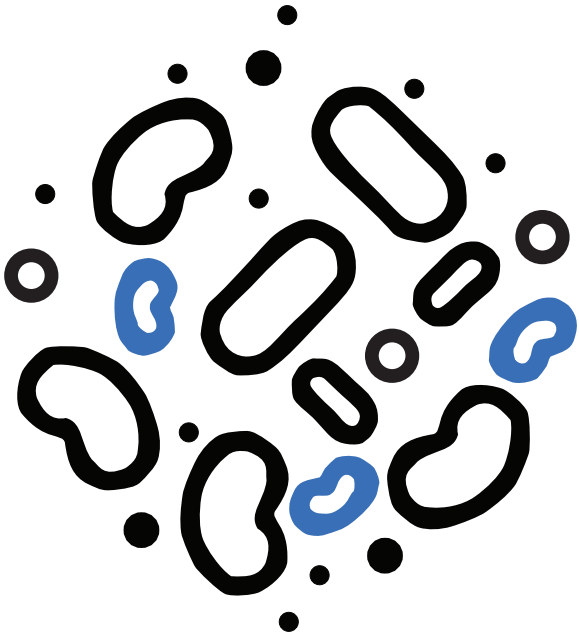

Slovenian Microbiome Network Symposium 2023

Defining a Healthy Microbiome

30. 11. - 1. 12. 2023, Maribor, Slovenia

Abstract Book

Slovenian Microbiome Network Symposium 2023
2023, Maribor, Slovenia

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Welcome note

Welcome to the Slovenian Microbiome Network Symposium 2023!

It is with great pleasure and anticipation that we extend a warm welcome to all participants joining us for this year's symposium. Under the overarching theme of "Defining a Healthy Microbiome," we are embarking on a journey to unravel the mysteries of microbial communities and their diverse applications.

This year, we are thrilled to host outstanding speakers from the region who will share their insights, expertise, and groundbreaking research. The symposium has attracted 100 attendees from 13 different European countries, underscoring the high level of interest and importance that researchers place on the field of microbiome studies. It is a testament to the growing significance of understanding the microbiome's role in health, ecology, and beyond.

Our two-day program features excellent talks, engaging poster presentations, a spacious sponsor exhibition area, and ample opportunities for networking. We encourage you to make the most of these two days — to not only delve into the scientific discussions but also to connect, share ideas, and build collaborations that transcend borders.

As we convene for this intellectual exchange, we hope the symposium serves as a catalyst for innovative thinking, fostering a deeper understanding of the microbiome and its impact on diverse facets of our lives. Thank you for being a vital part of the Slovenian Microbiome Network Symposium 2023. We look forward to the discoveries, connections, and shared experiences that will undoubtedly unfold over the next two days.

Let the exploration begin!

Warm regards,

Nejc Stopnisek

Organizer of the SMNS2023

Program

Day 1 | Nov 30, 2023

7:30-9:00 Registration

9:00-9:15 Welcome

9:15-10:00 Keynote: Christine Moissl-Eichinger (AT)

10:00-10:30 Coffee Break

Session 1: Environmental Microbiome: Linking Microbes to Ecosystem Health (Jozsef Geml)

10:30-11:00 Ulisses Nunes da Rocha

11:00-11:30 Petra Pjevac

11:30-11:50 Ines Svilicic Petric

11:50-12:10 Tijana Martinovic

12:10-12:30 Denis Kutnjak

12:30-14:00 Lunch Break

Session 2: Animal Microbiome: From Gut to Habitat (Suncica Bosak)

14:00-14:30 Marton Papp

14:30-15:00 Suncica Bosak

15:00-15:20 Adrian Wolfgang

15:20-15:40 Bojan Papic

15:40-17:30 Poster Session

19:00 Welcome dinner

Day 2 | Dec 1, 2023

Session 3: Human Microbiome: Insights into Health and Disease (Blaz Stres)

9:00-9:30	Marcus Claesson
9:30-10:00	Blaz Stres
10:00-10:20	Ursa Miklavcic
10:20-10:40	Klara Cerk
10:40-11:00	Matthias Schweitzer

11:00-12:00 Coffee Break and Poster Session

Session 4: Plant Microbiome: Harnessing the Power of Plant-Microbe Interactions (Nejc Stopnisek)

12:00-12:30	Tomislav Cernava
12:30-13:00	Omri Finkel
13:00-13:20	Carolina Lobato
13:20-13:40	Kristina Michl
13:40-14:00	Birgit Wassermann

14:00-15:30 Lunch Break

Session 5: One Health and Healthy Microbiomes (Gabriele Berg)

15:30-16:00	Emilia Hannula
16:00-16:30	Gabriele Berg
16:30-16:50	Vita Rozman
16:50-17:10	Isabella Kögl
17:10-17:30	Wisnu Wicaksono

Committees

Scientific

- Nejc Stopnisek, National laboratory of health, environment and food, Slovenia
- Gabrielle Berg, Technical University Graz, Austria
- Suncica Bosak, University of Zagreb, Croatia
- Joszef Geml, Eszterházy Károly Catholic University, Hungary
- Blaz Stres, Slovenian Institute of Chemistry, Slovenia

Organizing

- Nejc Stopnisek, National laboratory of health, environment and food, Slovenia
- Sandra Janezic, National laboratory of health, environment and food, Slovenia
- Natasa Sibanc, Slovenian forestry institute, Slovenia

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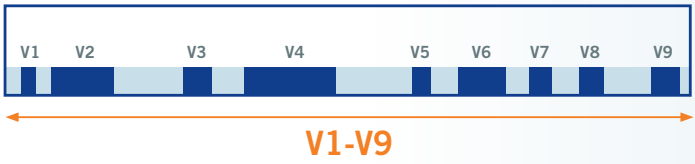

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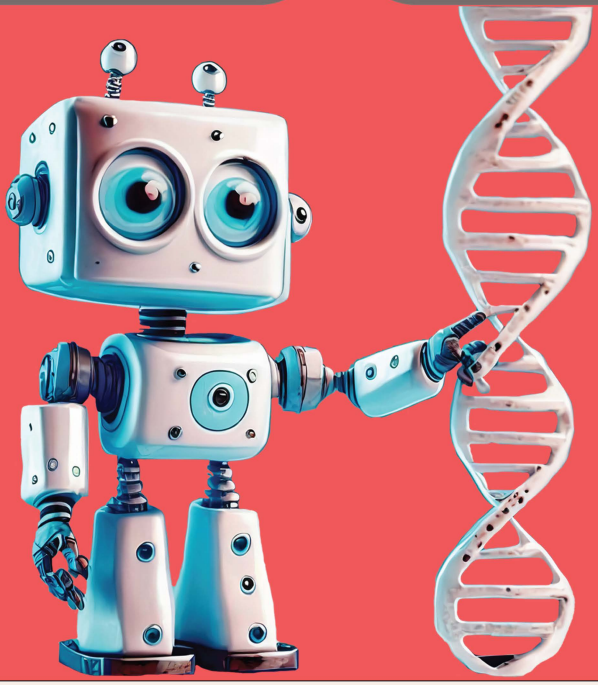

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Content

General information

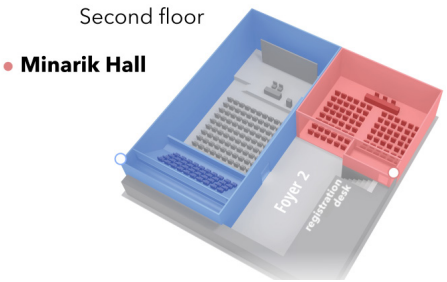
SMNS2023 will take place from November 30 to December 1, 2023, at Hotel Habakuk in Maribor, Slovenia.

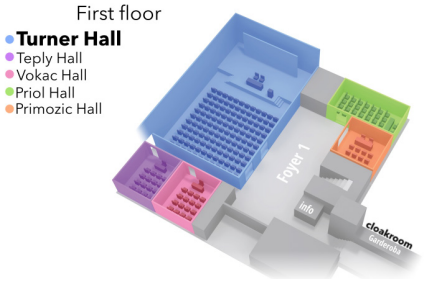
The registration desk will be operational from 7:30 to 9:30 on both days, with personnel available throughout breaks. You can find the registration desk on the 2nd floor.

The comprehensive program comprises five sessions covering various facets of microbiome research. All oral presentations will be held in Minarik Hall on the 2nd floor of the conference center. Invited speaker presentations are allocated 30 minutes, while selected oral presentations have a duration of 15 minutes. Kindly upload your presentations on the designated computer at least one session prior to your talk. The sponsor exhibition space and coffee breaks are situated in Foyer 2, just outside the lecture hall.

Two Poster Sessions are scheduled. The primary session takes place on day 1 between 15:40 and 17:30, and the second session occurs on day 2 between 11:00 and 12:00, both held in Foyer 1 on the 1st floor.

Registration includes lunch and the welcome dinner, both served in the hotel's restaurant located on the 2nd floor. Non-alcoholic beverages will be provided by the organizers at the venue. However, the option to purchase alcoholic beverages during the first poster session and the welcome dinner is available if desired.





Human-associated Archaea – Players in the healthy microbiome?

Christine Moissl-Eichinger

Medical University Graz, Graz, Austria

Our microbial world on planet Earth is composed of Bacteria, Archaea, viruses and small Eukaryotes. However, it is particularly the archaea that drive Earth's nitrogen and carbon cycles, and affect the speed of global warming by their methane production. Despite their importance and the fact that they have been discovered already more than 40 years ago, they remain understudied in many ecosystems – including the human.

Methane-producing archaea (methanogens) often co-exist with bacteria in complex microbiomes. There, Archaea are key microbes with respect to the efficiency of the bacterial fermentation, making the break-down process of fibres highly efficient by exploiting bacterial end products like hydrogen and carbon dioxide to form methane. Approximately twenty percent of the Western population innately exhale substantially higher amounts of this gas, and the underlying principle for differential methane emission and is effect on human health was not sufficiently understood.

In this talk, I will summarize our current knowledge on the (human) archaeal community, and discuss the role of archaea as a critical component of the gut microbiome, with physiological impact on the host.

Towards prediction of microbial phenotypes from genetic potential in large data sets

Ulisses Nunes da Rocha

Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

Since the advent of high-throughput sequencing, microbiology has seen a boom in data generated. A significant promise in the field was that more data would help us uncover mechanisms and eventually predict and control the function of microorganisms in pure culture or complex communities. Over 20 years later, we are still far from fulfilling that promise. In my talk, I will address the state-of-the-art of Prokaryotic genome recovery from metagenomes and indicate logical steps to interconnect (r- and K-)survival strategies to genome-scale metabolic reconstructions. In this discussion, I will use the CLUE-TERRA data, where a large consortium explores the boundaries of meta-analyses. I will also show a path for this type of analysis to be included in studies focused on different ecosystem functions.

Nitrification in Agricultural Systems

Petra Pjevac

Joint Microbiome Facility, Department of Microbiology and Ecosystem Science; Division of Microbial Ecology, University of Vienna, Vienna, Austria

Nitrogen fertilization is essential to support food production for the global population. But up to 70% of fertilizer nitrogen applied to agricultural soils is lost to the environemnt. A major driver of fertilizer nitrogen loss is nitrification, the transformation of urea and ammonium nitrogen to the easily leachable nitrate. To counteract this, synthetic and biological nitrification inhibition - SNIs and BNIs - are being investigated and applied. However, offten little is known about SNI and in particular BNI interactions with soil properties, about their specificity, their effective concentrations and their effect on other soil processes. We investigate the target and off-target effect of nitrification inhibitors in a range of agricultural soils, and in particular focus on the effect of soil pH on nitrification inhibitor activity. Overall, our results show that some BNIs could be a natural, effective alternative to the current wider used SNIs.

A Shotgun Metagenomic Approach for the Analysis of the Honey Bee (Apis mellifera) Gut Microbiome

Márton Papp[1], Adrienn Gréta Tóth[1], László Békési[1], Róbert Farkas[2], László Makrai[3], Maura Fiona Judge[1], Gergely Maróti[4], Dóra Tőzsér[1] and Norbert Solymosi[1]

1Centre for Bioinformatics, University of Veterinary Medicine Budapest, 1078 Budapest, Hungary, 2Department of Parasitology and Zoology, University of Veterinary Medicine Budapest, 1078 Budapest, Hungary, 3Department of Microbiology and Infectious Diseases, University of Veterinary Medicine Budapest, 1143 Budapest, Hungary, 4Plant Biology Institute of the Biological Research Center, 6726 Szeged, Hungary

Honey bee (Apis mellifera) is one of the economically most important arthropod species worldwide. Their significance is not only determined by the numerous goods they produce, but also by their importance as a pollinator of plant species of agricultural value. The microbiome of animals is an invaluable asset for the maintenance of their health and well being. Understanding the consequences of harmful agents and stressors on its composition could guide us in reverting these effects. However, to uncover such associations, we must understand its normal composition and variability.

We have collected samples from 20 apiaries in Hungary with a spatially balanced sample selection procedure. Two time points were appointed, one in March and one in May to account for the beginning of the foraging season and its course at high intensity, respectively. Furthermore, precipitation and temperature were also considered in sample selection to analyze the effect of these climatic factors on bacteriome composition. 20 worker bees were selected from 3 colonies at each sampling points and then a pool of 10 workers from each apiaries were prepared for next-generation sequencing. The resulting paired-end shotgun metagenomic reads were subjected to bioinformatic analysis.

Honey bees posses a relatively simple gut bacteriome, which was confirmed by our analysis as well. Furthermore, several species have shown shifts in their abundance from the beginning of the producing season. For example, based on the classification of Kraken2 and the NCBI nt database, two yet unclassified Gilliamella strains were found to increase in their abundance from March to May. Similar trend was observed in the case of Snodgrasella alvi, Lactobacillus helsingborgensis and Bifidobacterium asteroides. On the other hand, Lactobacillus apis, Bombilactobacillus bombi and Bombilactobacillus mellis have decreased in their abundance. Significant climatic association was found in the case of the March samples only. Bifidobacterium asteroides was less abundant in warmer areas in these samples, while Apilactobacillus kunkeei was less abundant in areas with higher precipitation. Several factors could account for the differences we observed. One of the most plausible is the difference and shift of the flowering plants bees forage on. Indeed we could observe major differences of Fabaceae and Salicaceae plant genome abundances in our samples. Furthermore, shotgun metagenomics made it possible to analyze the occurrence of non bacterial members of the microbiome as well. We assembled four Apis mellifera Filamentous Virus genomes from our bee gut samples.

In conclusion, our shotgun metagenomic analysis have provided valuable insight to the compisiton of the honey bee microbiome and its seasonal variation.

Tales of Adriatic loggerhead sea turtles and their microbial companions

Suncica Bosak, Klara Filek, Lucija Kanjer, Borna Branimir Vukovic, Marta Zizek, Romana Gracan

University of Zagreb, Faculty of Science, Department of Biology, Horvatovac 102a, 10 000 Zagreb, Croatia

Sea turtles are known for a long time as a habitat for numerous and diverse microbes that happily live on their shells and within their gut. These microbes can be specialized and found almost exclusively associated with sea turtles but can also be opportunistic and found elsewhere in the turtle environment. The main focus of our studies was to characterize the microbial communities found in both the external (skin and shell) and the internal (cloaca, oral cavities) habitats provided by loggerheads. We chose this species of sea turtle (Caretta caretta) as it is the most common turtle species in the Mediterranean Sea and there are ongoing strong conservation efforts focused on this flagship species. We collected samples from >120 animals from several locations mostly in the Adriatic Sea (Pula, Vis, and Lošinj in Croatia, Bari in Italy, and Amvrakikos Bay and Rethymnos in Greece) and analyzed them using amplicon profiling with 16S, 18S, rbcL and ITS as molecular markers. We aimed to describe the prokaryotic and microeukaryotic assemblages especially focusing on diatoms, cyanobacteria and fungi. We have also isolated and characterized ca. 10 cyanobacterial and ca. 200 non-axenic diatom strains, of which several were then used to investigate their associated bacterial community via cultivation and metagenomics approach. Our multidisciplinary investigations provide the first inventory of loggerhead endo- and epimicrobiota, revealing high diversity, different levels of host fidelity, and local biogeography of sea turtle-associated microbes.

Unraveling the microbiome of inflammatory bowel disease with machine learning across space & time

Marcus Claesson

School of Microbiology, University College Cork, Cork, Ireland; APC Microbiome Ireland, University College Cork, Cork, Ireland; SeqBiome Ltd., Moorepark Food Research Centre, Teagasc, Fermoy, Ireland

Inflammatory bowel disease (IBD) is comprised of two chronic inflammatory conditions, ulcerative colitis and Crohn's disease, which affects over 7 million people globally. While the condition can be controlled with drugs, the unpredictability of relapses is a major challenge for patients and is one of the reasons why the microbiome is so important in providing markers to predict relapse. This talk will present work from two published studies (Ryan et al. Nat Comms 2020; Clooney et al. Gut 2021) on the spatial and temporal microbiome variation of IBD along with some related unpublished work. Results from the recently completed COST Action ML4Microbiome will also be presented, aiming to optimise, standardise and disseminate best use of machine learning in human microbiome research. There will finally be an introduction to the spin-out company SeqBiome, which specialises on high-end microbiome sequencing and analysis for academia and industry.

Microbiome information layers: from constructed variables to single mutations

Boštjan Murovec[1], Leon Deutsch[2], Damjan Osredkar[3], Sara Rapuc[3], Maša Ošlak[4], Blaž Stres[4,5,6,7]

1Laboratory for Machine Intelligence, University of Ljubljana Faculty of Electrical Engineering, Tržaška cesta 25, SI-1000 Ljubljana, Slovenia, 2The NU, The NU B.V., J.H. Oortweg 21, 2333CH, Leiden, The Netherlands, 3Department of Pediatric Neurology, University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1525 Ljubljana, Slovenia, 4University of Ljubljana, Biotechnical faculty, Department of Animal Science, Group for Animal Breeding, Jamnikarjeva ulica 101, SI-1000 Ljubljana, Slovenia, 5National Institute of Chemistry, D13 Department of Catalysis and Chemical Reaction Engineering, Hajdrihova 19, SI-1000 Ljubljana, Slovenia, 6Faculty of Civil and Geodetic Engineering, Institute of Sanitary Engineering, Jamova cesta 2, SI-1000 Ljubljana, Slovenia, 7Jožef Stefan Institute, Department of Automation, Biocybernetics and Robotics, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

The term "Microbiome" contains various layers of information for which there is inherently vague understanding of their information content. Conversely, microbiome information is inherently linked to the vast amount of chemical and physical signatures that change over time and in space in response to variations in thermodynamic conditions of the environment, making them relevant in health and disease. In order to tackle this, machine learning is being increasingly utilized in analyses of data matrices, such as taxonomy, diversity, functional genes, enzymatic reactions, metabolic pathways, metabolomics, environmental chemistry (organic, inorganic), thermodynamic conditions, patient metadata, and other. In this study, we argue against utilizing historically constructed variables and demonstrate the usefulness of the concomitant use of information content present in all data layers all at once.

We reanalyzed 15,000 human gut microbiome samples encompassing 18 non-communicable disease classes plus the healthy utilizing the inhouse metaBakery pipeline.

Our analyses show that by increasing the number of individual samples (>15,000) the number of variables is increasing beyond 22 million variables in the final dataset. This number is far from final, as in parallel with human genome analyses, single point mutations in microbiome data and increasing resolution of organic and inorganic chemical space next to mapping physical forces in complex samples are increasing and are going to increase in the future even faster. Further, we envisage additional increase in the number of variables in analyses of microbiome in the future. This will enable us to further better explain disease mechanisms, uncover the timing of the onset of the diseases, next to reduce the unexplained variation in the data. Hands on experience also shows the drastic lag in the data processing capacities of modern HPC systems. Devoid of additional theoretical developments in heuristic computational approaches, HPC systems are also theoretically incapable of following the data surge in the microbiome and hence incapable to support the developments in the precision medicine.

We point out that the ensemble of constructed variables (taxonomy, diversity, enzymatic reactions, metabolic pathways) is highly informative once coupled to functional gene data and patient metadata, however currently the main need is to develop methods for data reduction and prioritization based on low variance, entropy or other tools for detection and removal of variables with noisy behaviour within class of samples, in order to enable these large datasets to be analyzed within machine learning algorithm and their hyperparameter space, for building biomarker ensmebles, mechanistic insight on the disease development, and building efficient classification tools for unknwon samples.

Harnessing beneficial functions of the microbiota to improve plant health

Tomislav Cernava

School of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, SO17 1BJ Southampton, United Kingdom

Food production is increasingly affected by various bacterial and fungal pathogens, posing a serious threat to the world's growing population. Current measures to control devastating plant diseases are primarily based on agrochemicals which often negatively affect non-target organisms. Research in recent decades has shown that various beneficial functions of the plant microbiota can be harnessed for sustainable improvement of crop production. Guided by microbiome studies, distinct bacteria were found within the microbiota of crop plants that confer disease resistance to their hosts. Although it is well known that the interplay between microbes and plants can result in improved plant health, the phenomenon of holistically disease-preventing bacteria is new. The general concept may even be extendable to additional groups of organisms beyond plants. Therefore, the introduction of the specific term 'soterobiont' is suggested in order to facilitate an unambiguous definition of disease-preventing microorganisms within the microbiota of higher organisms. Targeted studies will be required to discover further soterobionts and to harness their potential for biotechnological applications in agriculture. Such applications may include advanced microbe-carrier substance formulations that enrich agricultural soils with beneficial microorganisms.

Finding balance in the plant microbiome

Omri Finkel

The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

Soil microbiome and soil health as basis of crop quality and gut health

S.E.Hannula

Institute of Environmental Sciences, Leiden University

Soils are unexplored hotspots of (hidden) biodiversity. The soil biota perform many important ecosystem functions such as nutrient and carbon cycling, and interact with plants that bridge the belowground with aboveground.

Long-term intensive agricultural land management often comes at an ecological cost: it leads to reduced microbial diversity which negatively affects many soil functions. Reduced soil microbial biodiversity has important consequences for plant-associated microbiomes, which can thereby impact plant nutritional quality. On the other side, it is known that plant microbiome and quality affect gut microbiome of organisms feeding on the plant as well as their fitness.

In this talk I will first present the concepts of soil health, crop quality and (animal) gut health and then discuss the recent findings on the topic of linking soil health with gut health directly through transfer of microbes via plants but also indirectly through changes in plant quality. I will end by calling for interdisciplinary collaborations to find answers to the burning questions related to this field.

Innovations to support a healthy microbiome for one health

Gabriele Berg

Institute of Environmental Biotechnology, Graz University of Technology, Austria; Leibniz-Institute for Agricultural Engineering and University of Potsdam, Germany

Plant microbiomes are key components for ecosystem health in all terrestrial ecosystems. Human activities in the Anthropocene are linked to a significant shift of diversity and the symbiont/pathogen ratio of the plant microbiota (Berg & Cernava, Microbiome 10, 2022). Plant microbiomes can be managed either directly by applying (i) microbiome transplants, (ii) microbes with beneficial properties, or (iii) microbiota-active metabolites, or indirectly by changing environmental conditions in a way that microbiomes also shift their structure and function from dysbiosis into a healthy state. Examples for the different strategies for plant protection will be presented, and risk associated with the technology will be discussed. Beyond, the plant microbiome is connected across systems and, in frame of the one health concept, crucial for human and planetary health issues as well. This will be discussed in frame of the planetary boundary concept (Peixoto et al. Nature Microbiology, 2022).

Could microbial assemblages be a valuable (novel) indicators of marine ecosystem health?

Ines Petrić[1], Ana Ramljak[1], Ivana Babić[1], Jurica Žučko[2], Mavro Lučić[1], Martina Furdek[1], Maja Fafanđel[1], Slavica Matijević[3], Dimitrios Karpouzas[4], Sotirios Vasileiadis[4], Zlatan Morić[5], Nikolina Udiković-Kolić[1]

1 Institute Ruđer Bošković, Zagreb, Croatia; 2 University of Zagreb, Faculty of Food Technology and Biotechnology, Zagreb, Croatia; 3 Institute of Oceanography and Fisheries, Split, Croatia; 4 University of Thessaly, Larissa, Greece; 5 Algebra University College, Zagreb, Croatia

It is estimated that more than half of the population in the Mediterranean region lives in coastal areas. Consequently, these areas are under the strong influence of numerous human activities such as urbanization, tourism, wastewater discharges, agricultural and industrial pollution, and climate change. The increasing anthropogenic environmental degradation is already affecting the marine biota and the dynamics of the marine food web, although the consequences for this ecosystem as a whole are not yet known. The above challenges are an alarm signal for the urgent protection of the environment addressed by the Marine Strategy Framework Directive 2008/56/ EC (MSFD). The Directive aims to achieve and maintain good environmental status (GES) of marine areas through the monitoring of 11 different descriptors. Although microorganisms play a critical role in the overall health of marine ecosystems, they have not been considered as indicators of environmental status under the MSFD or other directives. Within the frame of the project MicroLink, funded by the Croatian Science Foundation, we aimed to determine potential of microorganism as indicators of marine ecosystem health. MicroLink integrates (i) a multi-domain approach where changes in microbial assemblages were tracked at different levels (bacteria, archaea, protists, fungi) and (ii) a multi-layer approach where studies were conducted at different levels of resolution (changes in structure, networks, functions and gene levels). During our sampling campaigns in the 7 selected marine areas under strong anthropogenic pressure (Pula Rijeka and Split harbors, Raša, Bakar and Šibenik bays and within the Vranjic basin), we collected 67 surface sediments using box corer or Van Veen grab.

Within the sediment samples, reflecting long-term exposure to anthropogenic pollution, we analyzed various physicochemical parameters: grain size analysis, concentrations of tributyltin compounds and metals, total C and -N, phosphorus content, and toxicity using Microtox© bioassay. Clustering analysis revealed that our sediment could be divided into 5 distinct groups representing classes with varying degrees of anthropogenic disturbance. Amplicon sequencing showed that, depending on the disturbance level, changes at both the bacterial, fungal, and protists levels are observed, with multiple populations emerging as potential indicators. The shotgun metagenomics approach revealed clear effects of anthropogenic pressure on the microbial gene pool. Finally, our intention is to provide answers to 2 main scientific questions: (1) can we find a general relationship between microbial community dynamics and anthropogenic pollution? and (2) at what level (different trophic and/or study levels) within microbial assemblages are changes best detected?

Using stable isotope probing to track the utilization of carbon in temperate forest soils

Tijana, Martinović, Tereza Mašínová, Rubén López-Mondéjar, Jan Jansa, Martina Štursová, Robert Starke, Petr Baldrian

Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Microorganisms mediate fundamental ecological processes in soil ecosystems, including their vital role in carbon cycling. Recent studies have revealed that bacteria possess the ability to break down complex plant polymers in soil, highlighting their significance in the carbon cycle, alongside fungi. However, there is limited research on the specific contributions of fungi and bacteria to these processes.

This study leveraged stable isotope probing (SIP) to track the utilization of carbon from compounds of different complexity in a temperate forest soil and carbon accumulation by individual microbial taxa. Using microcosms that contained a range of 13C-labeled substrates — including simple sugars, acids, and more complex plant polymers — we followed the respiration and biomass production (phospholipid fatty acids). Furthermore, through 13C -DNA isopycnic separation and Illumina MiSeq sequencing targeting bacterial V4 rRNA and fungal ITS2 regions, we identified 13C -enriched taxa (i.e. taxa accumulating 13C).

Results showed that microcosms supplied with labeled citric acid and glucose exhibited the highest respiration rates after one week of incubation, while those supplied with chitin showed the lowest rates. However, after three weeks, a significant increase in chitin respiration rate was observed, along with an increase in total microbial biomass and 13C-labeled microbial biomass. Notably, microcosms containing 13C-labeled chitin displayed a distinct microbial community, indicating the involvement of both fungi and bacteria in chitin decomposition.

The findings revealed the presence of specialized and generalist taxa in all microcosms, highlighting the capacity of numerous microbial taxa to utilize different carbon sources. Regardless, more specialization was observed in fungi compared to bacteria. Furthermore, low-molecular-mass compounds were more readily respired, whereas carbon from complex biopolymers was predominantly incorporated into the microbial biomass.

Our insights emphasize the collaborative and distinct roles of fungi and bacteria in soil carbon dynamics. Specifically, their intertwined relationship in chitin utilization highlights the intricate network of microbial interactions in soil ecosystems. As we move forward, a deeper exploration into these microbial consortia can pave the way for better soil management and understanding of carbon cycling, particularly in the context of changing environmental conditions.

Data mining-based discovery of (novel) viral sequences in various host organisms and environmental samples

Katarina Bačnik[1], Lana Vogrinec[1, 2], Timotej Turk Dermastia[1], Neža Pajek Arambašič[3], Tomaž Curk[3], Martina Bačič[4], Nataša Mehle[1,5], Denis Kutnjak[1]

1 National Institute of Biology, Večna pot 111, 1000, Ljubljana, Slovenia; 2 International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000, Ljubljana, Slovenia; 3University of Ljubljana, Faculty of Computer and Information Science, Slovenia; 4Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000, Ljubljana, Slovenia; 5School for Viticulture and Enology, University of Nova Gorica, Slovenia

The search for new viral sequences provides an opportunity to improve early detection of pathogens, to predict viral hosts and environmental reservoirs and to anticipate the influence they have on host population dynamics. The rise of high-throughput sequencing has led to an immense increase in the amount of publicly available sequencing data, which often remains unexplored. Here we present different examples of how data mining can be used to either detect a new species from a known group of pathogenic viruses in various samples, increase the knowledge on diversity of known viruses or identify novel viruses associated with a certain group of host organisms.

Emergence of tobamoviruses with significant impacts on agriculture has been observed recently. To prepare for possible future outbreaks of such viruses as pathogens of crops, we searched for known and novel tobamoviral sequences in a set of RNA shotgun sequencing datasets originating from various sources. Results show that novel tobamo-like virus sequences can be found in diverse sets of environments. We presented associations with source environments (e.g., irrigation water) and viral hosts (e.g., sugarcane) and performed phylogenetic analyses.

On the other hand, we mined publicly available macrophyte transcriptomes for viral sequences, utilizing data from the 1000 Plant Transcriptomes Initiative (1KP).

Macrophytes are taxonomically diverse aquatic plants, inhabiting numerous water-related ecosystems and as such represent an abundant group of potential viral hosts, however, very little is known about their association with viruses. We have found that many of the analyzed macrophyte transcriptome data sets contain known and novel plant viral sequences, including some of the recognized crop pathogens.

We have also explored the virosphere of microphytes i.e. aquatic unicellular microalgae, more specifically that of diatoms. To better understand the diversity and geographic distributions of diatom viruses we have combined the sequencing of diatom and virus cultures for detection of novel viruses with data mining of datasets obtained from other locations. We extended the data deficient field of diatom virus research by characterizing novel viruses that infect an ecologically and economically important diatom genus Pseudo-nitzschia and one of the most researched diatom-virus systems of Chaetoceros tenuissimus.

Data mining-based discovery of viruses offers a promising avenue toward a comprehensive description of the virosphere, shows the capacity to connect insilico and wet-lab research of known groups of viruses or host associated viromes and offers a way to address the uncontrolled presence of pathogenic viruses in the environment.

Could microbial assemblages be a valuable (novel) indicators of marine ecosystem health?

Ines Petrić[1], Ana Ramljak[1], Ivana Babić[1], Jurica Žučko[2], Mavro Lučić[1], Martina Furdek[1], Maja Fafanđel[1], Slavica Matijević[3], Dimitrios Karpouzas[4], Sotirios Vasileiadis[4], Zlatan Morić[5], Nikolina Udiković-Kolić[1]

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It is estimated that more than half of the population in the Mediterranean region lives in coastal areas. Consequently, these areas are under the strong influence of numerous human activities such as urbanization, tourism, wastewater discharges, agricultural and industrial pollution, and climate change. The increasing anthropogenic environmental degradation is already affecting the marine biota and the dynamics of the marine food web, although the consequences for this ecosystem as a whole are not yet known. The above challenges are an alarm signal for the urgent protection of the environment addressed by the Marine Strategy Framework Directive 2008/56/ EC (MSFD). The Directive aims to achieve and maintain good environmental status (GES) of marine areas through the monitoring of 11 different descriptors. Although microorganisms play a critical role in the overall health of marine ecosystems, they have not been considered as indicators of environmental status under the MSFD or other directives. Within the frame of the project MicroLink, funded by the Croatian Science Foundation, we aimed to determine potential of microorganism as indicators of marine ecosystem health. MicroLink integrates (i) a multi-domain approach where changes in microbial assemblages were tracked at different levels (bacteria, archaea, protists, fungi) and (ii) a multi-layer approach where studies were conducted at different levels of resolution (changes in structure, networks, functions and gene levels). During our sampling campaigns in the 7 selected marine areas under strong anthropogenic pressure (Pula Rijeka and Split harbors, Raša, Bakar and Šibenik bays and within the Vranjic basin), we collected 67 surface sediments using box corer or Van Veen grab.

Within the sediment samples, reflecting long-term exposure to anthropogenic pollution, we analyzed various physicochemical parameters: grain size analysis, concentrations of tributyltin compounds and metals, total C and -N, phosphorus content, and toxicity using Microtox© bioassay. Clustering analysis revealed that our sediment could be divided into 5 distinct groups representing classes with varying degrees of anthropogenic disturbance. Amplicon sequencing showed that, depending on the disturbance level, changes at both the bacterial, fungal, and protists levels are observed, with multiple populations emerging as potential indicators. The shotgun metagenomics approach revealed clear effects of anthropogenic pressure on the microbial gene pool. Finally, our intention is to provide answers to 2 main scientific questions: (1) can we find a general relationship between microbial community dynamics and anthropogenic pollution? and (2) at what level (different trophic and/or study levels) within microbial assemblages are changes best detected?

Using stable isotope probing to track the utilization of carbon in temperate forest soils

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Microorganisms mediate fundamental ecological processes in soil ecosystems, including their vital role in carbon cycling. Recent studies have revealed that bacteria possess the ability to break down complex plant polymers in soil, highlighting their significance in the carbon cycle, alongside fungi. However, there is limited research on the specific contributions of fungi and bacteria to these processes.

This study leveraged stable isotope probing (SIP) to track the utilization of carbon from compounds of different complexity in a temperate forest soil and carbon accumulation by individual microbial taxa. Using microcosms that contained a range of 13C-labeled substrates — including simple sugars, acids, and more complex plant polymers — we followed the respiration and biomass production (phospholipid fatty acids). Furthermore, through 13C -DNA isopycnic separation and Illumina MiSeq sequencing targeting bacterial V4 rRNA and fungal ITS2 regions, we identified 13C -enriched taxa (i.e. taxa accumulating 13C).

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1 National Institute of Biology, Večna pot 111, 1000, Ljubljana, Slovenia; 2 International Postgraduate School Jožef Stefan, Jamova cesta 39, 1000, Ljubljana, Slovenia; 3University of Ljubljana, Faculty of Computer and Information Science, Slovenia; 4Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000, Ljubljana, Slovenia; 5School for Viticulture and Enology, University of Nova Gorica, Slovenia

The search for new viral sequences provides an opportunity to improve early detection of pathogens, to predict viral hosts and environmental reservoirs and to anticipate the influence they have on host population dynamics. The rise of high-throughput sequencing has led to an immense increase in the amount of publicly available sequencing data, which often remains unexplored. Here we present different examples of how data mining can be used to either detect a new species from a known group of pathogenic viruses in various samples, increase the knowledge on diversity of known viruses or identify novel viruses associated with a certain group of host organisms.

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Data mining-based discovery of viruses offers a promising avenue toward a comprehensive description of the virosphere, shows the capacity to connect insilico and wet-lab research of known groups of viruses or host associated viromes and offers a way to address the uncontrolled presence of pathogenic viruses in the environment.

Understanding the wireworm microbiome for biocontrol strategies

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Wireworms are larvae of click beetle and an important agricultural insect pest in several root crops. Biocontrol of these insect larvae can be achieved by using entomopathogenic fungi, but their field efficacy and consistency of field effects of this approach do not meet farmer demands yet. We hypothesize these inconsistencies in biocontrol efficacy to be affected by wireworm-associated microbes derived from the given field soil. This study aimed to investigate temporal and spatial dynamics on and in wireworms to identify potential targets to increase the biocontrol efficacy of entomopathogenic fungi. We investigated bacterial and fungal wireworm microbiomes in four different species using a 16SrRNA and ITS amplicon sequencing approach. This included analyses of temporal dynamics and spatial differences in wireworm microbiomes in comparison to the surrounding soil microbiome. We further tested the dependence of entomopathogen virulence on preceding infestation with low-virulent entomopathogens, using mealworms (Tenebrio molitor, L.) as a model system. Similar microbial taxa colonized the surface (cuticle) and the interior (body) of wireworms, but the microbial communities significantly differed regarding alpha and beta diversity indices. The origin soil clearly affected wireworm microbiomes, but species-dependent bacterial communities were also observed in specimens from the same field soil. Fungal microbiomes were comparably stable across time, with low alpha diversity, and were dominated by Trichosporon sp.. We further frequently observed low abundances of entomopathogenic fungi in wireworms. We provide a first baseline survey on wireworm microbiomes. Low fungal diversity in and on wireworms indicates a strong antifungal selection pressure for soil fungi. Latent infections with entomopathogens could have immune priming effects in wireworms, with implications for biocontrol approaches in agriculture.

The effect of two different production systems on pig fecal microbiota composition

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Different production systems (conventionally and pasture-raised pigs) and co-rearing of pigs with other livestock have both been shown to influence pig fecal microbiota composition. In this study, we assessed 16S fecal microbiota composition of pigs kept at three different locations: (i) pigs reared on pasture with small ruminants and cattle (group L1), (ii) pigs reared indoors and without other farm animals and fed only organic commercial feed (group L2), and (iii) pigs reared on pasture with cattle (group L3). Fecal samples were rectally collected from 18 pigs in all four seasons of 2022. Pigs from groups L2 and L3 were raised on the same organic farm and maintained by the same livestock workers, whereas pigs from L3 were raised on another organic farm and were maintained by other livestock workers. All pigs were fed the same type of organic commercial feed. Total DNA was extracted from 72 fecal samples using QIAamp Fast DNA Stool Mini Kit (Qiagen). Sequencing of 16S rRNA gene (V3–V4 region) to a depth of 50,000 tags per sample was performed with Illumina paired-end (2×250 bp) technology. Operational taxonomic unit (OTU)-based microbiome analysis was performed using QIIME 1.9.1. Alpha diversity was comparable between study groups; the average number of observed OTUs ranged from 1,500 to 1,800 in all seasons and in all study groups. The two predominant bacterial phyla in all three groups were Firmicutes and Bacteroidetes. Significant seasonal changes in microbiome composition were observed between all seasons (nonparametric MANOVA, $p < 0.008$). Microbiome composition differed significantly between the study groups L1 and L2–L3, as demonstrated by principal coordinate analysis of weighted UniFrac distances and nonparametric MANOVA ($p = 0.001$), whereas groups L2 and L3 did not differ significantly ($p = 0.2$). Linear discriminant analysis Effect Size (LefSe) identified several bacterial taxa that were most differentially abundant between different study groups and different seasons.

Pig microbiota composition is not stable over time and is influenced by external (rearing-related) factors. In the present study, the production system or co-rearing with other animal species did not significantly affect pig microbiome composition. Rather, the main observed differences in microbiota composition could be explained by the farm and livestock workers.

Longitudinal changes in human gut microbiota vs. sporobiota

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The human gut harbors a complex and dynamic community of microbes. Among bacteria, approximately half can form endospores. This fraction of microbiota, termed sporobiota, can withstand disruptions such as antibiotic treatments, inflammation, and dis-eases. This facilitates its transfer between different hosts/environments and endows spore-formers with a broader distribution in the human population compared to their non-spore-forming counterparts. Our study aims to delve deeper into the human gut sporobiota in healthy individuals. Longitudinal studies of entire microbiota are com-mon, but here we are trying to understand how a fraction of bacterial spores in the hu-man gut changes with time. The study involved collecting stool samples from male volunteers every two weeks over a span of six months and additionally during predefined events, including antibi-otic intake, stressful events, dentist appointments etc. For isolation of DNA of sporobio-ta the stool samples were first treated with ethanol shock and ethidium monoazide (ef-fectively eliminating the DNA of vegetative cells), while for DNA isolation of entire mi-crobiota, stool samples were left untreated. Sequencing of the V3-V4 region of the 16S rRNA gene was performed on Illumina's NextSeq platform. The raw reads were pro-cessed in USEARCH to obtain operational taxonomic units (OTUs). Further explorato-ry and statistical analysis was carried out in R. We observed some pre-defined events such as antibiotic intake or migraines which cause a drop in the number of observed OTUs of microbiota but an increase in the sporobiota. Looking at beta diversity there is a distinction between microbiota and spo-robiota which is more pronounced than differences between individuals. Sporobiota samples are more dispersed than microbiota, both within and between individuals in the span of six months, which could indicate a higher turnover of spore-forming bacte-ria. The production of spores in the event of unfavorable conditions enables survival in the human gut despite the adversaries that kill vegetative cells, but also enables transmission.

Mucosodom: kingdom specific mucosal microbiota analysis in inflammatory bowel disease.

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The gastrointestinal (GI) microbial ecosystem is essential to the human host, and harbours bacteria that help in nutrient uptake, immune maturation, and pathogen defence. The mucosa represents an important interface between the gastrointestinal lumen, containing the microbes and food stream, and the gastrointestinal lining composed of host cells. In the case of diseases such as inflammatory bowels disease (IBD) with the two main subtypes ulcerative colitis (UC) and Crohn's disease (CD), the inflammation can spread from few localized patches up to the entire GI tract. The inflammation is associated to a decrease in mucosal thickness and increased bacterial. This is likely leading to a distinct dysbiotic signatures of the mucosal microbiome, which are very heterogeneous and individual specific. Furthermore, differences between the distal and proximal GI in nutrient uptake, luminal bacterial composition and localized inflammation in IBD suggest, that the mucosal ecosystems change as we move from the terminal ileum to the rectum. There are still significant knowledge gaps in our understanding of the mucosal communities. The few available studies that performed comprehensive integrated analyses of mucosa-attached microbiota, lumen microbiota and host intestinal-gene expression, show that indeed there are IBD-specific observations that warrant deeper investigation. This is due to studies being predominantly restricted to fecal sampling, and most studies that investigate the mucosa, are limited to amplicon (16S) sequencing since the high ratio of human to microbial DNA is making direct shotgun metagenomic sequencing unfeasible. To address this need, we developed an approach, to investigate colonization patterns of bacteria in the human mucosa and host–microbe interactions, while disentangling disease-, location- and inflammation specific associations.

To study those interactions, we performed host-transcriptomic (RNA-seq) and microbial (shotgun metagenomic-seq) profiling of >200 intestinal biopsies (from 7 colon sites), and stool samples, derived from 51 patients with IBD and controls.

Overall, we identified multiple bacterial species that are typically altered in prevalence in the IBD microbiome. With this data, we identified colonization patterns and colon site specific bacterial persistence, that differ between clinical pathogeneses. Furthermore, using host transcriptomics allowed us to build a network of bacterial taxa typically associated to particular gene regulation patterns.

At the current stage we aim to characterize the individual-specific IBD dysbiosis based on both dysregulated host transcriptomics and bacterial overgrowth. All this information may guide microbiota-directed personalised precision medicine in the future, since as we observed the mucosal microbiome signatures are individual-, and disease-specific.

The “Microbiome & Health” MOOC and a video game – innovative ways to disseminate microbiome research

Matthias Schweitzer[1], Birgit Wassermann[1], Florian Marcher[2], Maximilian Wlasak[2], Ahmed Abdelfattah[3], Tomislav Cernava[1,4], Johanna Pirker[2], Gabriele Berg[1,3,5]

1Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria; 2Institute of Interactive Systems and Data Science, Graz University of Technology, Graz, Austria; 3Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany; 4College of Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, United Kingdom; 5Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Microbiome research can be key for holistic and sustainable strategies to optimize the health of people, animals, plants and ecosystems in accordance with the One Health concept. In order to raise awareness, we must communicate the findings of microbiome research also to society. We decided to develop a massive open online course (MOOC) and a video game to innovatively teach about microbiome research. To create the MOOC, we conducted an extensive literature research and translated all the information gathered in a way that both the public and the academic community can benefit from. The “Microbiome & Health” MOOC offers a knowledge platform for many disciplines e.g., medicine, agriculture, food science, biotechnology and bioinformatics. The course is structured in six chapters: i) a definition of the microbiome, ii) an insight into techniques and methods, iii) latest findings in plant microbiome research, vi) facts about the human microbiome, v) knowledge about the resistome and the exposome, and vi) the potential of microbiome research for planetary health and the sustainable development goals. The prepared knowledge is conveyed in short, concise videos, underpinned by 3D visuals and animations. Each chapter can be completed by passing a small quiz to receive the course certificate.

The video game allows learning about the development of the human microbiome, from before birth to death. In scenarios, the player will make various choices that were shown to affect the microbiome and thus learn about potential drivers affecting the human microbiome. The learning success will be evaluated using feedback surveys based on the MEEGA questionnaire, an established model for the evaluation of educational games. With the “Microbiome & Health” MOOC we aim to disseminate findings, and also the importance of microbiome research. Gamification is a growing trend in education and could allow us to reach a different type of audience. The “Microbiome & Health” MOOC remains and the video game will be freely accessible and can be shared with everyone, therefore ideally suited to reach a broad audience.

Healthy Cannabis seeds harness a geno- and chemotype specific microbial signature

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The present study provides detailed insights into bacterial communities that naturally occur inside seeds of a broad range of Cannabis genotypes with distinct cannabinoid compositions and domestication status, in order to cover the high phenotypic variability within this plant genus. This was achieved by (i) characterising seed endophyte diversity, structure, and distribution across 46 Cannabis genotypes with healthy phenotypes, (ii) identifying shared and flexible endophytic taxa, and (iii) determining feature-specific biomarkers at the ASV level. All investigated seeds show low to intermediate bacterial richness, and harboured between 10 and 177 bacterial amplicon sequencing variants (ASVs). Gammaproteobacteria, Bacilli, Actinobacteria, and Alphaproteobacteria were identified as the dominant bacterial classes. The fraction of shared endophytes among all genotypes comprises only nine ASVs of mainly Gammaproteobacteria and account for a high proportion (67.26%) of the total amplicon reads. In addition, a flexible fraction of low abundant ASVs was identified that comprehends many known plant beneficial bacteria. The declining bacterial diversity in Cannabis selections > landraces > cross hybrids > inbred lines in low cannabinoid genotypes reflects the impact of domestication. Altogether, the Cannabis genotype was the main factor explaining variations in bacterial composition. In addition, the chemotype was identified as a driving factor, which can be an indicator of co-divergence in the seed microbiome during domestication.

Potential bacterial markers as indicators of domestication state and cannabinoid content were determined, e.g. Pantoea agglomerans, Rhodococcus erythopolis and Brevibacillus sp. indicating the differences between low and high cannabinoid genotypes. Understanding how different genotypes can impact microbial assemblages within Cannabis seeds can provide a basis for developing new strategies that improve plant growth and performance. This has yet to be explored in breeding strategies and could spur the reduction of chemical inputs without changing the genetic background of plants, by reintroducing missing plant symbionts.

Unveiling the Hidden Partners of Perennial Wheatgrass

Kristina Michl[1], Gabriele Berg[1,2,3], Tomislav Cernava[1,4]

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Grain production holds significant importance within agriculture, as the fundamental basis for global food security lies in edible plant seeds, contributing to nearly half of the world's food calories. However, modern agricultural practices have often proven unsustainable, posing harm to the environment and contributing to the decline of biodiversity. To address these issues, the adoption of perennial grain cultivation offers a viable alternative to conventional wheat farming. Perennial plants possess the advantage of enhanced water and nutrient utilization due to their more extensive and densely-rooted systems. This resilience becomes crucial for enduring the increasing frequency and severity of weather extremes caused by climate change. While strategies for cultivating perennial wheatgrass have been evolving since the 1980s, further research is necessary to enhance their productivity. The influence of ongoing intensive breeding approaches on the seed microbiome remains largely unexplored. The importance of seeds as a source of beneficial microorganisms has recently been highlighted and some of these bacterial endophytes remain associated with plants throughout their life cycles and even during postharvest storage. These attributes make them intriguing subjects for deeper investigation. Our study is the first to analyze the microbial composition within the seeds of perennial wheatgrass (Thinopyrum intermedium, L.) collected from three distinct sampling sites over three consecutive years. High-throughput sequencing of the 16S rRNA gene fragment was applied to unravel the taxonomic structure of bacterial communities. Our findings indicate that the predominant genera in the seed microbiome of perennial wheatgrass are Pantoea, Kosakonia, and Bacillus, with variations in bacterial community composition primarily driven by the sampling site, while the sampling year demonstrated a minor influence.

Additionally, we analyzed seeds from four breeding cycles and revealed a decline in microbial diversity across successive breeding cycles, accompanied by diminished interactions among the present bacterial species. Overall, this study sheds light on the composition of the perennial wheatgrass seed microbiome and emphasizes the significance of considering the impact of breeding on microbial diversity. Not only might our findings affect plant health and productivity, but also have an impact on future breeding strategies.

Analyzing native seed microbiomes to understand impacts of dormancy, storage, and early plant development

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Despite evidence for the importance of seed-associated microorganism for plant health, knowledge on how the microbiome is affected by seed dormancy and storage remains fragmented. Undisturbed environments offer valuable conditions to understand the intrinsic factors. In a protected Alpine meadow, we found highly diversified and abundant seed microbiomes consisting of bacteria, archaea, and fungi, with a higher degree of plant specificity than already discovered for crop seeds. Inter-microbial network analysis captured consistent patterns of co-occurrence between bacteria and archaea in contrast to exclusion within the fungal community, which may contribute to maintain soil microbial diversity and plasticity of the whole ecosystem. The factors that trigger microbial plant colonization after breaking of dormancy and early plant development stages were measured by transcriptomics, 16S rRNA amplicon sequencing, and confocal laser scanning microscopy. The impact of two different seed storage technologies, established in the Millenium Seed Bank (Kew, UK) and Graz Botanical Garden (AUT) on the survival of seed microbiota are currently being analyzed, which will help to advance international conservation strategies for seeds to preserve genetic diversity and a plant microbiome that fosters resilience to a changing climate.

Metagenomic insights into antibiotic resistance of the microbiota of probiotic products, starter cultures, and cheeses

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Antimicrobial resistance is a natural phenomenon in the environment, but extensive use of antimicrobials has accelerated its spread and led to a widespread dissemination of antimicrobial resistance genes. Commensal bacteria along the food chain may also serve as a potential reservoir of antimicrobial resistance genes, but the risk of these products in terms of resistance has not been well characterised.

With the goal of predicting antimicrobial resistance genes (ARGs) and comparing their abundance in different food samples, we performed shotgun metagenome sequencing of samples from probiotic supplements, starter cultures, and cheeses.

We isolated metagenomic DNA from 75 samples representing five product groups (probiotic supplements (Pr), starter cultures (St), cheeses from raw milk without starter cultures (R), and cheeses made with starter cultures from pasteurised (Tc) or raw milk (Rc)). To sequence DNA (Microsynth AG, Balgach, Switzerland), Illumina TruSeq nano DNA libraries were created and subsequently sequenced on the Illumina NovaSeq platform. After quality control, surviving pair-end reads were assembled de novo using publicly available programmes.

Coding sequences were mined for the presence of known ARGs. Bioinformatic analyses were performed in part by Microsynth AG and in part by the authors of this work. In addition, 10 selected acquired ARGs were verified by qPCR.

In silico analyses of metagenomic sequences led to the identification of a total of 539 ARGs, 129 of which were different. They are involved in resistance to clinically important antimicrobials such as tetracyclines, aminoglycosides, beta-lactams, macrolides, trimethoprim, glycopeptides, fosfomycin, phenicol, and quinolones. The most abundant ARGs were ANT(6)-Ia, APH(3')-III, erm(B), tet (L), tet(M), tet(U), tet O/W, lnu(A), and tet(S), however their abundance differed between product groups. Statistical analysis confirmed the significant difference between groups of isolates. Groups R and Rc were highly contaminated with ARGs compared to the probiotic supplements and especially starter cultures St and the group Tc, which were not rich in ARGs.

Our study suggests that starter cultures, probiotic supplements and cheeses made from pasteurised milk do not represent a significant reservoir of resistance and are therefore safe with respect to antimicrobial resistance, whereas cheeses made from raw milk can be considered reservoirs of resistance.

Changes in the fruit microbiome induced by domestication and air quality and implications for one health

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The plant microbiome is affected by factors driving the Anthropocene but this change is not well understood. Here, we studied the impact of two factors, air pollution and breeding, on the fruit microbiome of apples and cherries (Rosaceae). Air pollution has become an increasingly concerning issue, not only for humans but also for planetary health. Information on the impact of particulate matter (PM) pollution on fruit microbiomes is scarce. To analyse the effect of air pollution, apple pulp, skin and surface as well as dust were sampled at eleven locations, whereby the air quality of three sites was classified as very good (PM2.5<10), five as good (PM2.5 10<x<50), and three as moderate (PM2.5>50). Domestication is another anthropogenic influence whose impact on the Rosaceae microbiome we investigated in cherry trees. We collected cherry fruits, seeds, twigs and bulk soil samples from 20 Prunus avium trees during ripe fruiting time, including wild native trees, trees from private gardens and trees from an orchard to cover the whole spectrum of domestication. The bacterial and fungal communities of all samples were analysed via high-throughput microbial DNA barcoding. In both studies, the sample type was a major factor explaining variations in the microbial communities (P = 0.001).

The air quality had a significant effect (P = 0.001) on the epiphytic fungal community (R2 = 6.7%), and fungal communities of peel (R2 = 8.3%) and pulp (R2 = 6.3%) of apples. We found that 4.3%-49.7% of the bacteria and fungi potentially originated from the ambient air. Apple pulp was the compartment with the highest proportion of dust-derived fungi at 13.8%.

In cherry trees, domestication had a significant impact (P < 0.005) on the microbial community structure, explaining variations of 6.2%-20.4% across sample types. Domestication seems to enrich the bacterial community of cherries, as evidenced in the higher diversity observed in samples from highly domesticated trees compared to samples from wild native trees, which was significant in all sample types (Padjusted < 0.05) except twigs. In contrast, the fungal community in cherry pulp and seeds from highly domesticated trees showed lower diversity compared pulp and seeds from wild cherry trees, which was significant in pulp (Padjusted < 0.05).

Our research shows that anthropogenic influences such as air pollution and domestication are reshaping the microbiome of plants and also the fruits we eat, with potential implications for plant, human and One Health.

The edible plant microbiome and their importance for a healthy human microbiome

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Plant consumption i.e., fruits and vegetables can potentially serve as the main direct source of environmental microbiota to human gut microbiota. Here, we investigated the prevalence of plant associated bacteria in in 2,426 publicly available gut metagenomes. They were present in the human gut, but in low abundance - roughly 2.2%. Age of the host, the frequency of vegetable consumption, and the variety of plants ingested were the main factor influencing the diversity and composition of plant associated bacteria in human gut. Hence, the diversity of the human gut microbiota is likely affected by any variables affecting the fruit and vegetable microbiome. Therefore, we used functional assays, cutting-edge microscopy, and sequencing technologies to analyze fruits that had undergone food processing as well as those that were cultivated in various cultivation settings (commercial vs. home garden). Using apple as a model, we showed that various cooking processes resulted in a 63.3–86.7% decline in bacterial diversity and abundance. Cultivation and origin of the fruits had a strong impact on the diversity and composition of their microbiome. This result suggests that consuming fruits from various regions exposes us to various bacteria. The exposome and human health may be influenced by changes in fruit and vegetable microbiome. Therefore, it is crucial to take into account the effect of food processing and cultivation practices on the indigenous fruit microbiota, a factor that is frequently overlooked.

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P1: Challenges in isolating microbiome from foods

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Interactions between microbes, plants, and animals have created a diverse food microbiome. Most of the foods we consume are not sterile, making diet the largest source of microorganisms that are part of the microbiota in our digestive tract. With the advent of next-generation sequencing methods, sequencing of the microbiome has become available. Therefore, we evaluate the technical challenges of isolating the microbiome from food with the aim of demonstrating its applications in food safety production. First, we analysed the advantages of microbiome sequencing over traditional culture-based methods and also over 16S amplicon sequencing, which has been most widely used recently. The most important advantage is the time factor, because microbiome sequencing is faster than comparable methods. In addition, phylogenetic and taxonomic studies based on WGS results can accurately divide microorganisms into species and even strains. The method also allows us to obtain gene-level information, such as in the discovery of antimicrobial resistance genes. Despite the possibility of obtaining the entire DNA sequence of the microbiome, the technique is not yet used to the extent expected, mainly because of the lack of standardization in obtaining DNA of sufficient quality. Therefore, we have identified several important problems in isolating the microbiome that need to be addressed. First, the quantity and quality of isolated DNA for WGS must be higher than for Sanger sequencing. Second, it is important to shorten the time frame for isolation because the number or variability of microorganisms initially present changes rapidly, and thus different isolation kits and protocols have been tested. The final point is the isolation of the microbiome from food. A particularly important step is the depletion of host DNA. This includes the removal of any plant or animal DNA in the sample, as well as other components such as lipids or proteins that could be problematic for the purity of the isolated DNA. Keeping these points in mind, we have isolated DNA from various foods, both of animal and plant origin, to develop a standardized method that allows routine work and comparability between studies.

P2: Exploring provenance gaps between lab and computer analysis in genomics and metagenomic studies, a part of the NFDIMicrobiota consortium

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There is a growing need to make data from life sciences findable, accessible, interoperable, and reusable (FAIR), which is incredibly challenging in metagenomics. This field deals with the study of the functional potential of microorganisms in natural, host-associated, and constructed environments. It uses high-throughput sequencing data from total DNA isolated from microbial communities. There is often a disconnection between wet labs and computational analysis in this field, leading to provenance issues among scientists.

This study aims to improve FAIR principle usage in metagenomics by creating guidelines to connect the wet and dry lab parts of projects in the context of genomic sequencing. To this end, we explored provenance in over 100 projects involving more than 5,000 samples from multiple sources (e.g., bioreactors, agricultural and forest soils, freshwater, wastewater, and gut microbiome). We used short and long-read sequencing to explore the data provenance of wet lab sample preparation (sample storage and DNA extraction), library preparation, sequencing and data preprocessing before data analyses. We organized the guidelines into five parts: sample preparation, sequencing logistics, data downloading, integrity and quality checking of sequences, and preprocessing the sequenced data until assembly.

First, we analyzed provenance from sample preparation (sample storage and DNA extraction) for metagenomics.

Our data indicated that it is necessary to pay attention to the DNA yield and quality (particularly if interested in long-read sequencing) during sample preparation for sequencing. We observed that both low and high yields in the samples may lead to failed library preparation. DNA quality check must be performed in every sample in BioAnalyzer-like machines. We also highlight that memory configurations and resources required for sequencing projects to facilitate reproducibility, particularly for sequencing data preprocessing, must be considered, as it demands a high usage of resources. Reviewing and commenting must be implemented on automatic reports in existing data processing pipelines to improve the interoperability and reuse of metagenomics data.

In conclusion, small research groups and data stewards responsible for organizing data in local or large sequencing facilities may use our guidelines to bridge the gap between wet and dry lab researchers. Our guidelines may help to improve FAIR usage of metagenomics as we concentrate on data interoperability and reuse aspects in genome sequencing.

P3: Genome-centered metagenomics reveals a diverse microbial community participating in aromatics degradation under sulfate-reducing conditions

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This study aimed to analyze the functional potential of different species recovered from an enrichment culture regarding their ability to degrade aromatics under sulfate-reducing conditions. We cultivated an enrichment culture from coarse sand collected from an on-site reactor flushed with anoxic sulfidic groundwater in a benzene-contaminated field for over a decade. The culture was fed with benzene under sulfate-reducing conditions. We constructed metagenomes from the liquid and the solid phase of the culture to widen our genome pool. Using MuDoGeR, we retrieved metagenome-assembled genomes (MAGs, bins with completeness > 50% and contamination < 5%) from the two metagenomic libraries (64.2 and 35.3 Million reads). MAG quality was assessed using CheckM, and GTDB-tk was used to assign taxonomy. Furthermore, we identified operational taxonomic units (OTUs, a proxy for species) based on an average nucleotide identity of 0.95. We manually curated enzymes involved in aromatics degradation under sulfate-reducing conditions from KEGG, UniProt, and NCBI. Then, to perform functional analysis, we created a matrix of presence/absence from the annotated genes in our MAG set using StandENA.

Our analysis yielded 127 MAGs, 120 Bacteria, and 7 Archaea. Our MAGs affiliated with 22 Bacteria phyla, three of which were hitherto unclassified, and 4 Archaea phyla. After dereplication into OTUs, our MAG dataset comprised 48 Bacteria and 4 Archaea species. The most prevalent phyla were Desulfobacterota (22), Chloroflexota (21), and Patescibacteria (17). Methanogenesis genes were found in MAGs belonging to Candidatus methanofastidiosum. We found the genes for three enzymes required for dissimilatory sulfate reduction (sulfate adenylyltransferase, adenylyl-sulfate reductase, and dissimilatory sulfite reductase) in two phyla- Desulfobacterota and Firmicutes. Additionally, Desulfobacterota was the predominant phylum possessing some of the genes for the enzymes involved in the ATP-independent aromatic ring reduction of benzoyl-CoA. This compound is an intermediate in the anaerobic degradation of aromatics and subsequent ring cleavage. Our analysis also revealed the absence of the gene known to activate the benzene ring in Peptococcaceae members - benzene carboxylase abcA, suggesting that novel genes and degraders are likely responsible for benzene activation in this community. We identified the genes encoding the respective benzoyl-CoA degradation enzymes. Our research has shown that a diverse community is engaged in the sulfate-mediated breakdown of aromatic compounds and that the phylum Desulfobacterota is essential for respiration in sulfate-reducing environments. Further research is needed to uncover the processes and organisms that convert benzene and other aromatic compounds to benzoyl-CoA.

P4: Fungal diversity of Agaricus bisporus culture compost during organic cultivation

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The presented study aimed to evaluate the fungal diversity of Agaricus bisporus culture compost during organic cultivation in Belgrade, Serbia. Representative compost samples were collected at three timepoints (Day 0 (spawning), Day 14 (end-incubation, just before casing), and Day 33 (first A. bisporus fructification)) from 10 different, randomly chosen cultivation bags. Following total DNA extraction, targeted amplification of internal transcribed spacer 2 (ITS2) using universal fungal primers (ITS3-2024F and ITS4-2409R) was done and amplification products were sequenced using Illumina NovaSeq 6000 platform to generate 250 bp paired-end reads. Bioinformatic analysis was performed using QIIME 2 v2021.8, including the two quality control methods, ITSxpress followed by DADA2 to obtain 661 unique amplicon sequence variants (ASV). Taxonomic assignment was carried out using Naive-Bayes classifier and UNITE database (Version 8.3) for QIIME 2, with a minimum identity of 97%. Overall, the resulting ASVs are distributed across distinct taxonomic levels: 7 phyla (predominantly Ascomycota and Basidiomycota), 25 classes, 51 orders, 100 families, 143 genera, and 147 species, including those with uncertain taxonomic identification. The alpha diversity analysis showed that the observed fungal species richness significantly declined from spawning to the end-incubation stage ($p=0.020651$), followed by an increase until the fructification stage ($p=0.010903$). Moreover, similar fungal diversity dynamics was observed by Shannon index (spawning vs. end-incubation ($p=0.049366$); end-incubation vs. fructification ($p=0.000157$). The most prevalent (present in >60% samples at all cultivation stages) and abundant ASVs affiliated at the family level belonged to Chaetomiaceae, Agaricaceae, Pseudeurotiaceae and Leotiaceae.

At species level, Mycothermus thermophilus was expectedly the most abundant until the end-incubation stage, when A. bisporus started to overgrow the compost in the fructification stage. Notably, different Trichoderma species were anecdotally detected in spawning and end-incubation compost samples, highlighting the importance of their further species-specific functional characterisation to timely and effectively identify and mitigate contamination by Trichoderma and other harmful fungi at different cultivation stages. The beta diversity (pairwise PERMANOVA) analysis demonstrated clear separation and significant differences between fungal communities at each cultivation stage (spawning vs. end-incubation, $p=0.038$; spawning vs. fructification, $p=0.001$; end-incubation vs. fructification, $p=0.001$), verified with a principal component analysis. This study provides new insights on the dynamics, composition and structure of microbial communities of A. bisporus culture compost during organic cultivation, laying the foundation for further studies that could guide the best farming practices in mushroom cultivation.

P5: Defining functional metabolic diversity of microbiota during bioremediation of hydrocarbon polluted soil with Biolog® EcoPlatesTM

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The treatment of contaminated soil with the approach of excavation and removal is not in accordance with the zero-waste initiative, and if we want to take the latter into account, it is necessary to choose a different approach to the treatment of soil contamination. So, in that context, in 2022, the company Petrol d.d. agreed to remediate excavated soil, which was a product of construction activity in the petroleum products storage facility in Lendava, Slovenia. Excavated soil was contaminated with petroleum products that were a remnants of petroleum storage activity in that area. All, a total of 120m3 of soil, was relocated within storage facility and arranged in a landfarming unit for utilization of phytoremediation technique procedures. The purpose of this research was to determine effects of different plant species and plant communities on the native soil microbiota responsible for effective hydrocarbon degradation. The landfarming unit was divided into four experimental plots, with two plots seeded with a combination of grasses and two plots seeded with the combination of forbs. Functional metabolic diversity of microbiota during bioremediation process was evaluated with series of Biolog® EcoPlate™ analysis.

Soil in landfarming unit was sampled and analysed by three different approaches: bulk soil, rhizosphere soil and rhizoplane soil. We determined the effect of different plant species on the microbiota, during full-scale bioremediation process with community-level physiological profiling. We found significant differences for polymers and amino acids compounds in case of functional metabolic diversity between forbs and grasses. In case of Phacelia tanacetifolia, for rhizoplane soil, the highest functional metabolic diversity was determined. The lowest functional metabolic diversity was determined at rhizosphere soil for Matricaria chamomilla plant species. Since, this research was performed on full-scale bioremediation project it can provide a useful information in terms of choosing the most beneficial plant species for application of phytoremediation techniques for petroleum hydrocarbons contaminated soils at various spill sites.

P6: Efficiency of DNA extraction kits for microplastic-associated biofilms

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The production of plastics is increasing every year, but plastics can remain in the environment and break down into small fragments less than 5 milimeters in size, known as microplastics (MPs), which remain in the environment for centuries. One of the many properties of MPs is their ability to harbour biofilm communities composed of diverse microorganisms. Metagenomic and metatranscriptomic analysis of these biofilms can provide insight into their composition and functional potential, but due to the small particle size, the amount of DNA on each particle is very low. To facilitate research in this area, we evaluated four commercially available DNA extraction kits for their efficiency in isolating DNA from MP-associated biofilms.

Four different kits were selected for DNA extraction optimisation: Sigma Aldrich GenElute Bacterial Genomic DNA, ZymoBIOMICS DNA/RNA Miniprep, Qiagen DNeasy PowerSoil Pro, and Qiagen PowerBiofilm, kit. All extractions were performed in replicates, DNA was extracted according to the manufacturer's protocols, and DNA yield was quantified using a Qubit fluorometer.

Our results show that DNA yields differed between the tested kits. The Qiagen PowerSoil kit gave the highest DNA yield with minimal variability between replicates. The Qiagen PowerBiofilm kit yielded lower DNA amounts, followed by the ZymoBIOMICS DNA/RNA Miniprep and SigmaAldrich GenElute kits, which yielded approximately the same amount of total DNA. The choice of DNA extraction kit had a significant effect on the amount of DNA recovered from MP-associated biofilms. Qiagen's PowerSoil kit consistently outperformed the other kits, making it an optimal choice for metagenomic studies of MP-associated biofilms.

This study highlights the variability in DNA extraction efficiency between commercially available kits for MP-associated biofilms. Researchers should consider these results when selecting DNA extraction kits and conducting metagenomic studies on MP-associated biofilms to ultimately contribute to a better understanding of the ecological impact of MP pollution. Further optimisation and standardisation of DNA extraction protocols for these complex communities are essential for advancing research in this important area of environmental science.

P7: Climate Change Is Supercharging Extreme Weather Events: Story Threw The Soil Microbiome Perspective

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Global observations unequivocally show that there is a sharp increase in both the wettest and driest weather events in many regions around the world, and these trends will rise with the warming of the planet. The vulnerable agricultural sectors, directly dependent on the climate, is predicted to be significantly affected by climate change, with an expected decline in future crop yields. Project entitled “Potential of the rhizosphere microbiome in the adaptation of agriculture to climate change (PERSPIRE)”, funded by the EU Regional Development Fund, is focused on the effects of floods and droughts on both development of plants as well as the response of its soil microbiome. Project is based on the two separate experiments: (i) flood experiment was conducted under greenhouse conditions (temperature ranging 5-27 ˚C and 59-93% of relative humidity at day and 5-22 ˚C and 63-93% relative humidity at night) while (ii) drought in controlled growing chamber (16 h day/8 h night; 25 ˚C per day/20 ˚C per night; 60-70% relative humidity); with the cabbage (Brassica oleracea var. capitata f. alba) used as a model plant. Both experiments were run in replicates: flood -plants subjected to either one or two long-term flooding events (7 days duration of each flood); drought - plants subjected to one long-term drought event (14 days duration). At different time points (flood: after flooding and after recovery period; drought: after drought) whole plant as well as soil was removed from the pots and subsamples

were taken for culture-based and molecular-based microbiome analyses with the aim to determine flooding and drought disturbance effect on the structure of both total soil microbiome. Knowledge gained in this study represents the first step toward understanding potential negative effects of the extreme events on the fragile agricultural sector in Croatia. Our results will provide better insight on the impacts of flood as well drought stress on soil microbial communities with the final aim to propose new strategies that can help in crop adaptation to a changing climate future scenario.

P8: Advanced sequence detection of plant viruses in water samples: platform comparison between Ilumina and oxford nanopore sequencing

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Plant viruses pose a major threat to global agricultural production and food security. Accurate and timely detection of these viruses is crucial for effective disease control. Recent advances in high-throughput sequencing (HTS) technologies have revolutionized virus detection and enabled unbiased screening of viromes of different environmental niches, including irrigation water. In this study, we focused on plant viruses in water samples and performed a comparative analysis of two HTS platforms: Illumina and Oxford Nanopore Technologies (ONT). Here, we paid special attention to the detection of plant viruses, even in samples with expected low abundance of such targets. Our results showed similarities in detected viruses between the two platforms. In particular, well-known viruses such as tomato mosaic virus were consistently detected by both methods. ONT platform proved some advantages as being faster, offering opportunities for further protocol optimization. Our findings highlight the important role of advanced sequencing technologies in detecting the presence of plant viruses in water samples, paving the way for rapid and comprehensive monitoring of virus diversity in various environments. The comparison of the Illumina and ONT platforms shown here provides important information for researchers seeking to optimize strategies for virus detection in water samples. The development of new methods opens possibilities for progress in the field of plant virology and offers new possibilities for early detection of viruses and better management of diseases in agriculture.

P9: Impact of forest management on soil microbiome in calcareous and silicate bedrock forests

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Forest management practices, particularly tree cutting, substantially influence the soil microbial communities, crucial for nutrient cycling and tree health. These alterations can potentially diminish the diversity of ectomycorrhizal fungi, subsequently affecting tree growth and the key forest ecosystem functions. Recognizing these implications is not only crucial for sustainable forestry but also in the context of climate change, where forest ecosystems act as significant carbon sinks and can influence regional and global climate patterns.

Our study aimed to investigate the effect of different forest management practices on the soil microbiome of the common beech (Fagus sylvatica L.) dominated forests and discern potential variations in microbial community composition between forests rooted in calcareous or silicate bedrock.

Soil samples were collected from nine plots across Slovenia, Croatia, and Italy under the LIFE SySTEMiC project, all dominated by beech trees. We categorized these plots based on three management styles: close-to-nature forestry, medium combined objective forestry, and the undisturbed nature forest reserves. Furthermore, these sites stood on two distinct bedrocks: calcareous and silicate. In total, 45 composite samples were derived from five mature trees per plot. We used the Illumina MiSeq platform for amplicon sequencing, targeting bacterial V3-V4 16S rRNA and fungal ITS2 regions.

Our findings indicate a pronounced impact of the forest management regime on the bacterial and fungal species richness. Specifically, bacterial diversity was notably reduced in close-to-nature managed forests compared to both unmanaged and medium combined forestry. In contrast, fungal richness peaked in unmanaged forests.

Moreover, there were noticeable differences in bacterial and fungal community structures between forests based on management style. Calcareous bedrock, predominantly calcium carbonate leading to alkaline soils, fostered distinct microbial communities when compared to the more acidic silicate bedrock environments, irrespective of forest management.

Management strategies affect various factors including canopy structure, light access, and soil conditions, which in turn shape microbial habitats. For instance, selective cutting in close-to-nature practices modifies the canopy structure, leading to shifts in soil moisture and temperature compared to untouched forests. Furthermore, distinct management regimes alter litter deposition and decomposition rates, affecting soil organic content, pH, and nutrient profiles. Given bedrock's intrinsic role in determining soil nutrient availability, an integrated approach considering these interconnected factors becomes essential for future climate-conscious research with sufficient replication.

P10: Oligotrophic water systems affect the selection of fungi in drinking water

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Safe drinking water is a constant challenge not only due to global changes but recently also the rise of emerging pathogens. According to the latest WHO data fungi became the part of the list in 2021. Fungi in water are well documented, but the selection of fungi on different materials in contact with water is poorly investigated. In vitro testing on spores and pigmented fungal cells showed surviving of chlorine based disinfection, enabling the cells to form biofilms in water network systems. Due to the lack of uniform sampling and identification methods, fungi are not yet considered as a microbiological parameter in the European Drinking Water Directive. During the sampling of water at various stages of water treatment in Slovenia we isolated on the average 260 CFU/L from natural water, 49 CFU/L from water after cleaning and disinfection, 64 CFU/L at the first sampling point in water network, and 97 CFU/L at the last sampling point. The occurrence of fungi through drinking water supply system was mostly influenced by the location of the primary aquifer. Chlorination reduced fungal CFU on the average by 5-times, but its effect decreased with the time and distance of water network. At the final sampling points, CFU increased on the average 2-times when compared to freshly chlorinated water. Also, we noted positive selection of fungi on materials. The genera Aspergillus, Acremonium, Furcasterigmium, Gliomastix and Sarocladium were most common on cement, while Cadophora, Cladosporium, Cyphellophora and Exophiala dominated on metals. Plastic parts were more prone to colonization with Basidiomycota. Opportunistic pathogens of Aspergillus, Candida, Filobasidium, and Exophiala were isolated only sporadically from materials and water, posing little threat to human health.

P11: The bacterial microbiome of acute appendicitis in a Slovenian cohort

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While not much is known about the appendix microbiota in healthy people, a complex picture of acute appendicitis involving many of the known oral and some gut pathogens is emerging. We performed a preliminary investigation of inflamed appendix tissue samples of 80 adult patients without important comorbidities who underwent emergency appendectomy in the 2020-23 period.

The samples were obtained from inflamed appendix tissue that was removed during urgent surgical intervention and sent for a pathological examination. During pathological examination a piece of appendix (5-7 mm × 3 mm with the entire thickness of the wall) was preserved in a medium for stabilization of nucleic acids. One sample per patient was homogenized and a manual extraction with a ZymoBIOMICS 96 MagBead DNA kit was performed. The bacterial load was then estimated using the broad-spectrum 16S rDNA real-time PCR and Escherichia coli DNA as a bacterial standard for concentration estimation. The V3-V4 hypervariable region of the bacterial 16 S rRNA gene was subsequently amplified and sequenced using Illumina Miseq Reagent Kit v3 (600 cycle). The resulting reads were first filtered from human contamination using Bowtie2 and then processed with Usearch and R phyloseq package. Additional phylogeny analyses were performed using MegaX.

We uncovered vastly different bacterial microbiome compositions of the inflamed appendix samples: some were essentially monocultures of Escherichia, Haemophilus,

Salmonella and Bacteroides (fragilis), many were various mixtures of already known oral-derived pathogens involved in appendicitis (Fusobacterium, Aggregatibacter, Parvimonas, Prevotella, Porphyromonas) and commensal gut bacteria, while some were complex microbiota that contained no bacteria hitherto reported to be involved in acute appendicitis. There was a clear negative relationship between the alpha diversity as measured by the Shannon index and the share of proinflammatory bacteria of the microbiota. The bacterial concentration of the investigated tissue samples, however, did not show any connection to the above two parameters.

Further sequencing of samples deriving from other sites of the inflamed appendices is needed in order to see whether the above variability reflects spatial gradients relative to inflammation centre or the stage of inflammation at which the appendectomy was performed.

P12: Assessment of nanomechanical properties of Candida albicans as an element of the oral mycobiota in healthy subjects

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In a healthy physiological state, the mucous membrane of the oral cavity creates a suitable environment for the colonization of Candida spp. yeasts. The aim of the study was to analyze the nanomechanical properties of C. albicans cells derived from the oral cavity of healthy people in a biofilm produced in laboratory conditions.

Candida spp. were sampled from the oral cavity of healthy individuals. The process of biofilm formation was analyzed using classic microscopic observation enriched with SEM (scanning electron microscope) and the nanomechanical properties of the cells were assessed with the use of the atomic force microscopy technique (AFM).

From all isolated strains in the samples collected of the oral cavity healthy people was detected 79% C. albicans. Other isolated species belonged to the group „non-albicans". The observations of C. albicans carried out in 24-h cultures revealed a tendency of the cells to form a biofilm structure with multilayer cell systems.

The diameter of C. albicans cells in this structure was 5.75 µm, and the length of the pseudohyphae was 17.08 µm. The presence of an extracellular substance surrounding the C. albicans cells was detected. The mean value of the adhesion force determined for C. albicans cells was 4.01 nN. Areas with increased hardness (Force Modulation Mode signal; FMM signal) were found mainly in the zones of cells in contact with the glass substrate.

The analysis of Candida cells in liquid samples gives satisfactory results, as it prevents unfavorable changes in the cell surface and thus provides more reliable results. The quality of the biofilm is probably related to the nanomechanical properties of C. albicans cells and may consequently contribute to the stability of the biofilm structures and their susceptibility or resistance to antifungal drugs. The presence of Candida spp. especially in companion animals (dogs, cats) poses a risk of their transmission to the human organism. For this reason, it is advisable to undertake additional research to analyze the ability of zoonotic-origin Candida spp. to form biofilms with comparison of the biofilm-formation capacity of species isolated from humans.

P13: Short-term and midterm impact of probiotic supplementation on gut microbiota composition in neonates with early antibiotic exposure

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Postnatal antibiotic exposure plays a pivotal role in shaping the initial colonization of the neonatal gut microbiota. This study aimed to investigate the short-term and midterm effects of supplementing neonates, who had received antibiotic therapy, with Limosilactobacillus reuteri DSM 17938 on the composition of their gut microbiota.

This randomized, double-blind study enrolled 89 full-term neonates who had undergone antibiotic treatment for at least 5 days within the first 21 days of life. Upon the initiation of antibiotic therapy, neonates were randomly allocated to receive either a probiotic (L. reuteri DSM 17938, 1x108 CFU/day) or a placebo for a duration of 6 weeks. Stool samples were collected for microbiological analysis immediately after treatment and at 12 months following the commencement of antibiotic treatment. Gut bacterial community composition was assessed using 16S amplicon metagenomic sequencing.

No statistically significant impact of probiotic supplementation was observed immediately after treatment or at 12-month follow up. An exception was noted with an increased abundance of the Limosilactobacillus genus after treatment in the group receiving the probiotic, primarily attributed to the detection of the probiotic strain itself. Irrespective of probiotic treatment, the study revealed a correlation between obesity at approximately one year of age and an increased abundance of Lachnospiraceae, while a higher BMI at birth was associated with reduced microbiota evenness later in life.

In our study probiotic supplementation in neonates exposed to antibiotics during early life exhibited negligible short-term and midterm influence on gut microbiota composition. While our findings indicates that early-age probiotic use might not significantly influence microbiota development, they also indicate limited efficacy of chosen single strain probiotic in mitigating the adverse effects of antibiotics on neonatal gut microbiota.

P14: Alterations In The Microbiome Of Supragingival Dental Plaque During Fixed Metal Appliance Orthodontic Treatment

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Intraoral environment and use of oral hygiene products for dental biofilm control induce corrosion of metal orthodontic appliance which influences changes in microbiome. Fixed orthodontic appliances have many additional retention sites for plaque formation, long duration of orthodontic treatment, requires additional agents for oral hygiene such as chlorhexidine (CHX) digluconate or increased fluoride (FL) concentration gels.Changes in bacterial profile of supragingival dental plaque during orthodontic treatment were analyzed in this pilot study. We analyzed changes in bacterial profile in 3 different groups of participant during orthodontic treatment with fixed appliances. First group (control) performed standard oral hygiene, second group used the antiseptic chlorhexidine digluconate (CHX), and third used a gel with a high concentration of fluorides (FL) during orthodontic treatment with metal appliance. Twelve supragingival dental plaque samples were collected at the beginning of orthodontic treatment (T0) and after one month period of performing standard oral hygiene, use of CHX or use of FL (T1). The bacterial metagenomes were analyzed by 16s rRNA gene amplicon sequencing. Extracted DNA from each sample was used for amplification of V2-V9 hypervariable 16S rRNA regions and barcoded library preparation with Ion Plus Fragment Library kit.

Emulsion PCR and sequencing were conducted with Ion PGM™ Hi-Q View Sequencing Kit, on the Ion PGM™ System. In total, 176 different bacterial species were detected in analyzed samples. Bacterial abundance increment was detected in control group, while CHX and F group showed reduction. Bacterial diversity reduction was detected in all three groups (6 to 30 bacterial species less when comparing T1 to T0). Bacterial diversity changes on a species level: Actynomices sp., Lactobacillus sp., Prevotella sp., Porphyromonas sp., Rothia sp. and Veionella sp. were increased in control group and reduced in CHX and F group. Cardiobacterium sp., Fusobacterium sp., Corynebacterium sp. were decreased in all groups.

Metal orthodontic appliances increased overall bacterial abundance and stimulate the growth of bacteria associated with dental caries and periodontal disease, while the use of CHX and FL gel suppresses them. Additional measures for plaque control when undergoing fixed orthodontic appliances treatment should be advised.

P15: Slurry Resistome Of Dairy Farms

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Antibiotic resistance genes (ARGs) in the environment pose a threat to human and animal health due to the possibility of horizontal transfer. Cattle breeding in Slovenia is economically important but may represent a hotspot for the spread of ARGs. A comprehensive understanding of antibiotic resistance in the cattle production environment has yet to be explored. In the on-going project presented here, slurry and manure samples were collected from two large conventional farms with a total of 400 (farm 1) and 165 (farm 2) dairy cows. Total DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen). Library preparation and shotgun sequencing (Illumina, 2 × 150 bp, ~8 Gbp reads per sample) were performed. The presence of ARGs was determined using the ARGs-OAP v3.2.3 tool. Preliminary results showed the presence of ARGs conferring resistance to a very diverse group of antimicrobials. The main ARGs detected were the ARGs conferring resistance to aminoglycosides, beta-lactams and the macrolide-lincosamide-streptogramin (MLS) group. The most abundant ARGs detected were aph(3'')-Ib, aph(6)-Id and aadS for aminoglycoside resistance, cfxA2, cfxA3 and cfxA5 for beta-lactam resistance, and Inu(C) and Inu(H) for MLS resistance. The total number of ARG copies per cell was about 0.40 on farm 1 and 0.78 on farm 2. In the continuation of the project, the correlation between different farm-related factors (e.g. farm size, production system and antimicrobial use) and slurry resistome will be investigated and additional samples will be sequenced.

P16: Bacterial and fungal microbiome of dromedary camel milk

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Dromedary camel milk is produced by Camelus dromedarius and has a significant nutritional, therapeutic and economic value in regions of Asia and Africa. Camel milk is considered a functional food with significant health benefits. It is easily digested and safely consumed by people with a weak immune system. It also displays antimicrobial and antiviral effects (Rajput & Sharma, 2014; El Khasmi & Faye, 2019, Rahmeh et al., 2022). Milk microbiota assessment has traditionally relied on the culture-dependent approach, which includes isolation and culturing, using selective media, followed by subsequent analysis. Recent methods and data analyses have led to a new perception of the milk microbiota as a complex diverse microbial community. Rahmeh and co-authors (2022) used the V3-V4 regions of the16S rRNA gene for bacteria and the internal transcribed spacer (ITS) for fungi to assess the microbiota of raw dromedary raw camel milk from various regions in Kuwait during two seasons. They found four predominant phyla of bacteria: Pseudomonadota, Bacillota, Actinomycetota and Bacteroidota. The core bacterial microbiota of raw camel milk included Schlegelella, Paenibacillus, Lactobacillus, Comamonadaceae, Pediococcus, Moraxella, Acinetobacter, Staphylococcus, Enterococcus, Pseudomonas, Streptococcus, Micrococcaceae, Rothia, Sphingomonadaceae, Neisseriaceae and Sphingomonas. The fungal population comprised of genera mainly belonging to two phyla (Ascomycota and Basidiomycota): Penicillium, Cladosporium, Candida, Aspergillus, Alternaria and Fusarium. Kadri and co-authors (2021) used MALDI-TOF mass spectrometry and 16S rRNA gene multiplication of Moroccan raw camel milk samples and found that lactic acid bacteria (mainly lactococci and enterococci), Enterobacteriaceae, Aeromonadaceae, Pseudomonaceae and Streptococcaceae dominated the samples. applied have a significant impact on the composition and diversity of the dromedary camel milk microbiome.

Another study by Amrouche and co-authors (2020) focused on the fungal microbiome of camel milk from Algeria and utilised both culture-dependent methods and culture-independent molecular techniques, the latter being based on dHPLC analysis and metabarcoding of ITS region found yeasts belonging to Filobasidium, Naganishia, Malassezia, Mrakia, Rhodotorula and Yarrowia genera; and moulds belonging to Fusarium, Cladosporium and Penicillium genera. All the techniques used revealed that the fungal community was dominated by Filobasidium and Naganishia. High throughput 16S rRNA sequencing detected the presence of diverse bacterial communities dominated by Pseudomonas spp., especially in morning milk, followed by Enterobacteriaceae, the genus Janthinobacterium, Aeromonadaceae and Clostridiales. Lactic acid bacteria were found in the evening samples. We can conclude that geographical location, time of day, season as well as the identification method applied have a significant impact on the composition and diversity of the dromedary camel milk microbiome.

P17: The cloacal microflora of two tortoise species: Testudo graeca and Testudo hermanni - a potential source of opportunistic pathogenic bacteria

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It is well known that reptiles are capable of being asymptomatic carriers of various pathogens that could be transmitted to other animals or humans. Most studies have focused on reptiles in captivity or are subject of commercial interest as pets, and on a small subgroup of bacteria of zoonotic concern, primarily Salmonella. Extensive knowledge about the microbiota accompanying free-ranging hosts is still lacking. We aimed to explore the cloacal microflora of the two tortoise species, occurring in Bulgaria: the spur-thighed tortoise (Testudo graeca ibera Pallas 1814) and the Hermann's tortoise (Testudo hermanii boettgeri Mojsisovics 1889). Both species are included in the Red Data Book of the Republic of Bulgaria and the IUCN Red List of Threatened Species with status of endangered species. Potential pathogens believed to be one of the factors leading to population declines worldwide are understudied. Research on the wild animals-associated microbiota could have a positive impact on conservation efforts for vulnerable species. We report our preliminary data on the cloacal microflora of T. graeca and T. hermanni, studied for the first time in the country.

Sampled individuals were reared in a large enclosure, in a semi-free environment close to their natural habitat, at Tortoise Rescue Center.

Cloacal samples were collected from a total of 24 clinically healthy adults: 12 T. graeca and 12 T. hermanni, equal numbers of each gender. The cloacal sampling was done with sterile cotton swabs. Cultures were enriched in Nutrient Broth and Rappaport-Vassiliadis broth and plated on petri dishes with selective agars. The pure single colonies were identified morphologically and biochemically (FECAL WELL D-ONE; MICROLATEST ID: ENTERO 24N). Specialized software (ErbaExpert Identification Program (www.erbalachema.com)) was used to interpret the results.

Examination of cloacal swabs demonstrated a widespread presence of several Gram-negative bacteria of the Enterobacteriaceae family. The most common species include Salmonella enterica, Citrobacter braakii, Klebsiella spp., Enterobacter cloacae and Providencia/Proteus. No differences were found between species regarding the composition of the cloacal microflora, but the proportional presence of some bacteria was dissimilar. More heavy colonization with S. enterica, Klebsiella spp., Rachnella aquatilis, Pantoaea agglomerans, Escherichia coli were observed in T. graeca. E. cloacae, C. braakii, Morganella morgani prevailed in T. hermanni.

The testudinal cloaca was populated by various Gram-negative bacteria, which are opportunistic pathogens and could be hazardous as zoonotic agents. In certain cases, they could pose a potential risk for human health. It should be considered in close contact with animals.

P18: Type IV pili among non-pathogenic Gram-positive gut bacteria with diverse carbohydrate utilization patterns

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Type IV pili (T4P) are bacterial surface-exposed appendages that have been extensively studied in Gram-negative pathogenic bacteria. Despite recent sequencing efforts, little is known regarding these structures in non-pathogenic anaerobic Gram-positive species, particularly commensals of the mammalian gut. Early studies revealed that T4P in two ruminal Gram-positive species are associated with growth on cellulose, suggesting possible associations of T4P with substrate utilization patterns. In the present study, genome sequences of 118 taxonomically diverse, mainly Gram-positive, bacterial strains isolated from anaerobic (gastrointestinal) environments, have been analysed. The genes likely to be associated with T4P biogenesis were analysed and grouped according to T4P genetic organization. In parallel, consortia of Carbohydrate Active enZYmes (CAZymes) were also analysed and used to predict carbohydrate utilization abilities of selected strains. The predictive power of this approach was additionally confirmed by experimental assessment of substrate-related growth patterns of selected strains. Our analysis revealed that T4P systems with diverse genetic organization are widespread among Gram-positive anaerobic non-pathogenic bacteria isolated from different environments, belonging to two phylogenetically distantly related phyla: Firmicutes and Actinobacteria.

P19: Role of bacterial consortium in aerobic bioremediation of food waste

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Bioremediation is a green technology that deals with the degradation of various pollutants through the comprehensive action of microorganisms to restore ecosystems to their original state. Anthropogenic activities have become the source of numerous pollutants in ecosystems, with food waste being a specific human waste stream with major impacts. Microorganisms, especially bacteria, have impressive metabolic activity and therefore play an important part for overcoming environmental challenges.

The main focus of this study was the bioremediation of food waste stream with autochthonous microorganisms under submerged conditions, and the isolation and identification of microbial cultures with degradation capacities for organic pollutants. During the experiments, chemical oxygen demand (COD), total suspended solids (TSS), concentration of dissolved oxygen (DO) and pH value were monitored. Microbiological and MALDI-TOF analyses were performed to characterize the microbial isolates.

The average pH value was 7.20±1.57, while values of TSS ranged from initial 0.72 to maximal 3.55 g/L. In the 3rd day of the experiment, obtained COD efficiency removal was 68.3%. According to MALDI-TOF analyses, predominat genera of microbial isolates were identified as Sphingobacterium, Chryseobacterium, Microbacterium, Klebsiella and Pichia.

Biostimulation of autochthonous microorganisms from food waste can improve the biodegradation process and contribute significantly to environmental protection in a simple and economically viable way. The microbial isolates can potentially be used for bioaugmentation of food waste streams.

P20: National Research Data Infrastructure for the Research of Microbiota (NFDI4Microbiota) – Democratize access to microbiota data and high-end computational analyses

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Microbes (bacteria, parasites, viruses, protists, and fungi) influence our everyday lives and have a not-yet-determined impact on one health. Therefore, microbiology has had a considerable increase in research projects and applications in its various fields. Many of these projects are closely related and have the potential to be more than the sum of their parts. NFDI4Microbiota aims to facilitate that process with a digital transformation in the microbiological community by providing access to data, analysis services, training and infrastructure.

The NFDI4Microbiota was established as a central hub to support the microbiological community in Germany and abroad. It comprises ten well-established partner institutions. We are also supported by five professional societies and more than 50 participant institutions. Within the consortium, we aim to facilitate the digital transformation of the microbiological community to accelerate the generation of new and relevant knowledge.

The consortium has started to build a national and international network of mutual support and feedback. Within this frame, it provides specific training to accelerate research and customizes infrastructure and microbiome computational tools.

Further, we support high-quality research data management (RDM) and emphasize the importance of (meta)data to adhere to the FAIR principles (Findable, Accessible, Interoperable and Reusable). The consortium provides various tools, methodologies and resources to translate new and already existing data into new knowledge. Among others, tools such as AMBER (assessments for metagenome binners), BacDive (database for bacterial information), VirJenDB (virus genome database), BioAutoML (Automated feature engineering and Metalearning for classification of biological sequences), MuDoGeR (simultaneous reconstruction of Prokaryotic, viral and Eukaryotic genomes) and various Metagenome Metadata Databases (Terrestrial, Human, Marine and Animal). The NFDI4Micrbiota also provides various training programs for implementing FAIR principles and Open Science, RDM, databases, general programming and data science, data generation and analysis. To connect the resources mentioned above, we established an infrastructure that includes analysis tools, a knowledge base (GitHub contains material on RDM) and a cloud-based system for the analyses, integration and storage of microbiome data. An ambassador program was established to connect with the participants and identify the vast needs of microbiologists performing computational analyses.

By creating a central resource for German microbiota research, the NFDI4Micriota consortium aims to represent the central connection for the microbiological community. Also, it provides researchers with infrastructure, expertise and easy access to tools and training programs to enhance their research and make it FAIR.

P21: Fertilisation influences ammonia oxidisers and denitrifiers and nitrous oxide emissions in a long-term tillage experiment

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The aim of this study was to determine the effects of more than 20 years of differential tillage and fertilisation on the soil microbial community, particularly on the abundance of N cycling guilds, and to link them with the measured nitrous oxide (N2O) emissions.

The study was conducted during the 2021 maize vegetation period on a long-term combined tillage [no-till (NT) vs. conventional plough tillage (CT)] and fertilisation [unfertilized (CON), mineral (MIN) and compost (ORG)] field experiment, established in 1999. Gas and soil samples were collected throughout the maize growing season to perform physicochemical analyses and quantification of microbial genes by qPCR (16S, ITS, nirS, nirK, nosZI, nosZII, nrfA, bacterial and archaeal amoA). Samples from the last sampling point of the season were used to determine potential denitrification activity (PDA) and analyse taxonomic and functional community composition by 16S amplicon and WGS sequencing (nosZI and nosZII reads extraction) and later placing the reads phylogenetically.

Long-term mineral fertilisation increased the share of bacterial ammonia oxidisers in the total bacterial community (AOB/16S) which also coincided with the observed highest cumulative emissions in mineral fertilisation. This ratio was proven to be one of the most important variables explaining cumulative N2O emissions, possibly reflecting the role of bacterial ammonia oxidisers in minerally fertilised soil.

A higher genetic potential for N2O emissions was observed under NT than under CT, as indicated by an increased (nirK+nirS)/(nosZI+nosZII) ratio. Mentioned ratio under NT decreased in the order CON > MIN > ORG, indicating a higher N2O consumption potential in the NT-ORG treatment, which was confirmed in terms of cumulative emissions. The latter was indicated further by employing PDA assay where significantly lower (N2O)/(N2O+N2) product ratio was observed in NT-ORG compared to the other treatments. Sequencing of 16S rRNA gene showed substantial differences in community composition between the two tillage systems while the impact of fertilisation remains to be examined. Distinct patterns between different treatments were observed in terms of nosZI and nosZII phylogenetic placement analysis indicating an influence of long-term differential management on microbial community functional composition.

Our results suggest that even though the genetic potential of microorganisms involved in biological processes is important for elucidating the impact of agricultural practices on the underlying microbial community, its expression in real-life conditions depends on environmental factors. Understanding this link could provide novel insights and strategies for steering microbiome responsible for N2O emissions.

P22: Effect of abiotic stresses on microbial communities of the root environment of citrus rootstocks

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Drought or salinity are the most important environmental factors affecting agricultural productivity. The responses of plants to these stress conditions are highly complex and in turn influence the beneficial and detrimental plant-microbe interaction in the rhizosphere. To understand the effect of abiotic and biotic stresses on the root environment microbiome in trees, grafting is an important factor to consider, as rootstock roots directly interact with the soil recruiting microbial communities. In addition, to design and support microbiome-based agriculture, a crucial step is to understand factors that control microbial communities. Since in citrus the choice of the rootstock plays a crucial role in the overall responses to stresses, we investigated the influence of either water deficit or saline irrigation in two rootstocks, Carrizo citrange and Bitters, on the microbial communities associated to the root environment. Two root compartments, rhizosphere and endorhizosphere, were separately processed and total DNA was extracted from the samples with the DNeasy PowerSoil Pro Kit (Qiagen). Microbial communities were profiled by high-throughput amplicon sequencing using the NovaSeq 6000 system (Illumina). The differences in community diversity, structure and complexity were analysed with the Amplicon Sequence Variant (ASV) method through DADA2. Differential abundance was performed using DESeq2. The most representative phyla in the rhizosphere were Proteobacteria, Planctomycetota and Actinobacteriota, the latter being more abundant in the drought-stressed samples of both rootstocks. In the endorhizospheres, Proteobacteria, Actinobacteriota, Firmicutes and Planctomycetota were the most abundant phyla. In both rootstocks, Proteobacteria phylum was dominant under drought- and salinity-stresses whereas Firmicutes abundances were higher in the control plants.

Ascomycota, Basidiomycota, Rozellomycota and Glomeromycota were the most representative phyla across the rhizospheres. Some variability was observed according to the treatment and the rootstock. Beta diversity analysis of the microbial communities based on Bray–Curtis dissimilarity showed a significant division betweenthe two compartments. In addition bacterial, but not fungal, communities of the rhizosphere and endorhizosphere of the control plants as well as those subjected to drought and salinity stress were clearly delineated. Differential abundance analysed at the ASVs level highlighted that the stress, either drought or salinity, altered the microbial communities especially of bacterial communities. More in detail, Bitters as compared to Carrizo rootstocks presented more significant differentially ASVs. The same trend was observed for the fungal communities, although a lower number of ASVs was involved. Results will be discussed also in relation to morphological and physiological parameters of the rootstocks.

P23: Establishment and maintenance of potato phytobiome

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Understanding the interaction between plant and its surroundings is crucial to ensure long lasting and environmentally friendly system for plant protection. It is known that some microbes can stimulate plant immunity and promote its growth, however, little is known of mechanisms triggered in plant as a response in interaction with its microbiota.

A promising group when prospecting for such microbes are endophytes, which can modulate plant's response to the pathogen through priming of immune response, directly by antibiosis or competition for nutrients. Additionally, beneficial endophytes can directly promote plant growth through nitrogen fixation, synthesis of enzymes or peptides that provide nutrients or by production of phytohormones.

To get better insights into mechanisms of plant-microbe interaction we are: i) studying molecular mechanisms of colonisation by selected few bacteria and fungi isolated from potato phytobiome in controlled settings, as well as ii) collecting samples of potato roots and leaves epi- and endophytes in various environmental conditions to learn about general principles of community assembly.

This work presents new results on interaction between potato and B. subtilis. Using different fluorescently tagged strains, we determined the dynamics of root biofilm formation, studied the formation of biofilm in kin and non-kin interaction, and monitored responses of the plant to the colonialisation.

Potato roots do react to colonisation by B. subtilis, but no major differences in response were detected if the roots are exposed to two non-kin strains. Transcriptional response of the plant however revealed, besides expected and extensive regulation of receptor kinases, an interesting response in protein degradation and Ethylene Responsive Factor (ERF). In transgenic plants with its silenced activity, we detected increased abundance of bacteria in the leaves of potato plantlets, meaning that ERF is participating in stabilisation of interaction.

P24: Exploring the Carpospheric Microbiome Composition in Diverse Citrus Accessions: Implications of Field Treatments on Community Structure and Selection of Biological Control Agents Associated with Core Members

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Citrus, a globally cultivated major fruit crop, remains relatively understudied in terms of its carposphere microbiome composition and function. Furthermore, given the essential role of pesticide applications in citrus crop management, it is imperative to comprehend their impact on the beneficial microbiome associated with citrus. Here, we present a thorough examination of the structural composition of the citrus carposphere microbiome across three citrus accessions ('Tarocco Scirè' and 'Tarocco Tapi' oranges, 'Femminello Siracusano 2KR' lemon), and how microbiome is altered in response to various phytosanitary treatments (biological and copper-based antimicrobials) applied in the field, using both amplicon sequencing and culture-dependent techniques. Results revealed that the microbiota in the three citrus accessions had similar taxonomical composition but varied in relative abundance, with Proteobacteria, Firmicutes, Actinobacteriora, Ascomycota and Basidiomycota phyla being dominant in all the samples. Within the same accessions, data were analysed comparing different applied treatments (chitosan, sweet orange essential oil, their mixture and copper-based products) on citrus fruits.

While the cultural-method did not show relatable changes in microbial community, the cultural-independent method revealed substantial differences in the microbial composition and abundance profiles of the bacterial community when copper-based treatments were applied, whereas alternative products induced minimal or no differences. The fungal community structure appeared less sensitive to phytosanitary products applications. The core microbiome comprised 51 bacterial and 27 fungal Amplicon Sequence Variants, encompassing genera widely reported as plant beneficial microbes. In parallel, an extensive census of the cultivable microbiota was performed to collect a representative number of bacterial naturally occurring on the fruit surface to approach a selection of potentially biocontrol agents. A significant finding emerged from the 16S rRNA gene amplification of 120 bacterial antagonistic strains, as 75% of them matched with the core members. The present study represents an advancement of the current knowledge of citrus fruit microbiome and confirms that pesticides can cause measurable shifts in microbial communities. Moreover, our results further support the beneficial role attributed to the core microbiome and the integration of next-generation sequencing technologies with cultivable-dependent techniques as a powerful approach to elucidate the potential of core members as biological control agents, allowing for comprehensive profiling of cultivable microorganisms and enabling a deeper understanding of their roles and ecological dynamics within the context of biological control strategies.

P25: Seed aging affects bacterial diversity and community composition in Brassica napus seedlings

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Seeds harbour diverse microorganisms and it is thought that microbial composition and relative abundances have a strong impact on seed quality and subsequent crop performance. For example, seed microbes, either native or seed applied, can have a beneficial impact on seed germination and seedling's susceptibility towards phytopathogens. Seed germination performance and also seed vigor are highly associated with seed storage conditions. However, the impact of seed aging and storage on the seed microbiome has been rarely studied. Here, we investigated the effect of an artificially conducted accelerated aging stress given by increased temperature and moisture on the seed microbiota of four different oilseed rape (Brassica napus) genotypes derived from two different field sites in northern Germany. Oilseed rape seedlings were observed to germinate abnormally due to the accelerated aging stress, compared to the control seedlings. Amplicon sequencing of 16S rRNA genes revealed that bacterial diversity was reduced in artificially aged seedlings which was significant for all genotypes of the first field site. Regardless of the origin field site and the oilseed rape genotype, accelerated aging stress had the strongest impact on the bacterial community composition as revealed by Permanova analysis. Stressed seedlings were dominated by Firmicutes, whereas Proteobacteria prevailed in the control samples. More precisely, Bacillus, Tumebacillus and Paenibacillus were significantly more abundant in the seedling microbiota of artificially aged seeds. In control samples, mainly Pseudomonas, Pantoea and Sphingomonas showed increased abundance. The results will help to further understand the important role of the seed microbiome on seed vigor and may support strategies for improved storage conditions.

P26: Viromes Of Aquatic Plants

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Aquatic plants are a taxonomically diverse group of organisms inhabiting various marine and freshwater habitats. Due to their variety and widespread presence, they represent a substantial pool of potential viral hosts. Under various conditions, viruses can be released from plants into the surrounding environment and subsequently be transferred over longer distances via water. This is particularly concerning in the case of aquatic plant communities situated close to agricultural areas or water sources utilized for irrigation. Thus, aquatic plants represent largely unexplored viral reservoirs with a potential role in water-to-crop viral transmission. Despite that, their viromes remain understudied. The aim of this study was to expand the currently limited knowledge of viruses associated with aquatic plants. We explored the viromes of diverse marine and freshwater plant species by utilizing two distinct approaches. Firstly, we mined the transcriptome data generated during the 1000 plant transcriptome initiative to search for possible viral sequences. Secondly, we sequenced ribosomal RNA depleted total RNA of 17 aquatic plants. We have shown that aquatic plant species of various levels of complexity contain sequences of several known plant viruses. These include viruses unique to wild plants, as well as those that are known to infect economically important plants. Examples of the latter include tobacco streak virus, turnip yellows virus, and Cymbidium mosaic virus. Additionally, we have assembled multiple near-complete genome sequences with moderate similarity to known plant viruses, which represent potential novel species.Our findings demonstrate the presence of diverse viral sequences in aquatic plants, with some seemingly accumulating to high titers, as indicated by a large abundance of viral reads. However, additional experimental work is needed to confirm the frequency of viral infections in aquatic plants and their potential to release viruses into the water. Furthermore, it is essential to determine the ability of viruses to spread to adjacent plants through water transmission.

P27: Antimicrobial Efficacy of Bioactive Compounds Against Pseudomonas savastanoi pv. savastanoi: Strain-Specific Responses and Concentration-Dependent Effects

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The control of bacterial diseases in agriculture is challenging and worsened by the risk of bacterial resistance development. This is particularly emphasized for Gram-negative bacterial species due to their complex cell structures, which makes them less susceptible to antimicrobial agents. In this study, we investigated the antibacterial effectiveness of five essential oils (EOs) derived from plants within the Lamiaceae family and their selected EO components against Gram-negative bacterial species, specifically Pseudomonas savastanoi pv. savastanoi (Pss), a major threat to olive cultivation.

To determine the effect of EOs on Pss plant pathogen we used disk diffusion method and broth microdilution assay.

Our findings revealed that EOs from Mentha and Salvia species have limited antibacterial efficacy against Pss strains in vitro. However, EOs from Thymus vulgaris L. and Origanum compactum effectively inhibited the growth of all Pss strains. The relatively weak susceptibility of Pss strains to some tested compounds may be due to their inability to diffuse through solid growth media. Conversely, a broth microdilution assay indicated promising results for EOs of Origanum majorana L. and Salvia officinalis L. at lower bacterial density, indicating their potential for biopesticide formulations.

The concentration-dependent responses of EOs, particularly T. vulgaris, O. compactum and O. majorana EOs, suggest their potential as alternatives to control olive knot disease. Furthermore, we determined the concentration-dependent effects of tested EO components, where carvacrol emerged as the most potent inhibitor, showing effectiveness at low doses. This aligns with previous research highlighting the antimicrobial action of phenolic compounds like thymol and carvacrol.

Notably, all tested compounds exhibited antibacterial effects similar to antibiotic treatment at their respective MIC values, with exceptions noted for M. piperita L. and S. officinalis L. EOs. The antibacterial potential varied between bacterial strains, emphasizing the importance of understanding strain-specific responses when developing effective treatment strategies for plant host infections.

P28: Response of fungi colonising buckwheat grains to cold plasma treatment is species-specific

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Fungi are the leading cause of plant diseases worldwide and are responsible for enormous agricultural and industrial losses on a global scale. They are readily isolated from dried buckwheat grains. Cold plasma treatment (CPT) is a potential tool for eliminating or inactivating fungal contaminants from the surface of biological material such as seeds and grains. Plasma is a partially ionised gas, the fourth state of matter, that can be artificially generated by applying energy to a gas. It consists of free electrons, atoms, molecules, ions, radicals, and other reactive species together with (vacuum) ultraviolet radiation. This study used a low-pressure radiofrequency cold plasma system with oxygen as the feed gas to test the decontamination efficacy of different genera and species commonly colonising buckwheat grains. Firstly we sterilised the grains by autoclaving and then performed artificial contamination with ten selected fungal taxa previously isolated from buckwheat grains (Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium cladosporioides, Epicoccum nigrum, Fusarium fujikuroi, F. grraminearum, F. oxysporum, F. proliferatum and F. sporotrichioides) in concentration 106 spores per gram of grains. After different time exposures to CPT, we compared two widely accepted methods for evaluating the efficacy of fungal decontamination: direct cultivation method, expressed as a contamination rate (%) and indirect cultivation or colony-forming units (CFU) method. An efficient decrease in contamination levels with increasing CPT time was observed for most tested fungi. Fusarium graminearum was found to be the most susceptible to CPT, while the species from Fusarium fujikuroi SC seemed to be the most resistant to CPT. Our results show that fungi with smaller spores (microconidia) could have an advantage over species with larger spores (macroconidia) to CPT.

The observed doses of oxygen atoms needed for 1-log reduction ranged from 1024–1025 m−2. Our results indicate that the shape and colouration of the spores could also influence the decontamination efficacy. To our knowledge, this study reports the first work on responses of a broad range of fungi colonising grains to CPT, obtained by two evaluating techniques commonly used to determine decontamination efficacy.

P29: Biological control of fungal fruit diseases by Aureobasidium pullulans

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Fungal diseases such as grey mold or brown rot blossom blight (caused by Botrytis cinerea and Monilinia laxa, respectively) threaten many different fruits, under pre- and post-harvest conditions. Due to fungal infections, up to 25% of the yield can be lost. In order to reduce the amount of chemical fungicides in agriculture, biological control agents are needed to sustainably combat those pathogens. Aureobasidium pullulans, a ubiquitous yeast-like fungus, is a promising candidate. This beneficial fungus has gained significant attention in recent years due to its remarkable plant-beneficial properties. The antagonistic activity of A. pullulans has already been demonstrated for several strains and against a broad range of pathogens.

Here we demonstrate the distribution of A. pullulans across hosts and habitats around the world based on the “GlobalFungi” database. Further, we focus on two specific isolates, which were tested in in vitro assays for their ability to repress the growth of different plant pathogens using volatile organic compounds (VOCs). Interestingly, the growth of most of the tested pathogens was reduced compared to the control treatment. A combination of both tested strains performed best overall. We perform a VOC analysis using gas chromatography to clarify which compounds are produced by either of the strains or in co-culture and are responsible for their antagonistic activity. The results will give new valuable insights into the biocontrol ability of the specific A. pullulans isolates.

P30: Compost metagenome as a source of industrially relevant CAZYmes

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Second generation biofuels are suffering from a lack of efficient and cost effective hydrolytic enzymes which are crucial for lignocellulose pre-treatment. These enzymes are also gaining interest in textile, paper and chemical industries as environmentally friendlier alternative to harsh chemicals. Environmental lignocellulose degrading ecosystems are ideal sources for these biocatalysts since the polysaccharide hydrolysis occurs there naturally and is governed by the synergistic action of multiple proteins. Metagenomics is a powerful approach to explore the diversity and potential of microbial communities in such environments, revealing novel genes (enzymes) that may not be detected by traditional methods. In our study, a complement of bioinformatics and experimental approaches was used to characterize in silico predicted carbohydrate active enzymes (CAZymes) targets and evaluate their potential for lignocellulose pre-treatment in comparison to commercially available counterparts. Among 96 target genes which were cloned and expressed, 12 novel thermophylic xylanases were succesfully isolated and characterized. One of the promising targets was approximately 40 kD enzyme with a GH10 catalytic domain, active against beechwood xylan and arabinoxylan. The enzyme was optimally active at 50 °C and pH=8. Its xylanolytic activity increased in the presence of 5 mM Co2+, Fe3+, Mn2+ or Ca2+ ions or reducing agents such as 2 mM DTT or 10 mM ß-mercaptoethanol. Apart from its stability in different environmental conditions, the target enzyme also proved efficient in the degradation of natural hemicellulose-rich substrates, such as wheat or barley bran as well as considerable robustness in the presence of the most common chemicals used in lignocellulose pretreatments, showing promising potential for use in industrial applications, such as the production of biogas and bioethanol.

P31: Fecal microbiota diversity based on 16S rRNA sequencing of five syntopic lizard species from a low-mountain area in Western Bulgaria

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Investigations on gut microbiomes in free-living reptiles in Europe in general are still fragmentary. Research in such aspect has not been carried out in Bulgaria so far. We aimed to identify and compare the 16S rRNA gene profiles of fecal microbiota in five lizard species belonging to three families (Lacertidae, Scincidae and Anguidae): the European green lizard (Lacerta viridis), the Common wall lizard (Podarcis muralis), the Meadow lizard (Darevskia praticola), the European snake-eyed skink (Ablepharus kitaibelii) and the European slow worm (Anguis fragilis). In the particular region, which is low-mountainous and is located in western Bulgaria, they live in sympatry and syntopy. The co-occurrence of multiple species provides an opportunity to study their gut microbiomes in parallel. The relationship with their previously established diet has also been discussed.

Fecal samples were collected non-invasively, in sterile conditions. After sampling, animals were released at their natural environment. A high-throughput sequencing of the hypervariable V3-V4 region of the 16S rRNA gene was performed on the Illumina HiSeq 2500 platform (Macrogen Inc., Korea). A total of 86 specimens were captured: L. viridis n=15; P. muralis n=17; D. praticola n=26; A. kitaibelii n=26; A. fragilis n=2.

Highly divergent gut microbiomes in terms of composition and diversity among lizard species were observed. The richest alpha-diversity was found in D. praticola, and the least in P. muralis and A. fragilis. A dynamic phyla proportion between hosts was found. The predominant phylum was Bacillota with a relative abundance ranging between 35.4 – 86.4%, followed by Proteobacteria (0.4% - 36.8%), Bacteroidota (0.2 – 23%), Actynomycetota (2.5 - 18.7%), Veruccomicrobia (0.4 – 4.4%, except in A. fragilis) and Cyanobacteria (present only in L. viridis (30.8%). The core microbiota of each lizard species had a prevalence of a different phylum. The high predominance of Veruccomicrobia (55.2%) and the low alpha-diversity of the gut microbiota of A. fragilis could be a reflection of the uniformity of its diet. Within the three lacertids, the microbiota of D. praticola and L. viridis were more closely related than to P. muralis. Considering the relative abundance of shared taxa, the relatedness between the microbiota of D. praticola and A. kitaibelii was high.

The core microbiota of lizard hosts seems to be species-specific. Sharing a largely common trophic resourse (all species except A. fragilis are mainly insectivorous) was not an indication of similarity in their gut microbial communities. Our research sheds light on the still understudied animal-associated microbial communities in free-living hosts.

P32: Investigating the effect of intercropping on the rhizosphere microbiome of cultivated lettuce

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Microbial diversity has been shown to have an impact on plant growth and health by improving nutrient uptake as well as increasing tolerance to biotic and abiotic stressors. Considering this aspect in agricultural systems could lead to higher crop yields and resilience. Intercropping, as opposed to monoculture, consists of simultaneously growing more than one crop in the same field. The effect of this practice on soil, rhizosphere, and root microbiomes was investigated in various cropping combinations, often determining higher microbial diversity and enhanced plant growth. The aim of this study was to explore the effect of intercropping on the microbial rhizosphere composition of Lactuca sativa var. capitate L. Rhizosphere samples were collected from fields with different cultivation methods, namely monoculture with and without mulching and intercropping with narrow or wide rows, at three different time points during the growth season. High-throughput sequencing of the 16S rRNA gene fragment and ITS region was applied to unravel the taxonomic structure of bacterial and fungal communities, respectively. The relative abundance of the bacterial and fungal communities was mainly influenced by the sampling time point and to a lesser extent by the treatment. Additionally, the Shannon diversity index of both intercropping treatments showed a significantly higher diversity at the first time point compared to the mulched monoculture.

However, a significant increase in microbial diversity was also observable in mulched monoculture across sampling time points. PERMANOVA of Bray-Curtis dissimilarities revealed that both collection time and cultivation system explain a similar proportion of the observed variance. Visualization of non-metric multidimensional scaling (NMDS) plots by sampling time point highlighted a clustering behaviour of samples belonging to different cultivation systems, showing a shift in the bacterial and fungal communities due to different treatments. In conclusion, the most evident effect of intercropping on microbial diversity was observed during the early cultivation season, but it was also observed that mulching increased microbial diversity over time, although determining distinct communities compared to intercropping.

P33: Phyllosphere microbiota composition of tomato plants treated with Prosystemin-derived peptide

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The employment of growth or defense inducers, commonly used in agriculture to preserve crop health, may have an impact on both structure and behaviour of plant microbial population. In this context, plant phyllosphere is a flexible and challenging habitat involved in a dynamic microbial selection. Recent findings suggest that the application of protein hydrolysates or plant hormones can shape plant immunity responses, positively impacting the leaf microbiota. It is still unclear what molecular processes are responsible for the selection or the recruitment of beneficial microbes, but many efforts have to be made.
In this direction, we explored the impact of an elicitor peptide derived from Prosytemin protein, notably involved in tomato defense responses against biotic stress, on the epiphytic community of tomato leaves. The phyllospheres of peptide- and mock-treated tomato plants (cv. San Marzano Nano) were analysed for their 16S rRNA communities. According to our findings, the microbiota of leaves changes after peptide treatment compared to control plants, with the main variations occurring in bacterial abundance. For instance, peptide-treated samples showed an increase in the genera Photobacterium and Psychrobacter, which are linked to features that promote plant growth or that are antagonistic to plant pathogens. The present exploratory study emphasizes the importance of using tools derived by nature, such as plant-derived peptides, to safeguard plant health on a variety of levels, including the promotion of beneficial plant-microbe interactions.

P34: Transfer of Tuber aestivum truffle ascocarp microbiome to ectomycorrhiza in inoculated seedlings – testing the concept.

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Truffles are the fruiting bodies (ascocarps) of fungi belonging to the genus Tuber, best known for their aromas which are potentially shaped by the associated bacteria and yeast from the surface (peridium) in interior (gleba) of their fruiting bodies. In our recent study, we correlated the outcome of aroma analysis with the bacterial and fungal communities (Grebenc et al. 2023). The same ascocarps as analysed for bacteria and yeast were subsequently used to inoculate sterile hazel (Corylus avellana) seedlings to form mycorrhiza under pot conditions. We hypothesize that at least a part of the truffle microbiome is being transferred to the “next generation” truffle mycelium through inoculation, mycelium growth and mycorrhization of the host plant. With our experimental setup we will test how much of the initial bacteria and yeast community remains as a stable part of the truffle mycorrhiza after two years from seedlings inoculation.

P35: Combination of hosts and geographic origin affect the differentiation in Fomes fomentarius species complex

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Focus of this study is the examination of a versatile fungus known for its wood-decomposing capacity, as well as its exploration of parasitic and endophytic functions within weakened broadleaved woods. Previous research and our preliminary data indicate the influence of the host tree on the phylogenetic. To gain insights into the phylogenetic relationships within the genus, using comprehensive ITS sequence data, we conducted an extensive host sampling and phylogenetic analysis, exploring the potential influence of both host and geographic distance on Fomes spp. Sporocarps were collected in Slovenia and nearby countries in the years 2010 - 2023 using purposive sampling to target a diverse range of potential hosts and geographic areas. First, we sampled sporocarps for preliminary nr rDNA ITS barcoding of isolates for the identity confirmation and subsequent whole genome sequencing and functional genomics. The identity analysis indicated that host species/genus groups enforce differentiation within the Fomes fomentarius species complex. We identified two distinct clades within the phylogenetic tree. The first clade primarily consists of host species such as Betula and Fagus. In the second clade, the dominant host species are Tilia, Populus, Carpinus, and Quercus. From phylogenetic network the “Fomes fomentarious” group is relatively distant from nearest available genotype by 54 mutation events, while the two groups within the complex are separated by 18 mutation events. Genotypes within each group are separated by no more than 4 mutation events with no clear further corelation with host tree species.

The length of branches between both clades suggests relatively low genetic divergence among the “Fomes fomentarious”, indicating their close evolutionary relationship. We conclude that the host tree and potentially their postglacial migration routes have an influence on the genetic diversity of individuals within the Fomes fomentarious species complex and no additional cryptic species were detected from the area of SE Europe.

P36: Composition and ecology of soil fungal communities in the Significant Landscape of Donji Kamenjak (Istria, Mediterranean Croatia)

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Fungal communities mediate numerous ecosystem processes, such as the decomposition of organic compounds in the soil and they participate in the cycling of essential elements. Donji Kamenjak, located in the southernmost region of Istria (Mediterranean Croatia) has undergone historical changes due to human activities, such as timber harvesting and cattle grazing, altering its landscape from holm oak forests to its current mosaic of forests, grasslands, garrigue, and maquis. Designated as a Significant Landscape on the Croatian national level, and protected under the EU Habitats Directive, Donji Kamenjak hosts a remarkable biodiversity. The aim of this study was to explore the diversity of fungal communities and their ecological traits in the soils of the different habitats of Donji Kamenjak.

Covering the whole area of Donji Kamenjak, 49 soil samples were collected at 20 cm depth. DNA was extracted and the ITS2 region of fungal rRNA gene was amplified and sequenced on Illumina MiSeq platform (2 x 300 bp).

Our results indicated that the diversity and composition of fungal communities differed across habitats. Notably, the agricultural areas showed higher fungal diversity when compared to other habitats.

From the dominant phyla (Ascomycota and Basidiomycota), most abundant genera found in all habitats were Penicillium, Inocybe, and Hygrocybe. Putative ecological traits assigned to fungal genera revealed the prevalence of saprotrophic and ectomycorrhizal fungi in all habitats, except in the garrigue-maquis, where saprotrophic fungi and mycoparasites were predominant.

These results contribute valuable insights into the ecological status and conservation value of various habitats within Donji Kamenjak.

P37: Wastewater Sludge Pretreatment By Hydrodynamic Cavitation

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Large quantities of wastewater sludge (WWS) that are produced during wastewater treatment need to be stabilized for safe use and disposal. The most common method used for sludge stabilization is anaerobic digestion (AD). Here, microorganisms break down organic matter in the absence of oxygen while also producing biogas, mainly CO2 and methane. Biogas production is driven by complex microbial communities of bacteria, archaea, protozoa, and fungi and can be divided into four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. As hydrolysis is the known limiting step in the AD process substrate pre/treatment various attempts were made to alleviate its influence. Hydrodynamic cavitation was identified as one of the few highly effective tools where cell walls of microorganisms and organic materials are damaged and broken down by mechanical, thermal, and chemical effects of exploding cavitation bubbles, i.e., high local temperatures and pressures with simultaneous OH* production; biofilm matrices, exopolysaccharides, intracellular matter, DNA, are partly released into the bulk liquid, which increases the soluble components (sTOC and sCOD) of substrates amenable for hydrolysis. The actual physical, chemical and interactive effects of shear-induced hydrodynamic cavitation on the mechanisms of AD process were studied to understand the mechanisms in relation to biomethane production.

Different cavitation regimes, achieved by altering rotor-stator geometry on a novel Rotary Generator of Hydrodynamic Cavitation (RGHC), were optimized by means of Computer Fluid Dynamics (CFD) [1], high-speed imaging, OH* production, temperature, pressure, and volume flow rate measurements. Effects of cavitation on sludge matrix, its integrity, cell wall damage, intracellular secretions, extracellular polymeric substances (EPS), dewaterability and its overall effect on AD process are currently investigated and evaluated by analysis of physicochemical parameters, characterization of rheological properties, biomethane potential tests, microbiome (distribution of microbial groups, functional genes, antibiotic resistance, virulence genes) and spectroscopic analysis of water-soluble substances (UV-VIS, ExEm).

P38: Gut Microbiome Response Chip (GMRC)

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Human gut microbiome is inherently linked to the vast amount of chemical and physical signatures that change over time and in space in response to variations in thermodynamic conditions of the environment. Developing a mechanistic understanding of the impact of intial conditions and their complex interplay on microbiome physiological responses and resulting chemical space (metabolomic signatures) in real time is therefore of crucial importance. In this study, we present the first version of our newly developed Gut Microbiome Response Chip (GMRC).

In the analytical example presented here the actual gut conditions of each individual donor were separated in fecal surface and core, challenged with diet constituents (organic acids, sugars, fats, mucus) and data recorded at a rate of 1 readout per minute for up to 48 h, including the analysis of chemical stability of the reporters. Ten chemical compounds were utilized per challenge in four replicates per fecal specimen. In-house routines were utilized to deconvolute the data into biologically relevant descriptive summaries such as lag phases, rates of change, attained levels of signals, signal to noise ratios and other, next to curve fits, data restructuring and other derivatives, making them highly amenable for machine learning (numerous algorithms, hyperparameter search, reporting statistics, biomarker constellation search).

In total, 230,400 datapoints were collected within the 10h experiment and subjected to data analysis.

The results show clear, reproducible and significant differences in microbiome responses to chemical challenges, characteristic of individual test subject. This testifies as a proof of concept that the tool provides sufficient resolution and reproducibility to serve as a starting point in precision medicine.

Different sets of chemical compounds can be used (i) to challenge human gut microbiome, (ii) to derive data on microbial activities in real time, will be further expanded with metabolomics information and streamlined for high-throughput processing. This will allow us to shed the light into clinical background by determining the level of toxicity of resulting metabolites and their associations with human metabolism, identify the range of most affected metabolic pathways involved in the Non-Communicable Diseases.