicted prophage elements with known groups of Mobile Genetic Elements (MGEs) previously identified in laboratory strains. For the first time, we reveal their abundance, distribution, and potential contributions to shaping the *B. subtilis* mobilome. Our findings highlight complex interactions, including both synergistic and antagonistic relationships, among distinct prophage types, suggesting that mechanisms such as superinfection exclusion and co-infection significantly influence prophage distribution in *B. subtilis*.Furthermore, experimental studies demonstrate prophage activity. Although specific responses could not be attributed to individual prophages, the magnitude of the response to a phage-inducing agent correlates with the number of prophage elements present. This research provides novel ecological insights into the prophage elements of *B. subtilis*, laying a foundation for future studies to further explore their roles in the ecology and evolution of this bacterial species.

Poster Session

P141

Oasis in the Andean Steppe: Reactivation of dormant bacteria in the rhizosphere of *Pappostipa frigida*

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Question: To address the question of how different the total (those detected by DNA) and active (those detected by RNA) bacterial communities are between key below-ground microenvironments under the harsh conditions of the Andean steppe in the Atacama Desert, we conducted a field investigation on the rhizosphere (RZ) of Pappostipa frigida, a native perennial grass, and the surrounding bulk soil (BS). In addition, we used culture media to evaluate whether the inactive portion of the soil community (those not detected by RNA) could be reactivated.

Methodology: The site was located at 4,000 m.a.s.l. in Paso de Jama, near the Chile-Argentina border. Five RZ and five BS samples were collected, and DNA and RNA were extracted. The samples were amplified with primers 28F/519R targeting the 16S rRNA gene and sequenced on an Illumina MiSeq. In parallel, soil was used to inoculate culture media, and after 72 h, the plates were scraped to recover all bacterial growth. Then, the DNA was extracted, amplified and sequenced. The reads were processed into ASVs using Qiime 2 plugins, dormancy-related genes (sporulation and toxin-antitoxin) were predicted using PICRUSt2, and diversity analyses were conducted using the R package vegan.

Results: Diversity analyses showed no significant differences in richness and Shannon index in the total community of BS and RZ. However, these metrics were significantly higher in the RZ for the active bacterial population. In terms of beta diversity, the total and active community structure differed markedly between BS and the RZ. Furthermore, comparison of the relative abundance between the total and active bacterial populations showed a higher correlation in the RZ compared to the BS compartment. This finding is consistent with the high proportion of bacteria with no detectable activity in the BS. Notably, 79 % of these inactive bacteria were identified as active members of the RZ, 68 % were able to grow in culture media, and 16 % had genetic markers associated with dormancy.

Conclusions: These results show a clear distinction between the bacterial communities of the BS and the RZ of *P. frigida*, especially when their active communities are compared. Furthermore, a considerable proportion of active members of the rhizosphere appear to have been recruited from dormant BS bacteria, highlighting the role of the favorable conditions created by the plant in the recruitment of dormant bacterial populations in the Andean steppe environment.

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Poster Session

P142

From Cell to Community: Unlocking Microbial Diversity with Single-Cell Genomics

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Single-cell DNA sequencing complements the metagenomic analysis of uncultured bacteria by revealing cell-to-cell variation, linking host genomes to extrachromosomal DNA, and providing strain-level taxonomic resolution. However, current techniques are limited to processing fewer than 1,000 cells and produce single-amplified genomes (SAGs) of low completeness due to biases in whole genome amplification (WGA).

We present an innovative, cost-effective approach to sequence up to 10,000 SAGs with superior genome recovery. Individual microbial cells are isolated into 70 µm semi-permeable capsules (SPCs), enabling compartmentalized multi-step processing - including lysis, WGA, and barcoding - of all cells simultaneously at a cost of less than $1 per cell. Using well-characterized E. coli and B. subtilis, we demonstrate >90% genome recovery per SAG at sequencing depths below 10x and <1% cross-contamination. Additionally, we processed a commercially available microbial community standard and were able to detect all species within the mixture.

The advantages of SAG sequencing are especially valuable for uncovering the diversity and unique adaptations in the context of microbial ecology. We processed soil and aquatic samples to generate matched SAG and MAG datasets, with the latter obtained through bulk metagenomics. SAG assemblies produced longer contigs and revealed detailed genomic features, most notably the linkage between viral and plasmid sequences and their hosts. The linkage information was lost in the MAG dataset.

Our high-throughput SAG sequencing workflow provides a detailed view of microbial communities, offering unmatched resolution and scalability. This capsule-based single-cell sequencing technology opens new horizons for microbial genomics research.

The human microbiome in health and disease I

AL028

Horizontal Gene Transfer in the Gut and Its Impact on the Host–Microbiome Axis

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Gut environments, densely populated with microorganisms, serve as fertile grounds for horizontal gene transfer and microbial genome plasticity, with plasmids emerging as potent agents in this evolutionary process. This prompts inquiries into the driving forces behind plasmid dispersal across populations, the intricate factors shaping genetic material flow among them, and their impact on microbial interactions and host adaptation. In my presentation, I will talk about findings from our recent studies that probe these questions, offering deeper insights to these aspects of microbial ecology and evolution.

The human microbiome in health and disease I

AL029

Systems ecology of the human microbiome: from molecules to mechanisms

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The human microbiome, through its emergent properties, contributes essential functions to its host. Recent large-scale metagenomic studies have provided insights into its functional potential, but have primarily focused on taxon-centric views. However, the functional repertoire that actively contributes to human physiology remains largely unexplored. For example, the human microbiome produces a complex biomolecular cocktail in the form of small molecules, nucleic acids, and (poly-)peptides, recently defined as the expobiome. This cocktail possesses numerous bioactive properties, but these have yet to be systematically studied. This overall gap in knowledge is limiting our understanding of the role of the human microbiome in governing human physiology and how changes to the microbiome impact chronic diseases, including cancer as well as metabolic and neurological conditions, through the triggering and exacerbation of disease pathways. Furthermore, without a mechanistic understanding of the microbiome"s molecular complexity, we are unable to design microbiome-targeted therapies rationally. In this context, the microbiome also represents a treasure trove of leads for the development of future diagnostic and therapeutic applications for chronic diseases. I will describe the current state of understanding of the functional microbiome in contrast to taxonomic views with a specific focus on microbiome-derived molecules in neurodegenerative diseases. Ranging from systematic, integrated, multi-omic analyses of the microbiome-borne molecular complex to mechanistic studies in novel experimental systems, a clear roadmap will be drawn towards translating the functional ecology of the gut microbiome into novel diagnostic applications and drugs.

The human microbiome in health and disease I

AL030

Strain-level insights into the infant gut microbiome maturation across lifestyles

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[2]Experimental and Clinical Research Center, Berlin, Germany
[3]Charité–Universitätsmedizin Berlin, Berlin, Germany
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[6]Hannover Medical School, Excellence of Cluster RESIST, Hannover, Germany
[7]University of Trento, Department of Cellular, Computational and Integrative Biology, Trento, Italy
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[9]European Molecular Biology Laboratory, Structural and Computational Biology Unit, Heidelberg, Germany
[10]Berlin Institute of Health, Berlin, Germany
[11]German Centre for Cardiovascular Research, Partner Site Berlin, Berlin, Germany

Immediately after birth, the infant''s gut provides a vast, empty habitat for bacteria to colonize. As the infant grows up, its immune system matures, and its diet shifts from breast milk to solid food. This constantly changing habitat provides a model system to investigate ecological dynamics. However, a comprehensive understanding of microbial dynamics at different geographic and lifestyle scales remains scarce,despite numerous studies of infant gut microbial colonization. Large-scale meta-analyses benefit from the increasing number of available microbiome datasets to detect generalizable microbial dynamics across cohorts.

Here, we aim to investigatedifferences in the human gut microbiome colonization across geographic regions and lifestyles. Using a large longitudinal shotgun dataset comprising 1,944 infants under three years of age from 23 studies, we analyze microbial colonization patterns via strain-resolved metagenomics.

Infants with a non-Westernized lifestyle experience more strain replacement events during the maturation process compared to those with a Westernized lifestyle. Among bacterial taxa occurring in both lifestyles, we identified taxa with a lifestyle-specific phylogeny, where different strains occur in different lifestyles as well as generalist taxa where lifestyle is not linked to phylogeny.

Our findings suggest that the microbiomes of non-Westernized infants undergo a more dynamic maturation than those of Westernized infants, that is usually overlooked, and highlight the importance of diverse datasets for understanding universal and context-dependent longitudinal microbial dynamics. Our study contributes to a broader understanding of microbial succession in the infant gut and highlights the role of lifestyle in shaping early microbiome development.

The human microbiome in health and disease I

AL031

Investigating bacterial gene essentiality in context of the native intestinal microbiome

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For decades forward genetics has been used to identify new drug targets, conditionally essential genes or virulence factors in bacteria to help us define pathogenic traits. Using random mutagenesis these approaches cover whole genomes and allow for comprehensive genetic screens. However, several drawbacks hinder crucial discoveries especially in the context of natural host microbe interactions. Genome wide random mutagenesis relies on a substantial number of diverse mutants to identify essential genes. This limits our abilities to study events or compartments with tight bottlenecks or low colonization.

To address this problem, we have developed a system that allows for easy targeted gene knockout in bacteria via a CRISPR-Cas system called MULTICast. This system can not only be used in a genome wide approach to identify changes in abundance via standard sequencing techniques but also allows for fast easy and reliable targeted gene knockouts. MULTICast is based on a programmable transposon of *Vibrio cholerae* called Tn6677. It allows specific integration of the transposon at a target site determined by a CRISPR guide which in this case is located inside the transposon.

This guide can be easily tracked through sequencing analysis and enables the investigation of relative abundances of multiple guides in a pool. In preliminary studies, we successfully generated a knock-out pool of 50 *Vibrio cholerae* genes simultaneously. MULTICast reaches a saturated library with significantly fewer bacterial cells and can therefore be used to analyze small compartments of the intestine at various timepoints. This novel spatial-temporal approach to gene essentiality enables us to investigate infection processes in a more granular and holistic manner compared to previous methods. Furthermore, MULTICast has the potential to shed light on how probiotic or commensal bacteria flourish or fail to colonize diverse compartments of the intestine. In fact, our preliminary data suggest that several genes exhibit patterns of essentiality during infection, both temporally and spatially. Currently we are utilizing this system in a genome-wide approach, involving pools of several hundred genes in a diverse group of organisms. This enables us to understand patterns of essential genes during gastrointestinal infection or colonization and potentially lead to the development of new treatment approaches.

The human microbiome in health and disease I

AL032

Gut microbial metabolites as effectors and markers in chronic inflammatory disease

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[2]Technical University of Munich, Munich, Germany
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The human metabolome, with ~40% of unknown molecules, is susceptible to exposures, including the gut microbiota, which can drive inflammation. We design computational and experimental strategies for integration of clinical biomarkers and metagenomics. These guide isolation of disease-specific strains and culture metabolomics, pinpointing their metabolic capabilities.

By profiling 91 ulcerative colitis patients, we detected strains associated with fecal calprotectin, including Veillonella parvula [1]. We found its nitrate- and lactate-dependent products, including immunomodulatory tryptophan metabolites and signalling fatty acid amides (Fig. 1). V. parvula also metabolized thiopurine drugs through a xanthine dehydrogenase, potentially impairing the response to immunosuppressants.

In the context of cardiovascular disease, we profiled stool and serum from 1,429 healthy volunteers [2]. The abundant Oscillibacter genus was associated with decreased fecal and serum cholesterol (Fig. 2). AI-based protein language models of Oscillibacter isolates uncovered conserved cholesterol glycosyltransferases and dehydrogenases. The cholesterol metabolizing capacity of Oscillibacter is confirmed with in culture metabolomics. This establishes cholesterol metabolism as a broad trait of phylogenetically diverse Oscillibacter species, poised to benefit lipid homeostasis and cardiovascular health.

To aid these efforts, we developed AI methods for prediction of metabolites in liquid chromatography / mass spectrometry [3-5], tracking unknown metabolites and their sources, including the malleable microbiome as a therapeutic entrypoint.

[1] Stražar, M.*, Schirmer, M.*, ... , Xavier, R. J. (2024). Cell Host & Microbe, 32(2). DOI: 10.1016/j.chom.2023.12.013

[2] Stražar, M.*, Li, C.*, …, Xavier, R. J. (2024). Cell, 187(8). DOI: 10.1016/j.cell.2024.03.014

[3] Goldman, S., …, Stražar, M.,... ,Coley, C. (2023). Nature Machine Intelligence, 5(9). DOI: 10.1038/s42256-023-00708-3

[4] Vatanen, T., Sjoberg K, ..., Stražar, M., …, Xavier, R. J. (2022). Cell, 185(26). DOI: 10.1016/j.cell.2022.11.023

[5] Vatanen, T., Ang, Q., …, Stražar, M …, Xavier, R. J. (2022). Cell, 185(23). DOI: 10.1016/j.cell.2022.10.011

Fig. 1

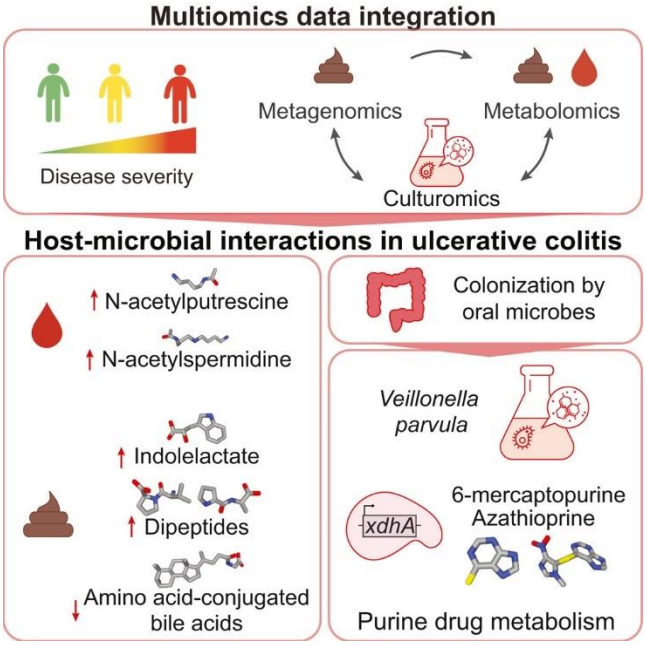
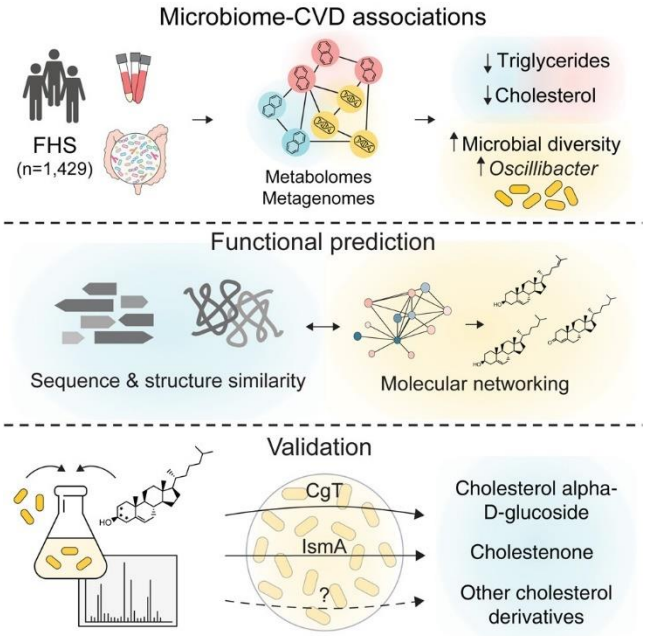

Multiomics data integration
Disease severity
Metagenomics
Metabolomics
Culturomics
Host-microbial interactions in ulcerative colitis
N-acetylputrescine
N-acetylspermidine
Indolelactate
Dipeptides
Amino acid-conjugated bile acids
Colonization by oral microbes
Veillonella parvula
xdhA
6-mercaptopurine
Azathioprine
Purine drug metabolism

Fig. 2


Microbiome-CVD associations
FHS
(n=1,429)
Metabolomes
Metagenomes
Triglycerides
Cholesterol
Microbial diversity
Oscillibacter
Functional prediction
Sequence & structure similarity
Molecular networking
Validation
CgT
IsmA
?
Cholesterol alpha-D-glucoside
Cholestenone
Other cholesterol derivatives

The human microbiome in health and disease I

AL033

The development of the infants microbiome

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Objective:

The establishment and maturation of the infants" microbiome play a crucial role in shaping lifelong health outcomes. While extensive research has focused on bacterial communities, the contributions of archaea and anaerobes remain relatively underexplored, ignoring their potential contribution to establishing a healthy oral microbiome. This study aims to unravel the dynamics of infant microbiome development with respect to anaerobes and archaea.

Methods:

We recruited a cohort of 30 infants and collected oral and fecal samples, starting from the neonatal period and extending monthly throughout the first year of life. Genomic techniques, including amplicon sequencing and metagenomics analysis, were employed to characterize microbial communities. Special attention here was given to archaeal and obligate anaerobic populations, employing targeted approaches. Our methodology aimed to provide a comprehensive understanding of the microbial diversity, abundance, and networks within the upper and lower intestinal tract.

Results:

We elucidate dynamic shifts in the composition of infant oral and stool microbiota over the first year of life. We are happy to be able to show that archaea can already be detected in samples of one month of age. Especially obligate anaerobes play a central role in microbial networks from the beginning on. The monthly sampling strategy has enabled the identification of critical milestones (first month and transition phase (months 4-7) when solid food is introduced) and potential factors (esp. breastfeeding) influencing the establishment of these microbial populations.

Conclusion:

Archaea can be detected already very early in life and anaerobic microbes play a key role in the establishment of microbial networks.

The human microbiome in health and disease I

FT037

Interactive AutoML-Driven discovery of human microbiome patterns across environmental contexts

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[4]Helmholtz Center Munich, Neuherberg, Germany

Despite sequencing advances, characterized profiles of the human gut microbiome across diverse populations remain fragmented, hindering microbial community classification and meta-studies. We standardized 514,932 metagenome-assembled genomes (MAGs) from over 22 thousand samples to create comprehensive microbiome fingerprints. Our resulting interactive platform comes with a AutoML-driven bioindicator detection, which can scan functional potential patterns (e.g., countries and disease conditions).

We selected 9,798 metagenomic samples (>20MM depth) from HumanMetagenomeDB and recovered 299,545 MAGs using MuDoGeR. Following, we included and harmonized 151,557 MAGs (from 8,625 samples) from Pasolli et al. (Cell, 2019) and 63,830 MAGs (from 3,638 samples) from Nayfach et al. (Nature, 2019) to create a unified, standardized dataset. After dereplication, we identified 6,794 species. All MAGs underwent gene annotation using the ISfinder, NCBI-RefSeq, UniProt, and HMM databases via Prokka. From these, we created a presence/absence gene profile for each MAG in adult gut samples (>18 years), yielding 426,648 profiles with 40,424 non-redundant genes. Next, we implemented an autoencoder with dense layers and ReLU activation, followed by DenMune clustering to capture metagenomic fingerprints. We analysed MAG distribution across taxonomy, geography, and host medical conditions, and developed an interactive platform with AutoML-driven bioindicator detection using the curated metadata.

The embedded space revealed distinct microbiome profiles, showing significant differences between control and colorectal cancerous samples and separate libraries by country (PERMANOVA < 0.05). Additionally, the MAGs clusters based on functional potential identified taxonomical groups diverging from their majority cluster, which could signal relevant functional shifts in specific strains. Our AutoML bioindicator detection system uncovered geographical markers, with *Agathobacter rectalis* and *Lachnospira eligens*, for instance, emerging as a promising bioindicator for differentiating Chinese and USA gut microbiomes. Specifically, *A. rectalis* have evidence to be involved in the immune dysregulation and the gut-brain axis. This study provides a standardized dataset, downloadable genome collection, and interactive webapp that can accelerate microbiome research across diverse disciplines, enabling faster hypothesis generation and more targeted experimental design.

The human microbiome in health and disease I

FT038

Pan-microbiome analysis on the metagenomes of the human Respiratory Tract (RT)

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[2]Harvard University, Biostatistics Dept., Boston, MA, United States
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[4]BioTechMed Graz, Graz, Austria

The human Respiratory Tract Microbiome (RTM) plays a critical role in respiratory health, with dysbiosis linked to conditions such as Asthma, Covid-19, Pneumonia, and Cystic Fibrosis. However, high inter-individual variability and dataset-specific biases challenge the identification of robust microbial signatures. To address this, we leverage a big data approach to integrate diverse datasets, enhance statistical power for biomarker discovery, and establish a comprehensive global view of the RTM across different respiratory conditions.

We analyzed >4,500 samples from >30 open-source Metagenomic Whole-Genome-Sequencing (mWGS) datasets spanning different regions of the human respiratory tract. After metadata standardization, uniform processing, and rigorous quality control, we retained 2,800 high-quality samples for downstream analysis. Using MMUPHin for batch correction and MaAsLin3 for microbial associations, we identified key taxa linked to respiratory diseases, offering novel insights into the RTM"s role in health and disease.

Our findings reveal distinct microbial diversity patterns across the respiratory tract, with alpha diversity peaking in oral samples and declining in the lower respiratory tract. Pneumonia primarily altered microbial prevalence in the lower respiratory tract (LRT), while Covid-19 and Cystic Fibrosis influenced both prevalence and abundance in intermediate respiratory tract (IRT) samples. Notably, Cutibacterium acnes and Rothia mucilaginosa exhibited significant prevalence shifts, with C. acnes being nearly absent in Pneumonia but common in healthy individuals.

These findings underscore disease-specific microbial disruptions and highlight the value of large-scale, stratified microbiome studies. Our approach improves reproducibility and statistical power, facilitating more robust biomarker discovery in respiratory microbiome research.

Fig. 1

Figure 1: Study design/ Dataset overview

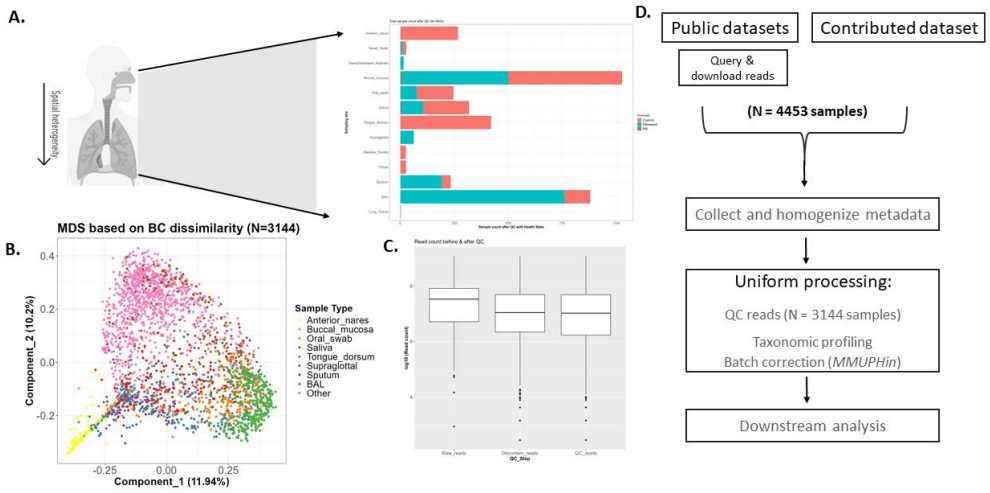
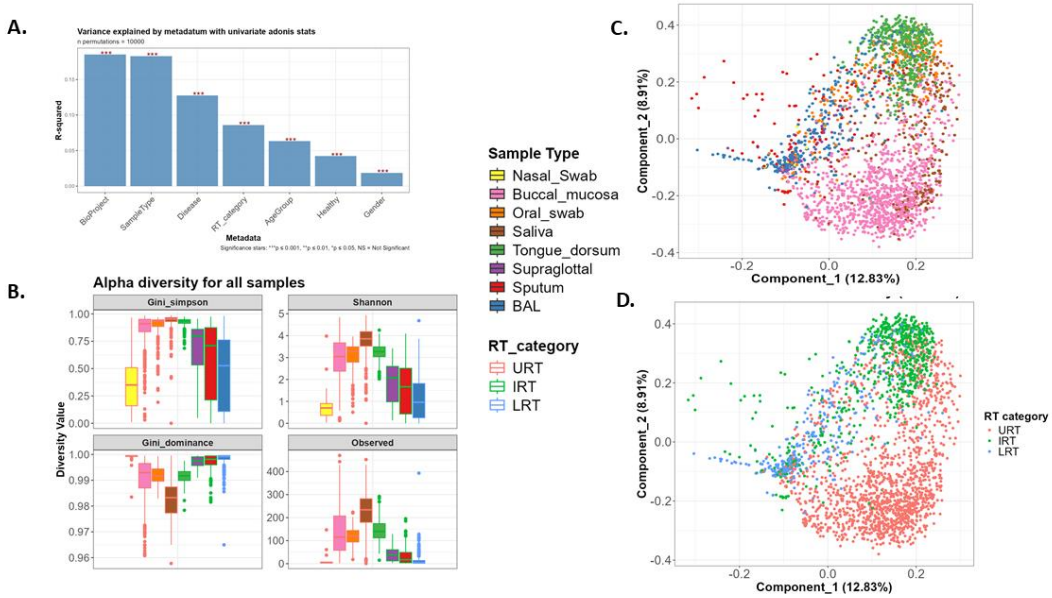


Fig. 2

Figure 2: Global microbiome structure



The human microbiome in health and disease I

FT039

OMV-mediated misfolded protein expulsion for alleviation of membrane stress in *Acinetobacter baumannii*

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The opportunistic pathogen *Acinetobacter baumannii* produces outer membrane vesicles (OMVs) to alleviate envelope stress, but the underlying mechanisms remain unclear. Quantification and imaging with lipophilic dyes, scanning electron microscopy, and transmission electron microscopy revealed increased OMV production with larger diameters at elevated temperatures in *degP* mutants, despite no growth defects. Proteomic analysis showed an enrichment of outer membrane proteins (OmpA, OmpW, BamABDE, and LptDE) in OMVs, but not in the outer membrane, under heat stress in *degP* mutants. The loss of DegP chaperone activity led to misfolded protein aggregation in the periplasm, triggering OMV formation. Fluorescence recovery after photobleaching confirmed the static nature of aggregated proteins. Western blotting indicated elevated levels of mislocalized LPS and misfolded OmpA in OMV lumens. Imaging of mCherry-tagged OmpA confirmed OMVs expelled misfolded proteins in *degP* mutants. Additionally, the lytic transglycosylase MltD was identified in OMVs, and its deletion reduced OMV production, highlighting its role in periplasmic homeostasis. These findings suggest OMV-mediated misfolded protein expulsion as a key mechanism for stress alleviation in *A. baumannii*.

Fig. 1

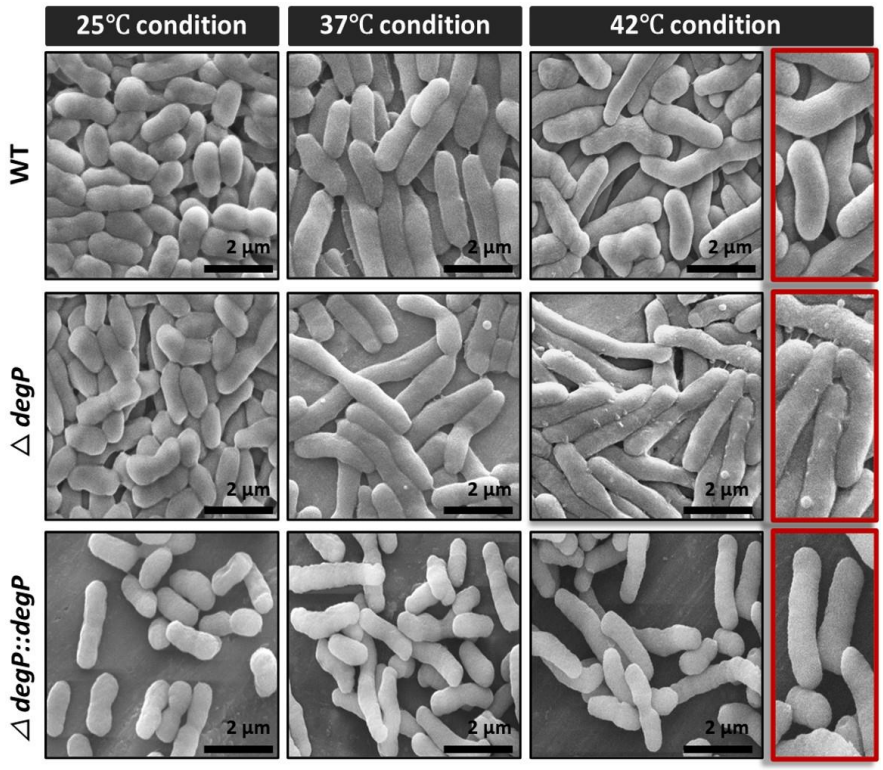
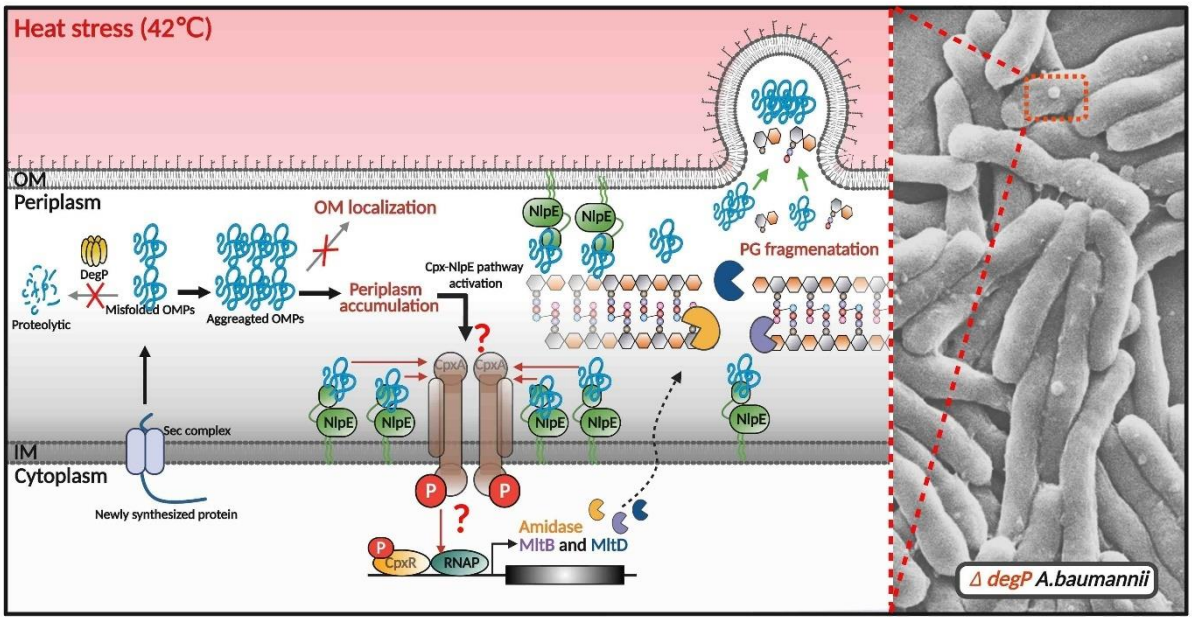


Fig. 2


Heat stress (42°C)
OM
Periplasm
OM localization
DegP
Proteolytic
Misfolded OMPs
Aggregated OMPs
Periplasm accumulation
Cpx-NlpE pathway activation
NlpE
PG fragmenation
CpxA
Sec complex
IM
Cytoplasm
Newly synthesized protein
P
CpxR
RNAP
Amidase
MltB and MltD
Δ degP A.baumannii

The human microbiome in health and disease II

AL035

Exploring Antimicrobial Resistance and Plasmid Diversity in the Early Life Gut Microbiome

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The escalating global challenge of antimicrobial resistance (AMR) alongside the enigmatic role of plasmids within the human gut microbiome constitutes a frontier for microbial ecology and public health research. This presentation synthesizes findings from two studies conducted by my group, shedding light on the intricacies of AMR and plasmid dynamics within the gut microbiomes of early life. The first study unravels the acquisition and determinants of antibiotic resistance genes (ARGs) in a cohort of 662 Danish children during their first year, revealing a bimodal distribution of ARG richness influenced predominantly by gut microbiome composition, particularly *E. coli*. Notably, environmental factors including antibiotic exposure play a significant role in shaping ARG profiles, which in turn correlate with gut microbiome maturity and potential health risks such as asthma. The second study pioneers a novel approach to plasmid analysis in the gut microbiomes of 34 mother-child cohorts, uncovering a previously underestimated plasmid diversity. This exploration not only expands our understanding of plasmid-host interactions but also illuminates the mechanisms through which plasmids enhance bacterial adaptability, especially in infants. Together, these studies offer novel insights into the microbial dynamics of the gut, emphasizing the critical need for innovative strategies to manage AMR and understand microbial gene transfer mechanisms in the context of human health.

The human microbiome in health and disease II

AL036

Modes of invasion – how opportunistic pathogens disrupt or integrate into the gut microbiome

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The gut microbiome, a complex ecosystem of microorganisms, plays a vital role in host homeostasis, including nutrition, immune system development, metabolism, and defense against pathogens. Disruptions in its composition can lead to the invasion and overgrowth of opportunistic pathogens, jeopardizing host health. The intrinsic factors that determine whether these pathogens can invade and cause dysbiosis are not well understood. We aim to characterize the growth characteristics of opportunistic pathogens and their mode of invasion in established communities. We grew ten common gut microbiome members under anaerobic conditions to determine their growth characteristics and in the supernatant of all nine other members to determine their interactions. A mathematical model fitted on those interactions could predict relative abundance in co-cultures of up to five species. Next, we simulated invasions of the opportunistic pathogens in co-cultures of different sizes and validated four cases experimentally. This revealed that the species had different modes of invasion or destabilisation: (1) displacement of native species, resulting in a reduction or replacement of a consortium member, and (2) integration into the existing microbiome without displacing other species or (3) destabilisation of the community resulting in multi-species dropouts. We show that these modes can be explained by their differences in growth characteristics and interactions with the other microbiome members. We conclude that opportunistic pathogens can have at least two sets of properties to invade an established community. Future research is needed to reveal whether most opportunistic pathogens fall in one of those categories or whether there is a continuous spectrum of growth properties. This research underscores the importance of simplified community models in studying gut microbiome dynamics. Our insights may inform future therapeutic strategies aimed at preventing or mitigating pathogen invasions in the gut microbiome.

The human microbiome in health and disease II

AL037

Not only bacteria matter! – How methanogens influence the gut microbiome of "Human Cows"

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Methane (CH_4) emissions are not exclusive to ruminants; the human gut also contains methanogenic archaea that produce methane, albeit in smaller quantities. Research has identified that approx. 40% of the general population can be classified as high CH_4 emitters (CH_4 > 4ppm; "human cows") which harbor a distinct microbial community associated with the archaeal genus *Methanobrevibacter*. This HE microbiome is shaped by dietary influences and has been linked to various health conditions, such as constipation and inflammatory bowel disease.

We hypothesize that *Methanobrevibacter* species depend on specific bacterial partners to thrive. The formation of this microbial community can be influenced by syntrophic interactions, dietary patterns, and the individual's overall health condition.

The aim of this project is to understand the microbial dynamics between the archaeal and bacterial communities in high and low CH_4 emitters (HE and LE) and if dietary habits or gut health influence the archaeal community. Therefore, fecal samples of 80 participants (40 HE and 40 LE) were collected and a multiomics approach (metagenomics, metatranscriptomics and culturomics) was performed.

Human cows (HE) have a significantly higher archaeal abundance in their feces compared to LE (*q=1.43e-06*). Even though both groups were dominated by one archaeal genus, *Methanobrevibacter*, they differed at species level. While HEs were dominated by *M. smithii* and *Cand.* M. intestini, *M.* sp900766745 was the representative in LEs. All three of them come with different bacterial partners and form their own network in the HE and LE gut, respectively. We were able to show a high activity of methanogenic archaea in particular in the human cows including transcripts that are involved in methanogenesis, incomplete tricarboxylic acid cycle (TCA) and gluconeogenesis. Pure culture experiments with isolated *Methanobrevibacter* strains could confirm these findings, as they also showed expression of the same genes.

In summary, this study sheds light on the complex interactions between the three identified methanogens and their bacterial networks in the human gut. Variations in microbial networks and differences in the expression of methanogenesis-related transcripts underscore the functional distinctions between HE and LE. Overall, these findings highlight the essential role of methanogens in the gut microbiome and point to potential strategies for influencing gut health and methane emission.

The human microbiome in health and disease II

AL038

Exploring organosulfonate degradation pathways by *Bilophila* wadsworthia – new substrates and insights from human gut metatranscriptomes

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The human gut pathobiont *Bilophila wadsworthia* has gained increased attention as a potential contributor to human-gut diseases such as irritable bowel disease and colorectal cancer (1,2). This association is thought to be due to increased production of sulfide (H_2S), e.g. from the degradation of organosulfonates, as H_2S can damage the intestinal mucus layer and DNA (3,4). Given the diversity of food-derived organosulfonates, we have evaluated potential organosulfonates for the degradation by the Human Microbiome Project reference strain *B. wadsworthia* 3.1.6. We can confirm and refine the degradation of known organosulfonates, i.e. sulfoacetate and cysteate. In addition, we can report on three new substrates for organosulfonate respiration and on potential degradation pathways used by strain 3.1.6. Finally, we have analyzed publicly available human gut metatranscriptomes from 201 individuals in order to assess the relevance of described and newly discovered organosulfonate substrates, as well as described electron donors for organosulfonate respiration by B. wadsworthia *in situ*.

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The human microbiome in health and disease II

AL039

Udderly Contagious – investigating zoonotic transmission pathways on dairy farms following a One Health approach

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Global demand for meat and dairy has increased in recent decades not only due to population growth, but also a growing global hunger for meat due to increased economic prosperity in developing economies. Long-term food security now relies on changes in farming practices, where farming operations are scaled up, resulting in higher livestock densities, increased antibiotic use and greater interaction between humans and livestock. These practices bring about new global health challenges, such as conditions that promote the transmission of zoonotic diseases.

Healthy dairy cattle can be colonized by pathogenic lineages of *Staphylococcus aureus*, a major human pathogen, without showing symptoms. *S. aureus* is also the cause of bovine mastitis, a potentially fatal mammary gland infection, leading to a significant economic burden on the dairy industry. Here, we investigated potential zoonotic transmission events and the virulence profile of *S. aureus* isolated from healthy cattle and humans from three dairy farms in Canterbury, New Zealand. This is of particluar importance due to the country"s strong economic reliance on milk and livestock production.

45 nasal carriage samples were taken from humans working at the farms alongside 55 inguinal swabs from cattle of various ages. Whole-genome sequencing was performed, followed by multi-locus sequencing typing and detection of antimicrobial resistance (AMR) and virulence genes. Transmission events were determined based on single nucleotide polymorphisms (SNPs). A total of thirteen transmission clusters were detected, with twelve clusters restricted to within species and one zoonotic transmission cluster. Transmission among cattle was mostly limited to a single age group, likely because different age groups are managed separately on farms. While the prevalence of AMR was low among both bovine and human isolates, of particular concern was the discovery of an extended spectrum beta-lactamase gene (blaTEM116) in a bovine isolate.

This research has provided evidence around frequency and patterns of transmission of *S. aureus* on dairy farms in New Zealand, identifying key transmission points. Better understanding of these contact patterns facilitates the development of effective intervention protocols to prevent livestock to human transmission of bacterial pathogens or vice versa.

The human microbiome in health and disease II

FT040

Proteomic and metabolomic profiling of archaeal extracellular vesicles from the human Gut

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Background/Aims: Extracellular vesicles (EVs) facilitate communication within the human microbiome, enabling interactions both among microbial species and between microbes and the host. While bacterial EVs (BEVs) are well-documented, the role of EVs from gut-associated archaea, particularly Methanobrevibacter species, remains largely unexplored. This study aims to characterize the EVs produced by Methanobrevibacter species to investigate their potential role in microbiome-host and microbe-microbe interactions.

Methods/Results: Archaeal extracellular vesicles (AEVs) were isolated from M. smithii and Cand. M. intestini for multi-omics characterization, including proteomic and metabolomic analysis. Structural analysis showed that AEVs are similar in size to BEVs (~130 nm) but possess unique proteomic profiles. A total of 229 proteins were identified in the AEVs, with a significant presence of adhesin-like proteins, suggesting roles in archaea-surface and archaea-bacteria interaction. Metabolomic analysis revealed the presence of glutamic acid and choline glycerophosphate, molecules potentially involved in gut-brain signaling. AEVs were efficiently taken up by macrophages and elicited species-specific responses in both immune and epithelial cells. They triggered chemokines, including CXCL9, CXCL11, and CX3CL1, implicating a role in immune cell recruitment. While AEVs of Cand. M. intestini strongly induced IL-8 in epithelial cells, others exhibited lower inflammatory potential, demonstrating distinct immunostimulatory properties among species.

Conclusion: This study represents a pioneering discovery in microbiome research, identifying the production of extracellular vesicles by gut-associated Methanobrevibacter species. Our findings reveal that their AEVs show distinct molecular compositions, capable of interacting with human cells. Given the limited research on archaea in the human microbiome, our characterization of AEVs opens novel avenues for therapeutic applications. This breakthrough underscores the importance of archaea as integral components of the human microbiota, reshaping our comprehension of interdomain interactions in health and disease. The characterization of AEVs may offer novel paths to modulate vesicle-mediated impacts on host health through an archaeal lens.

The human microbiome in health and disease II

FT041

Exploring the interconnections between indoor microbiomes and respiratory health in children

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In developed countries, individuals typically spend up to 90% of their time indoors, leading to continuous exposure to indoor air pollution. It is essential to understand the interconnectedness of indoor and outdoor air quality, as improvements in the outdoor environment may positively influence indoor air conditions. Poor air quality can pose serious health risks, especially due to biological agents linked to moisture and mold, which can act as allergens or worsen respiratory disease symptoms when airborne and inhaled.

Research indicates that high and diverse microbial exposure in young children is linked to reduced incidence of respiratory diseases. In urban areas where such exposure is insufficient, illnesses such as asthma are more common in children. As part of the EDIAQI project, we monitored the microbiome in the bedrooms of both asthmatic and healthy children. We specifically focused on bed dust, which serves as a primary source of indoor microorganisms. Utilizing high-throughput sequencing of the 16S rRNA gene fragment and the ITS region will allow us to analyze the taxonomic structure of bacterial and fungal communities, respectively. This will facilitate an investigation into the presence and abundance of microorganisms in relation to the lifestyle and health conditions of the children. Additionally, comprehensive data on both indoor and outdoor air quality will enable us to examine the relationships between air quality, the indoor microbiome, and the children's health status.

The human microbiome in health and disease II

FT042

Hold me tight! - Bacterial aggregation in the intestinal microbiota

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Bacterial aggregates, comprising a single or multiple species, have been observed across a variety of ecological niches, for both environmental and pathogenic bacterial populations. This suggests that bacterial aggregation may also be prevalent and ecologically relevant in human microbiomes. For example, in highly competitive environments like the human intestine, multicellular aggregates may provide protection, and hence a selective advantage. However, the specific role of bacterial aggregation in the context of the gut microbiome remains to be elucidated. To examine the effects of aggregation among human gut microbiota, Enterobacteriaceae were isolated from fecal samples taken from intensive care unit patients (from study MS-ICU) who had been treated with the antibiotics meropenem, piperacillin/tazobactam, or with no antibiotics. The aggregation capabilities of 75 isolates were evaluated. For these isolates, we also measured growth rates, biofilm formation abilities, and tolerance and resistance to the antibiotics administered to the patients, and assessed whether these characteristics correlated with aggregation capability. Motivated by the importance of bile acids in influencing the intestinal microbiota and disease progression during sepsis, we also investigated the effects of bile acids on our assays. Finally, we aimed to ascertain whether bacterial aggregates might evade phagocytosis or influence the host"s immune response. Our study reveals highly varied aggregate-forming capabilities among the isolated Enterobacteriaceae strains, with a strong influence of bile acids. Notably, in contrast to previous suggestions, aggregation and biofilm formation are negatively correlated and the aggregate-forming isolates do not exhibit enhanced tolerance or resistance to the tested antibiotics. Furthermore, aggregation did not offer protection from the immune system, since aggregates strongly trigger immune response, and are not universally protected from phagocytosis. Our work suggests that bacterial aggregation may not promote the survival of specific strains within the gut via biofilm formation, antibiotic tolerance or immune evasion. Further investigation is therefore required to elucidate the impact of aggregation on bacterial physiology and on host-pathogen interactions within the gut. Our work should help to elucidate the role of bacterial aggregation in the maintenance of a balanced human intestinal microbiota, and/or the loss of such balance in disease.

Microbial production systems and artificial intelligence

AL040

Insights into black boxes and traveling back in time – computer-inspired protein engineering for the synthesis of biobased olefines polymer precursors

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The on-going digitalization of biotechnology changes the way how enzymes can be approved for biocatalytic application. Two examples illustrate the enormous impact of bioinformatics and machine learning on the engineering of powerful biocatalysts.

A first example deals with an artificial pathway for the synthesis of Tulipalin A, a very promising polymer precursor. While Tulipalin A can be isolated from the flowers of tulips and alstroemerias, its biosynthesis remains unknown. We propose a synthesis from isoprenyl acetate, which itself can be produced via the microbial hemiterpenoid metabolism. The crucial reaction step is catalyzed by a membrane-bound monooxygenase. The difficulty to obtain accurate structure information has been a serious obstacle for their optimization by enzyme engineering. Here we show how de novo structure prediction tools and molecular modeling can guide the generation of tailor-made enzyme variants.

A second example presents enzymatic decarboxylation of bio-based hydroxycinnamic acids that gives access to phenolic styrenes for adhesive production. In biocatalytic reactions, the operational stability of enzymes is a crucial cost factor. Stabilization of enzymes is possible, but suffers from poor predictability and tedious screening campaigns. As practical alternative, we used ancestral sequence reconstruction (ASR) to generate thermostable decarboxylases. Investigation of a set of 16 ancestors resulted in the identification of a variant with an unfolding temperature of 78.1 °C and a half-life time of 45 hours at 60 °C. As no phenolic acid decarboxylases from thermophilic microorganisms are known, the ease to obtain thermostabilized enzymes by ASR is striking. This approach substantially improves productivity, rendering our approach a straightforward option for enhancing the industrial application of the process.

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Key Words: biobased polymers; alpha-methylene lactone; alkane monooxygenase, rational design.

Microbial production systems and artificial intelligence

AL041

Machine learning detects bioproduct treatment signatures in horticultural soil microbiomes

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Understanding how agricultural management practices shape the soil microbiome is essential for improving sustainable farming. In this study, we investigated whether the composition of the soil microbiome alone can be used to accurately predict treatment regimes in agricultural soils from apple, strawberry, and tomato production systems. We analysed approximately 2000 soil samples that were collected in the frame of the EU project EXCALIBUR from field and greenhouse environments, representing diverse treatment combinations, including organic farming and integrated pest management across Europe. The microbial profiles were generated using a standardized methodology for high-throughput sequencing of soil microbial communities, and taxonomic data served as the input features for predictive modelling. We implemented and compared four state-of-the-art classification algorithms, i.e., logistic regression, decision tree, random forest, and gradient boosting (XGBoost). These models were trained to classify soil samples based on their treatment status (treated vs. untreated), crop type, and cultivation system. Our results demonstrate that the microbial composition of soil contains robust signals that reflect underlying treatment histories. All models achieved classification performance significantly above chance level, especially Random Forest and XGBoost consistently outperforming simpler models in distinguishing treatment regimes. This work provides compelling evidence that machine learning models, can effectively capture the subtle microbial signatures imprinted by agricultural practices even at high taxonomic levels. These findings underscore the potential of microbiome-informed forensics as a non-invasive tool for monitoring soil management and guiding sustainable agricultural strategies. We gratefully acknowledge the EXCALIBUR consortium for providing access to the unpublished data used in this study. This work was supported by the EXCALIBUR project, funded under the European Union"s Horizon 2020 research and innovation programme (grant agreement ID 817946).

Microbial production systems and artificial intelligence

AL042

Hydrogen production by cyanobacteria – improving the yield

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Cyanobacteria are widespread in nature Gram negative prokaryotes with oxygenic photosynthesis. Great metabolic plasticity and biosynthetic capacity makes many cyanobacteria capable of producing compounds that can be used as the source of biofuels: hydrogen, lipids, bioethanol, etc. With their genomic and proteomic information becoming increasingly available, and the variety of suitable genetic tools, many cyanobacterial strains can be genetically engineered to further improve the yield of these products.

Cyanobacteria are ideal cell factories for hydrogen production because they have low nutrient requirements and are capable of using light to generate biomass from water and carbon dioxide. Cyanobacteria produce hydrogen through two key enzymes, nitrogenase and bidirectional hydrogenase (Hox), and oxidize molecular hydrogen by uptake hydrogenase (Hup). Increase of hydrogen production and inhibition of the hydrogen-oxidizing activity are important to maximize hydrogen yield from cyanobacterial cultures.

Question: How much of improvement in the production of molecular hydrogen can be achieved by genetic engineering of cyanobacterial strains?

Methods: The first approach was to generate knockout mutants of *hup* genes and test hydrogen production in mutant cyanobacterial cultures. The second approach was to overexpress the native *hox* genes in cyanobacteria using a series of alternative promoters. Hydrogen production in the mutant and wild type strains was measured by gas chromatography.

Results: Genetic inactivation of uptake hydrogenase in cyanobacteria affects strains in various ways. In *Anabaena* sp. PCC 7120 it increased hydrogen production by 4-7 fold while in *Anabaena variabilis* ATCC 29413 it reached a 5-fold improvement, compared to the wild type strain. Homologous overexpression of bidirectional hydrogenase produced an 8 to 11 fold increase of hydrogen production, depending on the parental strain and growth conditions.

Conclusion: Uptake hydrogenase knockout strains of cyanobacteria and the strains with overexpressed bidirectional hydrogenase can be the starting point for further genetic modifications for the purpose of enhancement of their hydrogen-producing capacity. Hydrogen can be used as an alternative biofuel, and cyanobacteria can generate it using the renewable energy of sunlight. Additionally, photosynthetic growth of cyanobacteria is consuming carbon dioxide and reducing carbon footprint - hence, it is environmentally friendly.

Microbial production systems and artificial intelligence

AL043

Exploring the propagation and function of the polyunsaturated fatty acid synthase system in bacteria beyond marine environments

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The bacterial production of long-chain polyunsaturated fatty acids and hydrocarbons (PUFA & PUHC respectively) is an elusive trait associated primarily with psychrophilic and piezophilic marine organisms. Since the discovery of their unique synthase genes, denoted as *pfa*, a special iterative type I polyketide synthase (PKS)-like system, there has been widespread interest towards harnessing this synthase system for industrial-scale production. This is because a bacterial producer of polyunsaturated fatty acids or alkenes is an attractive alternative to using marine or petroleum-based resources, and could supply oils for human nutrition or for industrial applications. However, two barriers remain in this effort. First, the breadth and distribution of bacteria who contain a functional *pfa* synthase is under-studied. Second, the physiological role of PUFA and PUHC among bacterial producers is not fully understood, with many conflicting observations. With this work, I present results of a comprehensive *pfa* synthase search among the largest collection of species-dereplicated bacterial genomes, using a sequence-naïve search strategy that instead leverages a distinctive domain architecture. I compare these results with those derived from the typical domain-feature-model searches to illustrate the novelty achieved by moving outside of purely phylogenetic inferences. Hierarchical clustering of the putative synthases using domain organization can predict the type of product for non-experimentally validated synthases. To inform about the functional role of putative synthases in the context of organism activity, I look at the synteny of neighboring genes using a pangenome analysis strategy. Similar genes found in similar arrangements relative to the *pfa* synthase thus relates organisms by their shared expression neighborhood, giving insight about cellular processes that positively correlate to *pfa* expression. Finally, I expand on the proposed physiological purposes for *pfa* synthase presence by analyzing gene expression in *Shewanella* during induced growth of outer membrane extensions (nanowires), and following changes in available terminal electron acceptors. These results help fill a critical gap in understanding the ecological context of *pfa* synthases in bacteria and build awareness about how to successfully incorporate them in biotechnological developments for sustainable industry.

Microbial production systems and artificial intelligence

FT043

Exploration of multiple protein language models and over 16 million open reading frames recovered from thousands of terrestrial metagenomes uncover protein domains and families in 13,486 previously unknown orthologs

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Annotation of open reading frames (ORFs) remains challenging for traditional alignment-based methods, especially for remote homologs or highly divergent sequences. We developed a framework leveraging protein language models (pLMs) for improved functional annotation. By integrating features from the Structural Classification of Proteins (SCOP) and Pfam databases, our approach enhances protein family detection and advances understanding of uncharacterised proteins. We assembled a dataset from the Collaborative Multi-domain Exploration of Terrestrial metagenomes (https://www.ufz.de/index.php?en=47300), comprising 23,336 high-quality metagenome-assembled genomes from 3,802 metagenomes. Over 16 million ORFs were extracted and dereplicated into 2.4 million orthologs. From these orthologs, we annotated 7.3 million representative ORFs using Pfam, KEGG, COG, and nr_NCBI databases, with 82.64% of the orthologs classified as hypothetical or unknown. After filtering, the 292,728 annotated ORFs were embedded using four pre-trained pLMs (DLM-LSTM, MT-LSTM, ProtBert-BFD, and ProtT5-XL-U50) and integrated into a hierarchical multi-label classification framework. We based our framework on the 1,375 SCOP-Pfam families mapped by employing the Local Classifier per Parent Node approach and evaluated our pLMs using Friedman and Nemenyi post-hoc tests. Hierarchical classification significantly improved the performance of smaller models, making them as robust as more complex models for prediction. While accuracy varied across models, their effectiveness depended on the specific protein family being classified. Notably, hierarchical approaches provided a distinct advantage for smaller models, enhancing their ability to classify certain families. From the 2.4 million orthologs, we identified 69,028 unique orthologs with Pfams using alignment-based techniques. With a high level of reliability (99% probability threshold), we identified 871 protein families in 13,486 unique orthologs (32,146 ORFs). Therefore, our pLM alignment-free approach identified 19.54% more orthologs beyond those detected with alignment-based methods. These orthologs spanned 89 bacterial phyla (e.g., Patescibacteria, Acidobacteriota, Verrucomicrobiota) and 14 archaeal phyla. Our findings emphasise that a long road exists for annotating novel protein functions and that integrating pLMs with unique architectures and datasets will uncover novel protein functions and provide new insights into microbial ecology.

Microbial production systems and artificial intelligence

FT044

Global DNA signatures of temperature and nutrient limitation in prokaryotes

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Microorganisms adapt to their environment through changes in both their genes and overall genome composition. However, identifying universal principles of genomic adaptation remains challenging because different environments present multiple, overlapping selective pressures. To overcome this challenge, we analyzed DNA composition patterns across diverse environments using machine learning. By examining tetranucleotide frequencies from 1,112 marine and soil metagenomic samples, we discovered that environmental temperature can be accurately predicted from DNA composition alone (R2=0.82). This temperature signal remained robust even when analyzing individual bacterial phyla and classes, suggesting a fundamental adaptive response. To examine this adaptation mechanism, we analyzed GC content relationships with temperature and found opposing relationships in different environments: positive correlations in soil but negative correlations in marine samples. We show that this inverse relationship in marine environments is driven by nutrient availability, as GC content increases with nutrient levels while nutrients decrease with temperature in marine ecosystems. Despite these contrasting GC patterns, we identified specific tetranucleotides composed of equal numbers of GC and AT bases (50% GC content) that showed consistent temperature correlations across all environments. These findings reveal a complex interplay between temperature adaptation and nutrient limitation in shaping microbial genomes.

Fig. 1

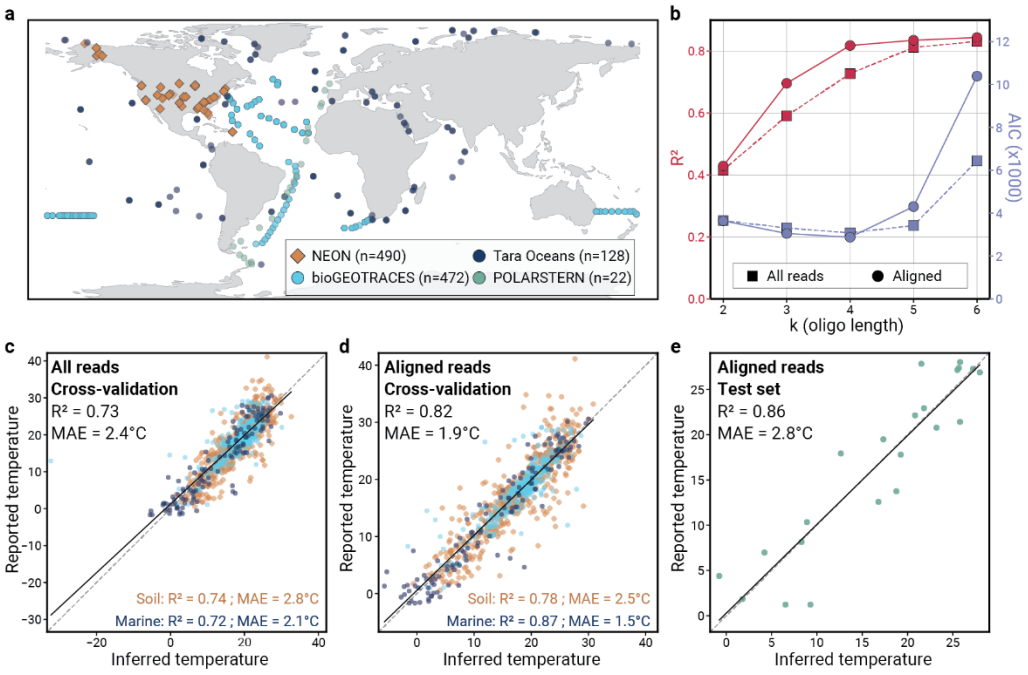
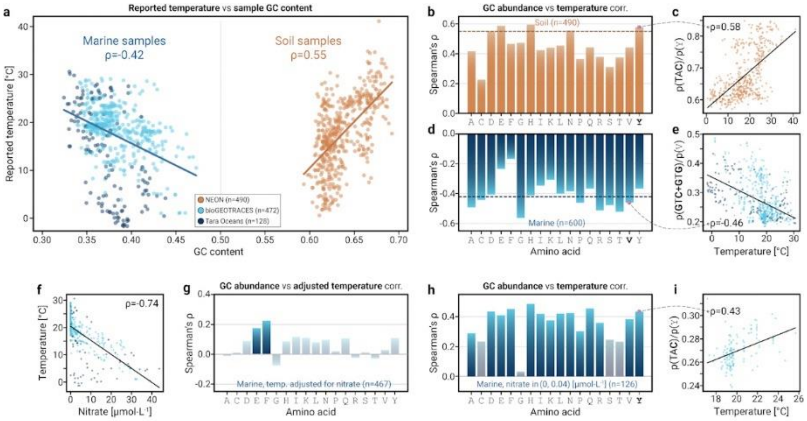


Fig. 2


a
Reported temperature vs sample GC content
Marine samples
ρ=-0.42
Soil samples
ρ=0.55
NEON (n=490)
bioGEOTRACES (n=472)
Tara Oceans (n=126)
Reported temperature [°C]
GC content
b
GC abundance vs temperature corr.
Soil (n=490)
Spearman's ρ
c
ρ=0.58
p(TAC)/p(·)
d
Marine (n=600)
Amino acid
e
ρ=-0.46
p(GTC+GTG)/p(·)
Temperature [°C]
f
ρ=-0.74
Temperature [°C]
Nitrate [µmol·L⁻¹]
g
GC abundance vs adjusted temperature corr.
Marine, temp. adjusted for nitrate (n=467)
Amino acid
h
GC abundance vs temperature corr.
Marine, nitrate in (0, 0.04) [µmol·L⁻¹] (n=126)
Amino acid
i
ρ=0.43
p(TAC)/p(·)
Temperature [°C]

Microbial production systems and artificial intelligence

FT045

Integrating machine learning to decipher rare microbial responses to arctic ocean warming

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The Arctic Ocean is highly impacted by climate change [1], yet its effects on prokaryotic communities, particularly the rare biosphere, remain largely unexplored. The rare biosphere plays a crucial role in ecosystem resistance and resilience [2], but is often overlooked in Arctic microbial studies.

To assess climate-driven shifts in the prokaryotic rare biosphere, we analyzed a standardized V4V5 16S rRNA gene amplicon dataset (n = 117) from 2016–2020, spanning epi-, meso-, and bathypelagic layers. Rare taxa were classified using the ulrb R package [3,4], and diversity patterns were examined through baseline statistical analysis and rule-based machine learning (association rule mining) [5].

Our results indicate that rising temperatures lead to a net loss of prokaryotic diversity, predominantly affecting the rare biosphere (~9 rare taxa lost per 1°C). This trend was not observed across years but along the water column, reflecting Arctic Ocean stratification. Within pelagic layers, temperature-driven diversity loss coincided with Atlantification, where Arctic taxa were replaced by Atlantic taxa. Machine learning revealed complex, non-linear relationships missed by traditional methods. Notably, it identified OM190 (Planctomycetota) as conditionally rare in the mesopelagic layer but always rare in the epipelagic layer—an undetected pattern in classical analyses.

Our findings demonstrate the negative impact of warming on the Arctic Ocean"s microbial rare biosphere and highlight the advantages of integrating unsupervised and rule-based machine learning for ecological assessments.

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Microbiome engineering for sustainable agriculture

AL044

The contribution of organic fertilizers and irrigation water to the burden of antibiotic resistance genes and plasmids in soil and plant microbiomes

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The acquisition of multiple antibiotic resistance genes (ARGs) by bacterial pathogens allows them to survive the exposure to antibiotics and thus increasingly hinders the successful treatment of bacterial infections in humans and animals. The exposure to mixtures of micro-pollutants in the presence of nutrients is the major driver contributing the proliferation of antibiotic resistant bacteria, the mobilization of ARGs and horizontal transfer of ARGs. The dissemination of ARG carrying bacteria between different habitats is also triggered by mixing human (biosolids, irrigation water) or animal (manure, digestate) gut microbiomes with the soil microbiomes and subsequently connecting to plants. For more than 30 years, my group investigated the contribution of organic fertilizers and more recently of irrigation water on the dissemination and transferability of ARGs using cultivation-independent and -dependent methods.

I will present recent work on antibiotic resistance plasmids captured into *E. coli* exogenously from biosolid bacteria of wastewater treatment plants (WWTPs). The molecular characterization (real-time-PCR, plasmid sequencing) showed that the majority of plasmids captured belonged to the IncP-1, IncN and IncU groups and carried complex ARGs often linked to class I integrons. Remarkably, we observed identical plasmids from different WWTPs. Biosolids contained not only ARG carrying bacteria but also a wide spectrum of antibiotics, heavy metals and disinfectants. The accessory gene load of plasmids captured likely contributed to a rapid adaptation of their hosts to survive in the presence of pollutants such as antibiotics. In the DFG-PARES project, we investigated the effect of irrigation water quality on the microbiome of soil, rhizosphere and phyllosphere of cilantro grown at four different sites in the Mezquital valley in Mexico with treated wastewater or spring water used for irrigation. Interestingly, the irrigation water quality was the main factor shaping the microbiome composition and the profile of the ARGs and mobile genetic elements (MGEs) in soil, cilantro rhizosphere and phyllosphere. In-depth analysis of the microbiome by 16S rRNA gene amplicon analysis revealed that in the cilantro phyllosphere irrigated with treated wastewater top abundant ASVs affiliated to *Acinetobacter* and *Enterobacteriaceae* were increased. Our data demonstrated that irrigation water quality shapes the microbiome and the ARG/MGE profiles of bulk soil, cilantro rhizosphere and phyllosphere. We suggest, ARGs connectivity between environments and the link to the human gut microbiome through produce.

Microbiome engineering for sustainable agriculture

AL045

Plant microbiome improvement – microbiome modulation, inoculation and future avenues

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Complex microbiota are found in association with different plant tissues, from below-ground organs like roots/rhizosphere to above-ground tissues and reproductive organs. Microbiome diversity and functions are driven by multiple parameters such as the tissue, vegetation stage, cultivar, agro-management and many other environmental factors. Accordingly, different approaches such as microbial inoculation and microbiome modulation are used to improve holobiont functions and increase the sustainability and productivity of plant production. This talk will discuss the potential of current and future approaches to improve plant holobiont functions.

Microbiome engineering for sustainable agriculture

AL046

Soil microbial communities regulate plant response to drought stress

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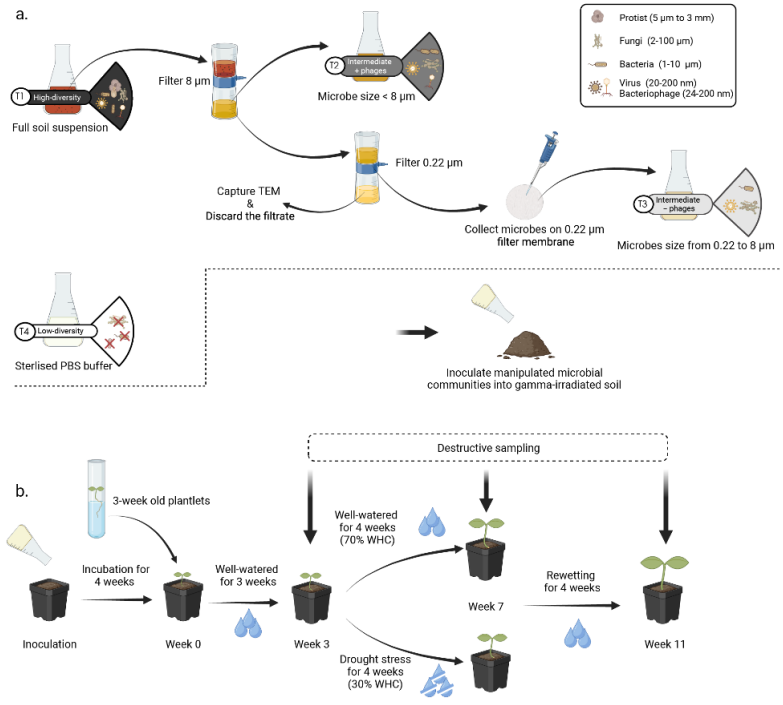
Background: Soil biodiversity, essential for ecosystem functioning and plant resilience, is increasingly threatened by climate change and agricultural practices. While the impact of soil biodiversity loss on plants like legumes and grasses has been demonstrated, its effect on potatoes remains underexplored. This study addresses this gap by investigating how different microbial diversity gradients, including the presence of phages, influence potato growth and resilience under drought stress in a controlled climate chamber experiment. Results: Our findings reveal that varying microbial diversity leads to distinct plant growth patterns, with low diversity treatments suppressing plant recovery post-stress. The presence of phages enhanced plant performance during drought stress, although no significant effect on recovery was observed. To our knowledge, this is the first study to demonstrate that the simplification of soil microbial diversity regulates the secretion of root exudate metabolites. Our results showed that soil biodiversity simplification significantly affects root exudate metabolite patterns in diversity and composition. Furthermore, correlation analysis revealed a positive correlation between soil biodiversity and plant productivity, particularly during the recovery period following drought stress, emphasising the critical role of microbial community diversity in supporting plant resilience. Conclusions: These findings underscore the importance of maintaining healthy soil microbial communities to enhance plant drought tolerance and sustain agricultural productivity in the face of climate change.

Fig. 1



Microbiome engineering for sustainable agriculture

AL047

Linking host root genes and belowground keystone microorganisms in the olive tree holobiont – the case of Verticillium wilt tolerance

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[2]University of Jaén, Department of Experimental Biology, Jaén, Spain

Microbial communities have been shown to enhance plant adaptation to abiotic stresses and to mitigate the impact of phytopathogens. Microbiome engineering approaches face challenges due to the complexity of microbial interactions. Advances in transcriptomics and metataxonomics now enable the identification of microbiome-associated phenotypes, co-occurrence networks, and host genes-microbiome interactions. We present a novel framework that combines co-occurrence network analyses and host transcriptome-microbiota correlations. Thus, we aim to identify microorganisms and host genes potentially involved in tolerance to Verticillium wilt olive (*Olea europaea* L.) (VWO), a devastating disease caused by the soil-borne, fungal vascular pathogen *Verticillium dahliae*. Both microbiome-regulating host genes and keystone bacteria and fungi could be eventually used as genetic and microbiological markers in olive breeding programs.

In the root endosphere, olive cultivars tolerant to VWO exhibited an enrichment of the bacterial genera *Actinophytocola*, *Kibdelosporangium* and *Nocardia*. Keystone taxa analyses revealed sharply different profiles when comparing microbial co-occurrence networks of the VWO-tolerant olive varieties with those ones displaying susceptibility to the disease. Thus, tolerant cultivars harbored bacteria predominantly displaying negative interactions with the mycobiome present in olive roots. In contrast, VWO-susceptible cultivars showed microbial hubs with positive fungal correlations. The analysis of the olive root transcriptome allowed identifying 1,143 differentially expressed genes (DEGs), with 309 upregulated genes in tolerant cultivars. Biological processes like defense response, carbohydrate metabolism, and amino acid transport were highlighted. Key bacterial taxa as *Actinophytocola*, *Kibdelosporangium*, *Nocardia*, as well as the fungi *Aquabispora* and *Fusarium*, strongly correlated with DEGs associated with plant defense.

These findings underscore the importance of studying keystone taxa along with essential host plant genes to understand plant-microbiota interactions and explore their potential in disease management. This holistic approach provides insights into the complex dialogue occurring between the host plant and its microbiota, offering potential targets for microbiome engineering to enhance olive resilience against VWO.

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Microbiome engineering for sustainable agriculture

AL048

The Evolutionary Role of Fruit in the Assembly of the Seed and Seedling Microbiome

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Fruits have evolved not only as vehicles for seed dispersal but also as ecological niches harboring complex microbial communities. Recent research suggests that these microbial assemblages play a crucial role in shaping the seed and seedling microbiome, with profound implications for plant health and adaptation. In this presentation, we explore the evolutionary significance of fruit as a mediator of microbial inheritance and recruitment. Drawing from comparative experiments in chili and tomato systems, we demonstrate that decomposing fruit tissues serve as microbial hotspots that influence the surrounding soil microbiome and, in turn, the microbiota colonizing emerging seedlings. By comparing fruit-derived and seed-derived microbial trajectories, we uncover distinct patterns of microbial transmission and highlight the potential of fruit decomposition as a selective filter or amplifier of beneficial microbes. These findings provide new insights into the co-evolution of fruit traits and microbial inheritance, suggesting that fruit-associated microbiomes may represent an overlooked mechanism of plant-microbe co-adaptation. We propose an expanded view of plant reproduction that integrates microbiome dynamics into the evolutionary framework of seed dispersal and seedling establishment.

Microbiome engineering for sustainable agriculture

FT046

Studying the evolution of soil bacteria to understand how the soil microbiome influences one health

B. Watson[1]

[1]University of Exeter, Penryn, United Kingdom

Soil microbiomes significantly influence the function and health of related ecosystems and are therefore interconnected with human health and disease. It is essential that we examine how microbes function and evolve in the soil so we can understand how the soil microbiome influences One Health, such as through the emergence of pathogens. Bacteria can rapidly adapt to new environments by acquiring traits from other bacteria on mobile genetic elements, which can lead to the spread of antimicrobial resistance and virulence traits. However, some elements may be costly, so bacteria carry defence systems to limit novel element uptake. Bacteria vary in the number and type of defence systems they carry, which will influence the types of mobile elements that are inhibited. Hence, in complex communities like the soil microbiome where bacteria are frequently exposed to mobile elements, differences in the repertoire of defence systems are predicted to drive variations in bacterial evolution. I will present my work examining bacterial evolution in soil, using *Azospirillum* spp. as a model. Azospirilla are ubiquitous free-living beneficial soil bacteria that can live on plant roots to support plant growth. These bacteria adapted to this niche by acquiring the necessary traits on mobile genetic elements from other soil bacteria. By comparing azospirilla to their relatives, I saw that azospirilla have much large genomes (on average 7 Mb, with 7 replicons). They also have more mobile elements and there is variation in the number and types of elements predicted. Crucially, azospirilla carry fewer defences than expected, plus they vary in their defence system profile. This suggests that certain defence systems shape bacterial evolution by limiting the ability of strains to acquire beneficial mobile elements, which is predicted to determine their success in soil as *Azospirillum* spp. vary in the number and type of genes that are involved in supporting plant growth. These findings have implications for understanding the evolution of bacteria that influence human health and the wider ecosystem.

Microbiome engineering for sustainable agriculture

FT047

Exploring microbial innovations for enhanced agricultural sustainability and productivity under climate change

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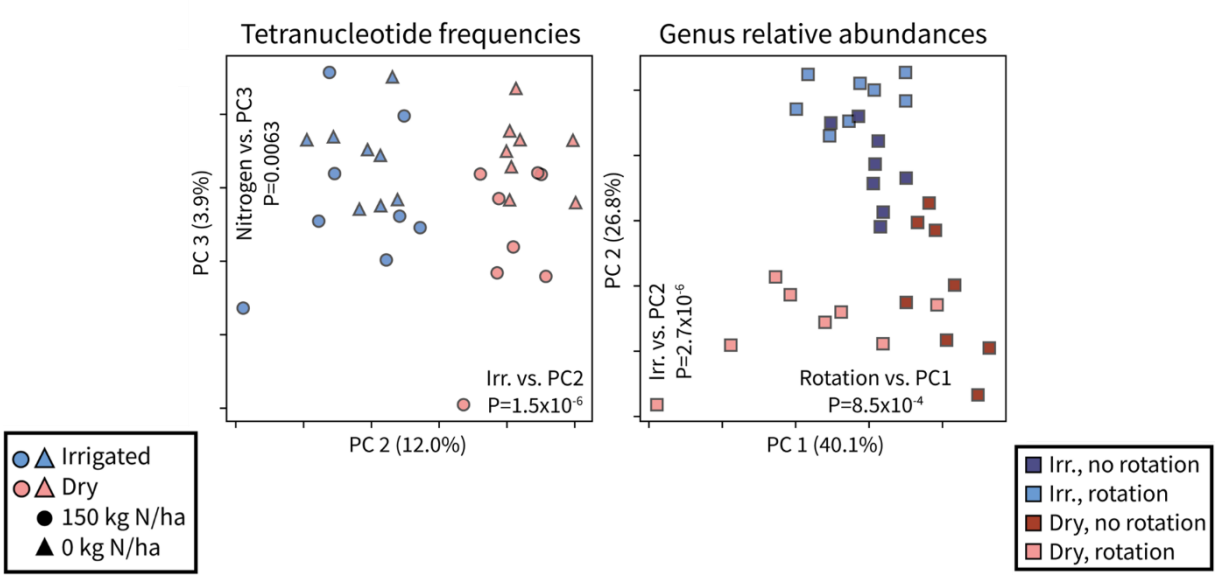
Global population growth and climate change are contributing to more frequent drought events, increasing the challenges of sustaining wheat production. Although decades of breeding and agronomic refinement have optimized wheat, continued improvements are necessary to meet the rising demand driven by a growing population under increasingly water-limited conditions. Soil microbial communities represent a largely untapped resource for enhancing crop resilience and yield, yet the intricate interactions within these systems remain poorly understood.

In this study, we harness the Gilat long-term wheat experiment in Israel—a unique field experiment that bridges real-world agricultural practices with the experimental controls typically found in greenhouse studies. Over decades, 384 plots have been maintained under defined irrigation, fertilization, tillage, and crop rotation regimens, allowing us to systematically dissect the impact of each management practice on soil microbial communities and wheat performance. Our approach integrates shotgun metagenomic sequencing with decades-long records of yield and weather data, with the forthcoming addition of soil chemistry as another critical data layer.

Preliminary principal coordinates analyses reveal significant clustering at the tetranucleotide, taxonomic, and gene-abundance levels in response to irrigation, rotation, and tillage treatments. This intriguing pattern underscores the sensitivity of soil microbes to specific agronomic practices, hinting at underlying biological processes that may drive differences in the microbiome community composition and crop performance.

Looking ahead, our focus will be on the functional aspects of these microbial communities and their metabolic interactions with wheat. By identifying beneficial microbial traits and clarifying their roles in nutrient cycles, stress tolerance (specifically drought), and soil characteristics, we aim to develop a mechanistic understanding of the processes that support sustainable crop production. Ultimately, these insights will inform strategies to a more resilient and sustainable global food system.

Fig. 1


Tetranucleotide frequencies
Genus relative abundances
Nitrogen vs. PC3
P=0.0063
PC 3 (3.9%)
Irr. vs. PC2
P=1.5x10-6
PC 2 (12.0%)
PC 2 (26.8%)
Irr. vs. PC2
P=2.7x10-6
Rotation vs. PC1
P=8.5x10-4
PC 1 (40.1%)
Irrigated
Dry
150 kg N/ha
0 kg N/ha
Irr., no rotation
Irr., rotation
Dry, no rotation
Dry, rotation

Microbiome engineering for sustainable agriculture

FT048

Enhancing phosphate-solubilising microbial communities through artificial selection

L. Faller[1,2], M. F. A. Leite[1,2], E. E. Kuramae[1,2]

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[2]Utrecht University, Institute of Environmental Biology, Utrecht, Netherlands

Microbial communities, acting as key drivers of ecosystem processes, harbour immense potential for sustainable agriculture practices. Phosphate-solubilising microorganisms, for example, can partially replace conventional phosphate fertilisers, which rely on finite resources. However, understanding the mechanisms and engineering efficient communities poses a significant challenge. In this study, we employ two artificial selection methods, environmental perturbation, and propagation, to construct phosphate-solubilising microbial communities. To assess trait transferability, we investigate the community performance in different media and a hydroponic system with *Chrysanthemum indicum*. Our findings reveal a distinct subset of phosphate-solubilising bacteria primarily dominated by *Klebsiella* and Enterobacterales. The propagated communities consistently demonstrate elevated levels of phosphate solubilisation, surpassing the starting soil community by 24.2% in activity. The increased activity of propagated communities remains consistent upon introduction into the hydroponic system. This study shows the efficacy of community-level artificial selection, particularly through propagation, as a tool for successfully modifying microbial communities to enhance phosphate solubilisation.

Microbiome engineering for sustainable agriculture

FT049

Conserving natural biodiversity – impacts of long-term seed storage on total and active bacteria of endemic alpine plants

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[2]Royal Botanic Gardens, Kew, United Kingdom
[3]Karl Franzens University of Graz, Graz, Austria
[4]Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
[5]University of Potsdam, Institute for Biochemistry and Biology, Potsdam, Germany
[6]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Despite the well-established importance of seed-associated microbes for plant health, little is known about their fate during long-term seed storage. In collaboration with the Millennium Seed Bank at Kew Botanical Gardens, which houses a collection of 2.4 billion seeds from around the globe, and the seed bank of Graz Botanical Gardens, we investigated the impact of seed archiving on the microbiomes of three endemic plant species from the Austrian Alps.

Utilizing DNA and RNA amplicon sequencing, we analyzed both total and active microbial communities in freshly harvested and archived seeds, as well as in plants that germinated after archiving. Additionally, we examined the total and active bacteria during six initial stages of seed germination: dormancy, post-vernalization, light exposure, germination, and 7 and 14 days after germination.

The RNA samples consistently demonstrated higher bacterial diversity than the DNA samples, indicating the presence of an active, low abundant, rare biosphere. The microbiota of fresh seeds were signficantly more diverse and primarily influenced by the plant genotype and population, while storage at both facilities homogenized the microbiota. Seeds from the Millennium Seed Bank exhibited higher bacterial diversity than seeds from Graz Botanical Gardens, a trend that persisted post-germination.

The six-stage germination assay revealed that bacterial diversity at the RNA level significantly increased after vernalization, remaining stable through subsequent developmental stages. However, community composition varied significantly, with certain taxa predominating at specific developmental stages; for example, *Pseudomonas* and *Erwinia* dominated the dormant seed, whereas *Methylobacterium* and *Aureimonas* increased after vernalization. Light activiated *Rhodococcus, Flavobacterium,* and *Caulobacter*, and during germination *Rhizobacter* and *Devosia* increased.

Our study illustrates that storage practices can influence the viability of microorganisms, potentially impacting plant-microbe interactions when seeds are reintroduced into their natural habitats. Optimizing seed bank storage conditions is critical for preserving both plant seeds and their microbial symbionts, thereby contributing to global biodiversity conservation and agricultural resilience.