

# **BiomeFUN 2025**

From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia

15 - 19 September Belgrade, Serbia



#### INTERNATIONAL WORKSHOP

# "From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia" – BiomeFUN 2025

September 15 – 19, 2025

#### **Edition**

Collection of the Faculty of Biology ISSN 3009-3740 (Online)

#### **Online Edition**

https://microbiology.bg.ac.rs/2025-workshop/

#### **Publisher**

University of Belgrade - Faculty of Biology Studentski Trg 16, 11158 Belgrade, Serbia www.bio.bg.ac.rs

#### For publisher

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#### **Editors**

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#### Technical Editor & Cover design

Vojislav Simić

#### Printing:

University of Belgrade – Faculty of Biology

Circulation: 100 copies

All submitted abstracts are original scientific papers

#### **International Standard Book Number**

ISBN 978-86-7078-192-4

#### Digital Object Identifier

doi: 10.5281/zenodo.17073767

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University of Belgrade – Faculty of Biology
Belgrade, 2025

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#### SUPPORTED BY



Federation of European Microbiological Societies (FEMS)



Ministry of Science, Technological Development and Innovation

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### INTERNATIONAL WORKSHOP

From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia **BiomeFUN 2025** 

# BOOK OF ABSTRACTS

September 15 – 19, 2025 Belgrade, Serbia

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# Message from the scientific organizers

Dear colleagues and friends,

It is our great pleasure to welcome you to the BiomeFUN 2025 Workshop – "From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia", held in Belgrade, Serbia, from September 15 to 19, 2025.

The Workshop is organized by the University of Belgrade – Faculty of Biology, in collaboration with the Delft University of Technology – Faculty of Applied Sciences, Department of Biotechnology. We are proud to count on the generous support of the Federation of European Microbiological Societies (FEMS) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (NITRA). We thank them for recognizing the importance of this event and for enabling a broader participation through more than 20 travel grants awarded to selected applicants from low- and middle-income countries.

BiomeFUN 2025 gathers researchers, students, and experts from around the world to explore innovative approaches in microbiome research and practical applications in biotechnology. In modern microbial ecology, the integration of high-throughput sequencing technologies, bioinformatic analyses and predictive modeling plays a pivotal role in revealing the structure, function, and dynamics of complex microbial communities. The scientific programme of BiomeFUN2025 offers participants a glimpse into both theory and application of cutting-edge tools and strategies for achieving this integration, including: Analysis of 16S rRNA and shotgun metagenomics data; Functional genomics, profiling, and visualization; Basic and advanced stoichiometric, kinetic and thermodynamic modeling, including tools such as COBRA; Meta-genome scale modeling of microbial communities; Structure-function landscape modeling of synthetic consortia & Applications of machine learning in microbiome research.

Through lectures, hands-on sessions, and discussions, the Workshop provides participants with the opportunity to acquire broadly applicable analytical and computational skills, and deepen their understanding of microbial community dynamics, functional interactions, and predictive modeling.

We strongly believe that BiomeFUN 2025 is an excellent platform to exchange ideas, share experiences, and initiate new collaborations. An overwhelming response has been received to our call, with numerous talented applicants, more than 60 participants from 25 countries (Benin, Bosnia and Herzegovina, Brazil, Chile, Croatia, Denmark, Estonia, Finland, France, Germany, India, Iraq, Ireland, Italy, Latvia, Morocco, Norway, Serbia, Slovakia, Spain, Switzerland, The Netherlands, Turkey, United Arab Emirates, and the United States of America).

We encourage you to take full advantage of the scientific programme and networking opportunities, and to enjoy the beauty of Belgrade and the warmth of Serbian hospitality. We wish you an inspiring and productive Workshop and look forward to engaging discussions and joint projects that will emerge from this event.

Sincerely,



**Ivica Dimkić** Scientific & Organizing Committee Chairperson



**Djordje Bajić**Scientific & Organizing Committee
Co-Chairperson

### General information

#### **SYMPOSIUM VENUE**

The BiomeFUN 2025 Workshop will be held at the Hotel Palace 4\*, located in the center of Belgrade, offering modern facilities for both scientific sessions and networking activities. Address: Topličin venac 23, 11000 Belgrade, Serbia.

# REGISTRATION OF PARTICIPANTS

The registration desk will be open on Monday, September 15, from 08:00 to 09:00 in front of the main conference hall of the Hotel Palace. Updated information regarding workshop sessions and social events will be available at the registration desk throughout the event. All participants and accompanying persons are kindly requested to wear their accreditation badges during all scientific sessions and social activities.

#### **LANGUAGE**

The official language of the Workshop is English.

# SOCIAL EVENTS AND INFORMATION FOR POSTER PRESENTERS

The Poster Party with catering and drinks will take place on Monday, September 15, from 18:00 to 23:00 in the Hotel Palace (Hall Club of the Hotel Palace, Ground floor, Topličin Venac 23, Belgrade), offering an informal opportunity for participants to present their research and engage in networking. On the second day (Tuesday, September 16), from 18:10 to 18:20, a group photo will be taken in front of the monument to Vojvoda Vuk on Topličin venac square. On Friday, September 19, a free walking guided tour of the Belgrade Fortress & Kalemegdan Park is planned from 16:00 to 18:00, allowing participants to explore Belgrade's cultural and historical landmarks. Later the same day, the Closing Celebration with dinner, drinks and live music will be organized from 19:00 to 00:00, with detailed venue information provided at the registration desk.

#### FEMS MICROBIOLOGY ECOLOGY Editor-in-Chief: Max M. Häggblom





#### **CALL FOR PAPERS:**

#### Thematic Issue on Genomic Analysis and Functional Models in Microbiomes and Synthetic Consortia

#### **EDITORS:**

Ivica Dimkić (University of Belgrade - Faculty of Biology) & Djordje Bajić (Delft University of Technology)

FEMS Microbiology Ecology invites submissions to a thematic issue associated with the BiomeFUN2025 Workshop 'From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia', taking place September 15-19, 2025, in Belgrade, Serbia.

Website for more information: https://bgmicrobiomes.github.io/

This thematic issue will explore microbial communities related to the health and functionality of ecosystems and the development of sustainable alternatives in different economic sectors. The rapidly evolving field of microbial community ecology and biotechnology requires advanced computational and modeling approaches. To address the growing interest in microbial communities and to utilize the accumulating data — from genome sequencing, environmental studies and metagenomic datasets – advanced computational systems are essential to make sense of this complexity. These systems, which combine bioinformatics with predictive modeling, are at the core of modern efforts to rationally modify microbiomes to achieve specific functional goals. Genomic and metagenomic sequencing are providing unprecedented insights into

the composition and diversity of microbial communities. Recently, new statistical models such as structure-function mapping or various types of machine learning are being developed to predict community behavior and engineer synthetic communities with tailored functional properties.

FEMS Microbiology Ecology welcomes original research articles, mini-reviews, and perspectives that address these topics. All submissions should present complete and novel findings supported by robust experimental data or theoretical insights that contribute to the understanding of microbiomes and synthetic consortia. All manuscripts will undergo standard peer review by members of the Editorial Board and selected experts. Pre-submission inquiries may be directed to the Guest Editors or the Editor in Chief.

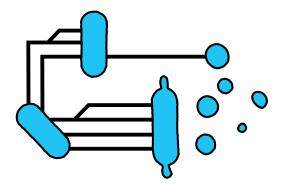
#### SUBMISSION TARGET DATE: 30 December 2025

Authors should specify in their cover letter that their paper is submitted for consideration for the 'Thematic issue on Microbiomes and Synthetic Consortia'. For instructions on submitting a manuscript, please consult FEMS Microbiology Ecology journal instructions at:

#### https://academic.oup.com/femsec/pages/instructions\_for\_authors

Accepted manuscripts will be published in regular issues of the journal upon acceptance, and the Thematic Issue will be compiled and made available online upon completion.





### **BiomeFUN 2025**

From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia

15 - 19 September Belgrade, Serbia









#### DAY 1: FUNDAMENTALS IN BIOINFORMATICS AND DATA PROCESSING

**MONDAY, SEP 15** 

Chairpersons: Marcel van den Broek & Milan Dragićević

- 8:00 9:00 Registration of participants
- **©** 9:00 9:30 Welcome and Orientation

Ivica Dimkić & Djordje Bajić

Introduction by organizers and workshop objectives

Branko Jovčić FEMS introduction

**©** 9:30 – 10:15 INVITED LECTURE: Introduction on Sequence Technologies, Common

Bioinformatic File Formats and BASH for Genomics

Marcel van den Broek

- **10:15 10:30** Preparation break for the practical session
- **Quality Control, and Filtering**

Marcel van den Broek, Katarina Kruščić

- **11:30 12:00** Coffee break
- **©** 12:00 13:00 Practical 1.1 (part II): Intro Command Line, Cloud, Intro Sequencing, **Quality Control, and Filtering**

Marcel van den Broek, Katarina Kruščić

13:00 - 14:00 Lunch
 14:00 - 17:00 Practical 1.2: Amplicon Data Analysis
 Milan Dragićević, Alfonso Esposito
 18:00 - 23:00 Poster Party with Catering and Drinks
 Opportunity for participants to present

#### DAY 2: GENOME AND METAGENOME ANALYSIS

their research and network

**TUESDAY, SEP 16** 

**Chairpersons:** João Pedro Saraiva & Alfonso Esposito

**©** 9:00 – 09:45 **INVITED LECTURE: Metagenomic Sequencing Approaches** for Microbial Communities João Pedro Saraiva **09:45 – 10:00** Preparation break for the practical session ♦ 10:00 – 11:30 Practical 1.3 (part I): Metagenomics Assembly Alfonso Esposito, João Pedro Saraiva **11:30 – 12:00** Coffee break ○ 12:00 – 13:00 Practical 1.3 (part II): Metagenomics Assembly Alfonso Esposito, João Pedro Saraiva 13:00 - 14:00 Lunch ♦ 14:00 – 14:45 INVITED LECTURE: Functional Annotation of Metagenomes: From Contigs to Community Function Prateek Shetty **14:45 – 15:00** Preparation break for the practical session 🐧 15:00 – 18:00 Practical 1.4: Functional Genomics and Data Visualization Prateek Shetty, Bogdan Jovanović

#### DAY 3: FUNDAMENTALS OF MODELING: FROM THEORY TO DATA

**18:10 – 18:20** Group photo in front of the monument to Vojvoda Vuk

on Topličin venac square

WEDNESDAY, SEP 17

Chairpersons: Alexandre Jousset & Djordje Bajić

<b>⑤</b> 10:00 – 10:45	INVITED LECTURE: Modelling Microbial Communities Across Scales to Understand Complexity and Guide Engineering Timothy Páez-Watson
10:45 - 11:00	Preparation break for the practical session
<b>⑤</b> 11:00 – 11:45	Practical 2.1 (part I): Introduction to Theoretical Models in Microbial Ecology and Python Practice Timothy Páez-Watson, Djordje Bajić
11:45 - 12:00	Coffee break
<b>⑤</b> 12:00 – 13:00	Practical 2.1 (part II): Introduction to Theoretical Models in Microbial Ecology and Python Practice Timothy Páez-Watson, Djordje Bajić
13:00 - 14:00	Lunch
<b>⑤</b> 14:00 – 14:45	INVITED LECTURE: From Kinetics to Stoichiometric Models in Metabolic Networks Karel Olavarria
14:45 - 15:00	Preparation break for the practical session
<b>5</b> 15:00 – 18:00	Practical 2.2: Practical Introduction to Metabolic Network Modeling Timothy Páez-Watson, Minke Gabriëls
	Timothy Paez-watson, Minke Gabriets
	ME AND METAGENOME-SCALE METABOLIC MODELING
THURSDAY, SEP	ME AND METAGENOME-SCALE METABOLIC MODELING
THURSDAY, SEP	OME AND METAGENOME-SCALE METABOLIC MODELING
THURSDAY, SEP Chairpersons: Ka	ME AND METAGENOME-SCALE METABOLIC MODELING  18  Invel Olavarria & Prateek Shetty  INVITED LECTURE: Thermodynamic and Kinetic Analysis of Metabolic Networks
THURSDAY, SEP Chairpersons: Ka	OME AND METAGENOME-SCALE METABOLIC MODELING  18  Trel Olavarria & Prateek Shetty  INVITED LECTURE: Thermodynamic and Kinetic Analysis of Metabolic Networks  Karel Olavarria  Preparation break for the practical session
THURSDAY, SEP Chairpersons: Ka    9:00 – 09:45  09:45 – 10:00  10:00 – 11:30	ME AND METAGENOME-SCALE METABOLIC MODELING  18  Invited Olavarria & Prateek Shetty  INVITED LECTURE: Thermodynamic and Kinetic Analysis of Metabolic Networks  Karel Olavarria  Preparation break for the practical session  Practical 2.3: Thermodynamic Analysis of Metabolic Networks
THURSDAY, SEP Chairpersons: Ka    9:00 – 09:45  09:45 – 10:00  10:00 – 11:30	ME AND METAGENOME-SCALE METABOLIC MODELING  18  Invited Olavarria & Prateek Shetty  INVITED LECTURE: Thermodynamic and Kinetic Analysis of Metabolic Networks  Karel Olavarria  Preparation break for the practical session  Practical 2.3: Thermodynamic Analysis of Metabolic Networks  Karel Olavarria, Minke Gabriëls
THURSDAY, SEP Chairpersons: Kd  \$\tilde{\Sigma}\$ 9:00 - 09:45  09:45 - 10:00  \$\tilde{\Sigma}\$ 10:00 - 11:30  11:30 - 12:00	INVITED LECTURE: Thermodynamic and Kinetic Analysis of Metabolic Networks  Karel Olavarria  Preparation break for the practical session  Practical 2.3: Thermodynamic Analysis of Metabolic Networks  Karel Olavarria, Minke Gabriëls  Coffee break  INVITED LECTURE: Resource Allocation and Dynamic Metabolic Modeling of Microbial Communities  Djordje Bajić

**14:45 – 15:00** Preparation break for the practical session

₫ 15:00 – 18:00 Practical 2.4 (part II): Resource Allocation and Dynamic Metabolic **Modeling of Microbial Communities** Djordje Bajić, Uroš Gojković

#### DAY 5: STATISTICAL APPROACHES AND AI IN MICROBIAL ECOLOGY

FRIDAY, SEP 19

Chairpersons: Juan Díaz-Colunga & Sascha Patz

INVITED LECTURE: Structure-Function Mapping for Microbial Consortia **5** 9:00 – 09:45 Juan Díaz-Colunaa **09:45 – 10:00** Preparation break for the practical session 🐧 10:00 – 11:30 Practical 3.1 (part I): Structure-Function Landscape Analysis and Prediction Juan Díaz-Colunga, Minke Gabriëls **11:30 - 12:00** Coffee break 🐧 12:00 – 13:00 Practical 3.1 (part II): Structure-Function Landscape Analysis and Prediction Juan Díaz-Colunga, Minke Gabriëls, Uroš Gojković **13:00 – 14:00** Lunch ♦ 14:00 – 15:00 INVITED LECTURE: Introduction to Machine Learning in Microbiome Science

Sascha Patz

**♦ 15:15 – 15:45 Closing Discussion** 

Course Board & Participants

Reflection on workshop outcomes, future directions in microbiome research, and opportunities for collaboration

16:00 – 18:00 Free Walking Tour: Kalemegdan Park and Belgrade Fortress

**19:00 – 00:00** Unforgettable Gala Dinner

Join us for live music, delicious food, Serbian drinks, engaging conversations, and a celebration of outstanding workshop achievements!

# **KEYNOTE LECTURERS**



#### KL1

# Scalable digital twin framework for microbiome engineering and functional prediction

Alexandre Jousset
Cybiome GmbH, Switzerland
contact: alexandre.jousset@cybiome.com

Microbiomes drive critical ecosystem functions from human health to agricultural productivity, yet translating descriptive microbiome data into actionable interventions remains a major bottleneck. Moving beyond characterization to manipulation requires predictive frameworks that can reliably forecast community responses to targeted perturbations. We developed a scalable digital twin framework that transforms standard amplicon metabarcoding data into predictive models for microbiome engineering. Our approach integrates phylogenetic inference with mechanistic community ecology models, enabling quantitative prediction of both individual taxa dynamics and emergent community-level functions. The framework generates auditable predictions by linking taxonomic composition to functional outcomes through validated ecological principles. We validated this approach across diverse environments including wastewater treatment systems, agricultural soils, and plant-associated communities. In wastewater applications, the model successfully predicted optimal microbial consortia for enhanced pollutant removal with 85% accuracy. For plant microbiomes, we demonstrated selective enhancement of disease suppressive functions while maintaining community stability. The framework identified keystone taxa whose targeted manipulation produced predictable cascading effects on community structure and function. Our digital twin approach bridges the gap between genomic analysis and functional applications by providing researchers and biotechnology companies with an accessible interface for rational microbiome design. We are currently developing user-friendly software implementation to democratize access to predictive microbiome engineering across academic and industrial settings.

**KEYWORDS:** Predictive microbiome engineering; Individual taxa dynamics; Emergent community-level functions; Digital twin approach



# INVITED LECTURERS



# Modelling microbial communities across scales to understand complexity and guide engineering

#### Timothy Páez-Watson

Delft University of Technology, Department of Biotechnology, the Netherlands contact: timothypaezw@gmail.com

Microbial communities are central to ecosystem function, biotechnology, and human health, yet their complexity poses challenges for prediction and control. Modelling provides a powerful lens to disentangle this complexity, offering frameworks to interpret interactions, anticipate dynamics, and ultimately design or engineer communities. Different modelling approaches capture distinct aspects of microbial systems, each with specific advantages and limitations. At the ecological level, conceptual models describe population-level changes over time based on simplified processes such as substrate uptake, growth, and mortality. These models highlight the role of competition and coexistence but remain limited in mechanistic depth. Moving toward more detailed descriptions, kinetic models of individual species integrate time-resolved parameters to quantify metabolic rates and interaction strengths, allowing stronger predictions of activity under dynamic conditions. Finally, genome-scale metabolic models offer mechanistic insights into intracellular processes, enabling the study of metabolic plasticity, niche specialization, and emergent community-level interactions through metabolite exchange. Results from my research and teaching across these modelling levels demonstrate how each scale provides complementary understanding. Simple ecological models emphasize general principles of stability and resilience, kinetic models capture functional responses and cross-feeding dynamics, and metabolic models uncover trade-offs in resource allocation and the metabolic flexibility of community members. Together, these perspectives reveal how combining modelling approaches can bridge scales, from abstract ecological interactions to molecular mechanisms, thereby informing strategies for microbial community engineering. In conclusion, understanding the contribution and limitations of each modelling layer is essential for making informed choices. Rather than applying all approaches at once, researchers can select the level of modelling that best aligns with the question at hand, ensuring both efficiency and relevance when studying or engineering microbial communities.

KEYWORDS: Microbial communities; Modelling; Ecology; Kinetics; Metabolism

# Practical session on shotgun metagenomics, assembly and binning

#### Alfonso Esposito

Department of Biotechnology, University of Verona, Italy contact: alfonso.esposito@univr.it

Recent computational and algorithmic advances have enabled the reconstruction of metagenome-assembled genomes (MAGs) directly from complex environmental sequence data. This process relies on a sophisticated pipeline involving co-assembly of metagenomic reads, mapping back to contigs, and binning—a critical step that considers both sequence composition (e.g., k-mer frequency, GC content) and differential coverage profiles across multiple samples to group contigs into putative genomes. Within the framework of the BiomeFUN 2025 workshop, we will provide a hands-on introduction to this bioinformatic workflow. Participants will be guided through a comprehensive analysis, beginning with quality control and trimming of raw reads, followed by co-assembly, read mapping for coverage evaluations, and binning of the contigs using up-to-date softwares. At the end, the resulting MAGs will be evaluated by estimating their genome completeness and redundancy (a proxy for contamination) and will be taxonomically annotated. Each student will be provided with a pre-configured Amazon Web Services (AWS) cloud instance and s/he will independently apply the pipeline to a dataset drawn from the Tara Oceans expedition. The expected outcome upon completion is that attendees will gain the practical expertise required to assemble, quality-check, and taxonomically annotate genomes from metagenomic data.

**KEYWORDS:** Next Generation Sequencing; Shotgun Metagenomics; Assembly; Binning of contigs; Taxonomic annotation

## Metagenomic sequencing approaches for microbial communities

#### João Pedro Saraiva

Department of Applied Microbial Ecology, Helmholtz Centre for Environmental Research – UFZ, Germany contact: joao.saraiva@ufz.de

Metagenomics has transformed our ability to interrogate complex microbial communities by bypassing the need for cultivation and directly sampling the collective genetic repertoire of environmental or host-associated microbiomes. Early efforts relied on targeted marker-gene surveys—most notably 16S rRNA amplicon sequencing—to infer taxonomic composition, but offered limited resolution into functional potential. The advent of whole-genome shotgun (WGS) sequencing in the early 2000s marked a paradigm shift: initial clone-library Sanger projects revealed rich pools of novel genes, but throughput and cost constraints restricted its use. The emergence of high-throughput, short-read platforms such as Illumina enabled deep WGS surveys across diverse habitats, catalyzing development of specialized assembly algorithms, binning strategies to reconstruct metagenome-assembled genomes, and annotation pipelines to predict metabolic pathways and ecological interactions. These innovations have illuminated microbial roles in biogeochemical cycles, human health and disease, and biotechnology. More recently, long-read and hybrid sequencing strategies have begun to resolve strain-level variation and complex genomic architectures, while single-cell and spatially resolved metagenomics enrich ecological context. Concurrent advances in machine learning-guided functional annotation and scalable graph-based assembly promise to deepen our understanding of community dynamics and function. As sequencing costs decline and analytic tools mature, WGS-based metagenomics is poised for real-time environmental monitoring, precision microbiome therapeutics, and the rational design of synthetic microbial consortia. To fully harness this potential, researchers must gain proficiency in experimental design, rigorous data generation protocols, bioinformatic workflows, and robust statistical interpretation—ensuring that metagenomic investigations yield accurate, reproducible insights into the microbial world.

KEYWORDS: Whole-Genome Shotgun; Sequencing; Functional potential

# Introduction on sequence technologies, common bioinformatic file formats and BASH for genomics

#### Marcel van den Broek

Department of Microbiology, Delft University of Technology, the Netherlands contact: marcel.vandenbroek@tudelft.nl

Advances in sequencing technologies have revolutionized modern biology, enabling high-throughput analysis of genomes, transcriptomes, and epigenomes. Next-Generation Sequencing (NGS) platforms generate vast quantities of raw data at high speed and low cost, producing millions of short DNA or RNA reads per experiment. To manage and interpret these data, standardized bioinformatic file formats have been developed. The FASTQ format stores raw sequence reads alongside quality scores, while FASTA provides reference sequences without quality information. Alignment results are typically stored in SAM/ BAM, which balance human readability and computational efficiency. Downstream analyses often rely on formats such as VCF for genetic variants and GFF/GTF for genome annotations. Efficient handling of these files is essential, as datasets frequently scale to hundreds of gigabytes. In this context, the Bash shell plays a central role in bioinformatics workflows. Bash provides a flexible command-line environment for automating repetitive tasks, integrating specialized tools, and managing large-scale data processing. Common operations include file manipulation, text processing with utilities such as grep, awk, and sed, and the orchestration of pipelines through shell scripting. By chaining commands with pipes and redirects, researchers can construct reproducible workflows that efficiently process sequencing data from raw reads to biological insights. Together, sequencing technologies, bioinformatic file formats, and Bash-based computational workflows form the backbone of modern genomics. Mastery of these elements empowers researchers to transform raw sequencing output into meaningful biological interpretations, accelerating discoveries in medicine, agriculture, and evolutionary biology. This is an example of a one page abstract fitting to the needs for publishing in the collection of abstracts. Please do not forget to delete the instructions for writing the abstract.

KEYWORDS: Illumina; Nanopore; Fastq; BASH; Linux

# Functional annotation of metagenomes: from contigs to community function

#### Prateek Shetty

JSMC Postdoctoral fellow, Friedrich Schiller University Matthias Schleiden Institute, General Botany, Am Planetarium 1, 07743 Jena, Germany contact: prateekshettys@gmail.com

Soil microbiomes represent Earth's most complex microbial communities and serve as critical reservoirs of microbial diversity for crop plant associations. Understanding their functional potential is essential for sustainable agriculture. This workshop module provides a comprehensive pipeline for transforming assembled metagenomic contigs into meaningful functional profiles specifically tailored for soil systems. We present an integrated annotation framework that combines structural gene prediction using Prokka with comprehensive functional characterization through eggNOG-mapper. Participants will learn to leverage the complementary strengths of three major annotation systems; i) KEGG Orthology (KO) for pathway reconstruction, ii) Clusters of Orthologous Groups (COG) for evolutionary classification, and iii) Gene Ontology (GO) for detailed functional themes. The theory part of the workshop also features three carefully selected case studies from recent agricultural research, allowing participants to examine real-world applications, understand methodological decisions, and critically evaluate the benefits and limitations of different approaches. These examples demonstrate how to adapt established pipelines for specific research questions. The practical session provides hands-on experience with command-line annotation tools, R-based statistical analysis for functional abundance data, and strategies for biological interpretation of complex soil metagenomes. Special emphasis is placed on addressing soil-specific challenges, including the strategic use of specialized databases such as CAZy for carbohydrate metabolism and CARD for antimicrobial resistance genes. By bridging the gap between unannotated contigs and ecosystem-level understanding, this module equips researchers with robust methodologies to derive functional insights from soil/plant associated microbiomes.

**KEYWORDS**: Metagenomics; Functional annotation; Soil microbiome; Agricultural systems; Pathway reconstruction

#### Thermodynamic and kinetic analysis of metabolic networks

#### Karel Olavarria

Department of Bionanoscience, Delft University of Technology, the Netherlands contact: k.olavarriagamez-1@tudelft.nl; kogamez@gmail.com

The increasing availability of DNA/RNA/protein sequences, along with the proliferation of tools for analyzing this data, provides a wealth of information. However, this information is often fragmented and static. To connect these data and understand temporal changes in microbial populations, the study of the enzyme-catalyzed reaction rates is critical. Microbial growth, substrate consumption, and product generation (in cultures or isolated communities of single species) are governed and limited by these rates. These rates can be determined by studying isolated enzymes, networks of enzyme-catalyzed reactions, or whole organisms. At the same time, these rates are limited by chemical and thermodynamic laws. The purpose of the lectures and exercises in this section is to review the most important kinetic and thermodynamic laws connecting enzyme activity, metabolic fluxes, and microbial growth. Given the large number of parameters involved in these processes, the use of computational models is fundamental.

**KEYWORDS:** Rate equations; Haldane's relationship; Flux-force relationship; Enzyme cost

#### **ACKNOWLEDGEMENT**

Dutch Organization for Scientific Research (project BBE.2017.013) and the Sao Paulo Research Foundation (project 2017/50249-6); and Gravitation Grant (project 024.002.002) from the Dutch Ministry of Education, Culture and Science.

#### Introduction to machine learning in microbiome science

#### Sascha Patz

Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Braunschweig, Germany contact: sascha.patz@julius-kuehn.de

Artificial intelligence (AI) has reshaped many scientific disciplines, including medicine, ecology, engineering, and microbiome science, among many others. The complexity and scale of microbiome data demand approaches that can go beyond conventional statistics, making machine learning (ML) and deep learning (DL) indispensable tools for advancing our understanding of host–microbe systems. This lecture introduces the basics of ML in microbiome research, with a strong focus on both technical advancements and biological insights enabled by AI-driven approaches. We will explore how ML and DL support key analytical tasks such as pattern recognition, feature selection, and predictive modeling. Examples will illustrate applications across human and plant microbiomes, from taxonomic profiling and functional prediction to microbial interaction networks, metabolic modeling, and precision interventions for health and environmental resilience. In particular, attention will be given to the choice of data transformations and models, strongly shaping feature importance and downstream predictions of microbiome – phenotype relationships, and to multi-omics data integration. At the same time, the session will address current challenges. Microbiome data are inherently compositional, sparse, and high-dimensional, which raises concerns about generalizability, fairness, and reproducibility of AI-based models. We will discuss the impact of method and data selection on model performance, the limitations imposed by cohort size and diversity, and strategies to mitigate bias and improve interpretability. By spanning topics from ecological community profiling to predictive applications in precision health and agriculture, this lecture aims to provide a balanced perspective on both the promise and pitfalls of ML and DL in microbiome science. Participants will gain a conceptual roadmap for AI integrations into their own research, as well as a comprehensive insight into the factors that ensure reliable and biologically meaningful discoveries in this rapidly advancing field.

KEYWORDS: Microbiome; Machine and deep learning; Data transformation; Model selection; Challenges

#### Structure-function mapping for microbial consortia

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Microbial communities carry out many important functions in both natural and biotechnological settings, such as nutrient cycling in the soils, food fermentation, or biofuel synthesis in industrial biorefineries. Our ability to rationally design communities that optimize these functions is limited by the enormous complexity of microbial interactions: The contribution of a particular species to the function of a community often depends on the presence or absence of additional community members, owing to multiple molecular, physiological, and/or population-level mechanisms of interaction between species. This work demonstrates that, despite this potential complexity, the function of a community is often predictable using very simple statistical models. These models mirror the patterns of global epistasis reported in quantitative genetics, which allow us to predict the fitness/phenotypic effect of a mutation despite the potential complexity of the interactions between genetic components. This observation represents a crucial step towards unifying the task of predictively linking biological structure and function across scales: from molecules and organisms to entire communities. From a practical standpoint, these results illuminate a new path for the rational design of microbial communities that optimize a variety of biotechnologically-relevant functions.

**KEYWORDS:** Synthetic microbial communities; Microbial interactions; Community function; Global epistasis

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#### Microbial community analysis using amplicon sequencing

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Amplicon sequencing has become a cornerstone technique in microbial ecology, enabling high-resolution profiling of complex communities. This workshop introduces participants to the principles and practical implementation of 16S rRNA gene sequencing using the Illumina MiSeq platform, with a focus on amplicon sequence variant (ASV) inference via the DADA2 pipeline. Participants will learn to process raw FASTQ files, remove primers using cutadapt, filter and trim reads, model sequencing errors, and infer true biological variants. The workshop emphasizes the statistical rigor of DADA2's self-consistent error modeling and partitioning, and guides users through taxonomy assignment using the IdTaxa classifier trained on GTDB reference data. Integration with the Phyloseq package enables downstream ecological analysis, including diversity metrics, taxonomic aggregation, and phylogenetic tree construction. The training uses real-world data from *Drosophila melanogaster* and *D. subobscura*, providing a biologically relevant context for hands-on learning. By the end of the session, participants will be equipped to perform robust microbial community analysis and interpret results with confidence.

KEYWORDS: Amplicon sequencing; DADA2; Microbial ecology; 16S rRNA; Phyloseq

#### **ACKNOWLEDGEMENT**

We thank the GTDB and NCBI BioProject PRJNA616141 for providing reference data and sequences used in this workshop.

# Resource allocation and dynamic metabolic modeling of microbial communities

#### Djordje Bajić

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Microbial communities colonize nearly every habitat on earth, extracting energy from their environments through myriad metabolic strategies. They are also becoming increasingly interesting as a sustainable alternative across industry and agriculture, for example as bio-fertilizers and bio-pesticides. Despite a growing accumulation of empirical data, a systematic mechanistic understanding of how metabolism and environment interact to shape the composition and function of microbial communities remains elusive. Stoichiometric modeling of microbial metabolism has proven a powerful tool for predicting the behavior and physiology of microorganisms from their genomic data. Metabolic models also hold great potential as a tool for understanding predictively and mechanistically the metabolic interactions that structure multi-species microbial communities and determine their function. In this workshop, I will explore the application of metabolic models to microbial communities by introducing two core topics. First, I will explore the concept of global resource allocation constraints (e.g. related to proteome or limited membrane surface). I will show how these constraints can be ncorporated into metabolic models, enhancing their physiological realism and leading to better predictive capabilities. Second, I will introduce the technique of dynamic flux balance analysis, a popular tool that allows us to use stoichiometric modeling of metabolism to simulate multi-species communities. I will discuss state of the art applications of these models and give participants the computational tools to apply them independently in their research. To conclude, I will discuss the potential of these modeling techniques for addressing fundamental questions across microbial ecology, as well as help for engineering communities with desired functions in many fields of application.

**KEYWORDS:** Metabolic modeling; Metabolic resource allocation; Computational biology; Microbial ecology

# PRACTICAL LECTURERS



#### PL<sub>1</sub>

# Functional characterization of top-down community assembly in a simple environment

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Microbial communities are ubiquitous, and they perform essential functions that have shaped the world as we know it. Functions that range from biogeochemical processes to supporting the function of associated hosts. Besides that, we have a rising number of synthetic microbial consortia that are designed and used for targeted applications across medicine, environmental engineering, food and beverage industries and biotechnology. All of these functions depend on the composition of the community. However, the lack of a predictive understanding of community assembly hinders our ability to engineer these communities for desirable outcomes. This research aims to develop a predictive understanding of microbial community assembly processes, enabling the manipulation of microbial communities to optimize their functional outputs. Using a top-down approach, we conducted sequential batch experiments in bioreactors. Bioreactors allow us to precisely characterize community dynamics at high functional resolution, providing insights into the reproducible and complex metabolic activities that stabilize community structure. By starting with a highly diverse sample and looking deeply into the processes that shape it towards a stable community we can reveal a general rule that can be later applied in various fields. Preliminary results reveal significant shifts in metabolic profiles and microbial composition across experimental runs, highlighting the complexity of microbial dynamics.

This research demonstrates that microbial community assembly is governed by complex, reproducible metabolic principles. By integrating bioreactor experiments with high-throughput analyses and simulations, it aims to develop a predictive framework for engineering microbial ecosystems across diverse applications.

**KEYWORDS:** Microbial community; Top-down community assembly; Microbial ecology; Community dynamics

#### **ACKNOWLEDGEMENT**

We gratefully acknowledge Delft University of Technology for funding this PhD research, and the UNLOCK project for providing access to state-of-the-art laboratory infrastructure that made this work possible.

#### PL<sub>2</sub>

# Emergent patterns in mechanistic metabolic models of microbial community assembly

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Microbial communities play an essential role in the functioning of most global ecosystems and they have myriad applications across biotechnology and environmental science. Both in nature and in the laboratory, the diversity of microbial communities exhibits simple emergent patterns. For example, communities often converge towards a similar functional composition when assembled in similar environments. Coexistence within microbial communities has also been shown to be an emergent property of their diversity, as often species that coexist within diverse communities fail to coexist in isolated pairs. Although it seems clear that these patterns emerge from the complex and dynamic network of interactions structuring microbial communities, it is still unclear precisely how. Computational models of microbial metabolism represent a promising theoretical tool for quantitatively describing how these emergent patterns in microbial communities arise from the mechanistic complexity underlying their assembly. We use dynamic Flux Balance Analysis to assemble hundreds of enrichment communities in silico starting from diverse pools of "core" metabolisms. These metabolic networks, generated by simulating reductive genome evolution in different environments, attempt to statistically simulate the natural metabolic diversity of microbes. The resulting stable in silico communities reproduced many of the patterns observed in empirical microbial communities, such as convergent composition in similar environments or emergent patterns in species coexistence. Our simulations further show that community diversity leads to a progressively richer carbon source environment, suggesting a mechanistic explanation for the "diversity begets diversity" hypothesis. Our simulations allow us to explore how stoichiometric and eco-evolutionary constraints shape the properties of microbial communities.

**KEYWORDS:** Microbial communities; Community Assembly; Metabolic modelling

#### PL3

# Seasonal dynamics and agricultural management of soil bacterial communities across distinct soil types

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Soil health, composition, and microbial activity are crucial for sustainable agriculture and ecosystem functioning. Here we investigated how microbial communities respond to seasonal variation and agricultural management. Soil samples were collected in spring and summer from five locations, comparing nutrient-depleted and nutrient-overloaded soils. Microbial composition was assessed using the QIIME2 platform with the RESCRIPT plugin and the IDTAXA classifier in R to achieve improved taxonomic accuracy. The results showed that alpha diversity at the ASV level did not differ significantly between sites, while beta diversity analyses showed clear clustering by soil type and sampling location rather than by nutrient treatment or season. Sandy loam soils (SL) had the lowest relative abundance of Gaiella, Solirubrobacter, and Nitrososphaera, while clay and sandy clay (C/SC) soils displayed the highest abundance of Nitrososphaera. Light clay soils (LC) showed consistently higher abundances of Gaiella, Solirubrobacter, Vicinamibacter, and Blastococcus, regardless of the season. Differential abundance analysis highlighted substantial microbial shifts between soil types, and identified 233 significantly different taxa between SL and LC soils, 135 between SL and C/SC soils and 206 between LC and C/SC soils (FDR-adjusted p < 0.05). Among many others, LC soils were consistently enriched with genera such as Microbacterium, Lysobacter, Acidibacter, Priestia, Bacillus, Chryseobacterium, Novosphingobium, and Parviterribacter. Contrary, C/SC soils showed higher abundances of Pseudalkalibacillus, Vallicoccus, Rhizobacter, Ellin6055, Metabacillus, Flavobacterium, Allostreptomyces, and Brevibacillus, reflecting the influence of higher clay content, neutral pH, and increased moisture retention. SL soils were enriched in Acinetobacter, Cutibacterium, Caedimonas, and Pseudomonas, likely due to their sandier texture, improved aeration, and lower water-holding capacity. Overall, these results suggest that soil type has a stronger influence on bacterial community composition than nutrient status or seasonal variation, emphasising the central role of edaphic properties in shaping microbial ecosystems and pointing to the robust and adaptive nature of soil bacteriobiota.

**KEYWORDS:** Microbial ecology; Soil health; Agricultural management; Seasonal variation; Edaphic factors

#### **ACKNOWLEDGEMENT**

This research was supported by the Science Fund of the Republic of Serbia, PRIZMA, #GRANT No. 7455, TERRA\_MADRE and Ministry of Science, Technology and Innovation of the Republic of Serbia; #Grant Nos. 451-03-135/2025-03/200178 and 451-03-136/2025-03/200178.

#### PL4

# metaRNAseqEffect: A mixed-effects model framework for RNA-Seq meta-analysis

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The vast number of RNA sequencing (RNA-seq) studies available in public repositories creates an opportunity for integrative analysis across datasets. Meta-analysis of RNA-seq results can increase statistical power, improve reproducibility, and reveal consistent molecular signatures that may be obscured in individual studies. However, most existing approaches to RNA-seq meta-analysis rely on combining  $\rho$ -values, which has important limitations. These approaches neglect effect sizes, do not fully capture the direction of change across studies, and provide little insight into study-to-study heterogeneity. We present metaRNAseqEffect, a novel R package for RNA-seq meta-analysis that focuses on effect sizes rather than p-values, by integrating log2 fold changes and their variances across datasets using mixed-effects meta-analysis models. This strategy directly quantifies biological effects while also providing estimates of confidence intervals and heterogeneity metrics, allowing assessment of both the magnitude and consistency of gene expression changes. Because each dataset is analyzed independently before integration, the method avoids batch-effect correction issues and remains robust across diverse experimental designs. The framework is universally applicable, supporting both eukaryotic and prokaryotic transcriptomic datasets, and is compatible with standard RNA-seq analysis pipelines. Beyond gene-level integration, meta hits can be subjected to pathway- or gene set-level enrichment analyses, facilitating biologically meaningful interpretation. By adapting well-established statistical methodology to high-throughput transcriptomics, this approach provides a rigorous and versatile tool for integrative RNA-seq studies. It has the potential to uncover robust, reproducible expression signatures and to advance our understanding of complex biological systems.

**KEYWORDS:** RNA-seq; Meta-analysis; Mixed-effects models; Transcriptomics; Functional genomics

# GRANT AWARDEES & POSTER PRESENTERS



# Effect of processing variations on the microbiota and quality characteristics of maize starch for traditional ogi production in Benin

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Traditionally fermented foods often suffer from inconsistent quality related to uncontrolled processing conditions, variations in processing technology and unpredictable composition and contribution of the microbial species present. Fermented maize starch called "ogi" in Benin, and popular in West Africa, is an intermediate food product suffering such inconsistency. Significant differences were found in the microbial composition and functional properties of the oqi as a result of technological processing variations. However, little is known about how these variations affect the characteristics of maize starch before fermentation. To fill this gap, our study evaluated the physicochemical characteristics, volatile organic compounds and microbial communities of maize starch samples from 11 production processes, each of them characterised by specific processing variations discriminating the traditional maize ogi technological variants found in Benin. Results showed significant connections between processing variations, abundance of distinct microbial groups and maize starch quality variability. Some processes led to samples with low pH values (4.4 - 4.5) and high lactate concentrations (5.5 - 19.6 mM), dominated by lactic acid bacteria. The samples with high pH values (5.9 - 6.4) and low lactate concentrations (0 - 2mM) were dominated by Enterobacteriaceae. Other processes induced high concentrations of butanoic acid (66.9 - 89%), correlated with a high abundance of undesirable bacteria from the family Clostridiaceae. A closer look at the composition of the bacterial communities of replicated samples from every production process showed little to high variability in the abundance of certain bacterial families, e.g., Streptococcaceae varying between 3.8, 12 and 49% in the biological replicates from a same process. This suggests the existence of cofounding factors causing the variability in maize starch and ogi quality. In-depth investigation is needed by determining and evaluating all the biotic and abiotic factors that may affect maize starch quality in maize ogi production process.

KEYWORDS: Processing technology; Cereal starch; Microbial community; Bacteria; Lactic acid fermentation

#### **ACKNOWLEDGEMENT**

Funding: INREF - Wageningen University and Research. Contributions: Stefano Renzetti, Jolanda Henket, J. C. M. Wolkers-Rooijackers

### GR<sub>2</sub>

# Microbial functional ecology in African soils: responses to microplastics and environmental stressors in agricultural systems

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My research lies at the intersection of soil microbial ecology, environmental sustainability, and agricultural resilience, with a strong geographical focus on Morocco and the broader African context. I investigate how soil microbial communities contribute to key ecosystem functions such as nutrient cycling, soil fertility, and plant health, particularly under the pressures of climate change, land degradation, and emerging agricultural pollutants. A growing focus of my work involves exploring the ecological impacts of microplastics in agroecosystems. These pollutants, increasingly prevalent due to plastic mulching and agrochemical packaging, pose unknown risks to soil microbiota and soil function. I am currently investigating how microplastics influence microbial diversity, community structure, and functional traits, and how microbial communities adapt or respond to such persistent contaminants. This research aims to provide a microbial-based understanding of plastic pollution in soils, an underexplored issue in African agricultural systems. I employ a combination of metagenomic and metatranscriptomic analyses, ecological modeling, and bioinformatics to link microbial diversity with function. My research also explores the development of functional and predictive models to assess the resilience and response of soil microbiomes to environmental disturbances, with the goal of informing sustainable land-use practices. Rooted in local challenges but globally relevant, my work contributes to developing data-driven, microbiome-informed strategies for improving soil health, enhancing agricultural productivity, and mitigating the impact of pollutants in Africa's vulnerable ecosystems. Ultimately, I aim to bridge microbial ecological theory with applied solutions to advance climate-resilient and pollution-aware agriculture in Morocco and across the continent.

KEYWORDS: Microbial ecology; Microplastics; Agroecosystems; Metagenomics; Soil health

#### **ACKNOWLEDGEMENT**

I would like to express my sincere gratitude to OCP Group for their support of this research.

### GR<sub>3</sub>

### Genomic insights into *Klebsiella* from hospital wastewater: antimicrobial resistance and virulence factors

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Hospital wastewater is a recognised source of antibiotic resistance genes (ARGs) and resistant bacteria, contributing to their release into the environment. Within the One Health framework, Klebsiella spp. are considered high-priority pathogens due to their capacity to carry extended-spectrum β-lactamases (ESBLs) and carbapenemase genes. This study aimed to characterise Klebsiella spp. from untreated (UWW) and hospital wastewater treated with a combination of ozone and UV (TWW), with a focus on resistance and virulence genes, biofilm formation, and persistence after treatment. Samples were collected from the Cancer Centre at Ullevål Hospital and cultured on selective media for ESBL and carbapenemase-producing bacteria. (KPC). Klebsiella spp. were identified using MALDI-TOF MS, and 35 isolates were selected to be tested for antibiotic susceptibility by disk diffusion and for biofilm formation using microtiter plates. Resistance and virulence genes were analysed using PCR and whole-genome sequencing (Illumina). Sequence typing, as well as identification of virulence and ARGs, was performed using Kleborate (v2.4.1). Moreover, genomes of K. pneumoniae ST147 were run on a phylogenetic analysis, using the core genome track of ALPPACA. A total of 35 Klebsiella isolates were analysed, including K. pneumoniae, K. oxytoca, and  $\it K.\ michiganensis.$  All isolates carried multiple resistance genes, including  $\it bla_{\rm OXA}$  and  $\it bla_{\rm CTX-M15}$  and 63% of  $\it K. pneumoniae \, harboured \, bla_{\scriptscriptstyle {
m NDM}}. \, Most \, \it K. pneumoniae \, belonged \, to \, ST147, \, a \, high-risk \, clone. \, Virulence \, was$ generally low; however, the siderophore gene yersiniabactin was found in 37% of isolates, and several isolates demonstrated strong biofilm-forming ability. Notably, some clones survived combined ozone and UV treatment, suggesting potential for post-treatment regrowth. Multidrug-resistant and potentially virulent Klebsiella spp. were detected in both treated and untreated hospital wastewater. Their presence, even after ozone and UV treatment, highlights the need for targeted monitoring and the development of effective hospital-specific wastewater treatment strategies within the One Health approach.

**KEYWORDS:** Bacteria genomes; Resistome; Virulome; Gene prediction

# Genomic and functional adaptations to anthropogenic environments in *Yarrowia lipolytica*

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Yarrowia lipolytica is non-conventional yeast increasingly used as a microbial chassis in various industrial processes, from food additives to biofuels. While its biotechnological potential is well recognized, its ecological roles and evolutionary dynamics in natural and human-associated environments remain underexplored. In this study, we integrate whole-genome sequencing and high-throughput phenotyping across a globally representative collection of strains to uncover patterns of intraspecific diversity in Y. lipolytica. Our results reveal distinct genetic lineages tightly linked to different anthropogenic environments. In particular, the Eur2/Dairy population — dominated by strains from dairy products — and the So-HC population — from soils and hydrocarbon-polluted sites—exhibit clear genomic signatures of adaptation, coupled with specialized phenotypic traits. These adaptations likely reflect selective pressures imposed by human-driven environments, shaping metabolic versatility and ecological preferences. The identified lineages provide a window into the evolutionary responses of yeast populations to human influence, offering insights relevant to both microbial ecology and applied biotechnology. Building on this framework, our data has also enabled the identification of key traits of biotechnological interest, such as lipid accumulation, along with the strains exhibiting the best performance in this regard. In upcoming work, we aim to further optimize this trait by exploring the combinatorial effects of four carbon and four nitrogen sources, each previously shown to individually modulate lipid accumulation. Participating in this workshop will be instrumental in developing the skills needed to integrate our existing genomic dataset with the large-scale phenotypic data to come. Additionally, the solid theoretical background offered by the course will represent a significant step forward in my thesis project and help consolidate the direction of my early research career.

KEYWORDS: Yarrowia lipolytica; Population genomics; Yeast ecology; Niche adaptation

#### **ACKNOWLEDGEMENT**

Sergio Izquierdo-Gea acknowledges his predoctoral grant FPU21/06830 funded by the Spanish Ministry of Science, Innovation and Universities.

## Microbial profiling of trout skin and eggs: associations with Saprolegnia parasitica infection

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Saprolegniosis, caused by Saprolegnia parasitica, presents a major threat to salmonid aquaculture, resulting in significant economic losses in fish farms and hatcheries. Saprolegniosis can adversely affect all life stages of fish, from eggs to adults. Previous research has demonstrated the importance of host-associated microbiomes in pathogen defence across various systems. However, there is limited knowledge about how S. parasitica infection influences microbial diversity and community structure on the external surfaces of salmonids. This study addresses this gap by investigating bacterial community changes associated with S. parasitica infection, particularly on trout skin and egg surfaces. Microbial samples were collected from injured and healthy trout (N = 12) across three fish farms in Croatia and from laboratory-infected trout eggs (N = 12). High-throughput sequencing of the V4 region of the 16S rRNA gene revealed that microbial diversity was significantly reduced on infected eggs, indicated by lower Pielou's evenness and Shannon's diversity indices. Taxonomic analysis showed a notable decline in some bacterial genera, i.e. Pseudomonas and Flavobacterium, on infected egg surfaces. Compared to Acinetobacter and Janthinobacterium which were increased in abundance on infected eggs, while Aeromonas was prevalent on injured trout skin. Further, beta diversity metrics demonstrated that both infection status and sampling location significantly influenced microbial community structure. These findings suggest that infection with S. parasitica leads to microbial dysbiosis, altering both the composition and diversity of host-associated bacterial communities. However, since sampling location significantly influenced microbial community structure, trout skin and egg microbiome is also shaped by environmental factors. Overall, the results indicate that S. parasitica infection disrupts the native microbial communities on trout skin and eggs. These insights could help to develop microbiome-targeted strategies for disease prevention and control in aquaculture systems.

KEYWORDS: Saprolegniosis; Trout; Aquaculture; Host-associated microbiome

### GR<sub>6</sub>

## The impact of raw and extruded dog diets on canine gut microbiota and pathogen carriage

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Globally, zoonotic diseases cause millions of cases of illness and death in humans. There are various routes of transmission for zoonotic pathogens, including contact with pet food. Both conventional pet foods, such as dry, wet, and semi-moist diets, and non-conventional diets, such as raw food, have the potential to become contaminated with pathogenic bacteria like Salmonella and Listeria monocytogenes, acting as vehicles for their transmission. Understanding the structure and function of microbial communities in companion animals is essential for improving both animal and public health since the interaction of gut microorganisms can favor or inhibit the colonization of pathogens. Pet gut microbiota is shaped by multiple factors, including age, sex, health status, physical activity and diet, among others. As unconventional diets, such as raw meat-based diets (RMBDs), gain popularity among pet owners, there is a growing need to investigate how these new feeding practices might impact the microbial composition and pathogen carriage of pets. In this study, we applied 16S rRNA gene sequencing to characterize the fecal microbiota of clinically healthy dogs fed either commercial extruded diets (n = 14) or RMBDs (n = 10) and explored the impact that pathogen carriage might produce on the fecal microbial composition of dogs. Our results showed significant differences in alpha and beta diversity between the dietary groups. Dogs consuming RMBDs harbored more diverse microbiotas, enriched with less common taxa, compared to those fed extruded diets. Importantly, within the raw-fed group, six dogs were identified as carriers of the zoonotic pathogens Salmonella and Listeria monocytogenes. Pathogen-carrying dogs exhibited distinct shifts in microbiota composition compared to raw-fed dogs that did not carry pathogens. These findings suggest that pathogen carriage may alter host-associated microbial communities, even among animals consuming the same diet.

KEYWORDS: Canine fecal microbiota; Pathogens; Pet food; Raw meat-based diets; Extruded diets

# Insights on the role of the microbial communities in plant responses to biotic and abiotic stresses

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Plant-associated microbial communities are recognized as integral components of plant health and resilience, representing a valuable resource for developing sustainable alternatives to chemical inputs, with potential applications in crop productivity and disease resistance. This abstract provides a synthetic overview of multiple studies using high-throughput amplicon sequencing to explore microbiome dynamics in two key Mediterranean crops: citrus and tomato (Solanum lycopersicum), under abiotic and biotic stress conditions. In citrus, root-associated microbiota of two rootstocks (Bitters and Carrizo Citrange) subjected to water and salinity stress showed distinct community responses, with bacterial profiles shaped by both genotype and stress, while fungal communities responded primarily to stress. Microbial network analysis identified rootstock-specific bacterial taxa as central nodes, suggesting possible roles in host adaptation. The effect of Plenodomus tracheiphilus, the causal agent of Citrus Mal Secco, a destructive disease occurring in the Meditarranean basin, was assessed in controlled infection trials on sour orange seedlings. The plant microbiome of rhizosphere, endosphere and xylem revealed a compartment-specific impact of pathogen inoculation, exhibited shifts in composition, diversity and structure. Tomato studies focused on the functional potential of a core microbiome-derived SynCom composed of ten bacterial strains. Genomic screening identified traits linked to growth promotion, stress mitigation, and plant colonization. Subsequent trials evaluated the performance of selected SynComs in tomato plants challenged with Xanthomonas euvesicatoria pv. perforans (Xep), the causal agent of Bacterial Spot. Syn-Com treatments induced consistent changes in microbial community composition. RNA-Seq analyses of leaf samples at multiple time points (12 hpi, 3 dpi, 6 dpi) showed that Xep strongly reprogrammed host gene expression, particularly in pathways related to plant-pathogen interaction, hormone signaling, and photosynthesis. Collectively, these findings underscore the relevance of an integrated microbiome-based approach to understand plant-microbe-environment interactions, paving the way for the development of microbial inoculants for specific crops and stress conditions.

**KEYWORDS:** Amplicon-based metagenomics; SynComs; RNA-Seq; Citrus; Tomato

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# Diversity of psychrophilic bacteria isolated from cold alpine lakes in High Tatras, Slovakia

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Psychrophilic bacteria are adapted to thrive at low temperatures through specialized cellular mechanisms, including the production of cold-active enzymes, antifreeze proteins and modified membrane lipids. These traits make them promising candidates for biotechnological applications in cold-chain industrial processes, biodegradation of pollutants in cold ecosystems and bioremediation of contaminated areas under low-temperature conditions. This study explores the bacterial diversity in selected glacial lakes of High Tatras, Slovakia, with a focus on the isolation and characterization of psychrophilic microorganisms with potential biotechnological applications. Water and sediment samples were collected from five alpine lakes and cultured at two temperatures 20 °C and 4 °C to differentiate. Using MALDI-TOF mass spectrometry, 107 bacterial isolates were identified. At 20 °C, the predominant genera included Serratia, Bacillus and Pseudomonas, with a notable abundance of potentially pathogenic Enterobacteriaceae in Slavkovské pleso, suggesting anthropogenic influence. In contrast, cultivation at 4 °C yielded a distinct spectrum of psychrophilic taxa, including Pseudomonas antarctica, Exiguobacterium sibiricum and Janthinobacterium lividum — species known for their ecological resilience and functional potential in cold environments. The observed microbial profiles also provide insights into how environmental and anthropogenic factors shape microbiome composition in fragile alpine ecosystems. By highlighting the functional traits of psychrophiles, this work contributes to the broader understanding of microbiome adaptation, resilience and their prospective use in sustainable biotechnology.

KEYWORDS: Psychrophiles; Anthropogenic impact; High Tatras; MALDI-TOF MS; Nioremediation

#### **ACKNOWLEDGEMENT**

This research was supported by the project UGA-11-DS-2025: Potential of Extremophilic Microorganisms in the Bioremediation of Contaminated Environments in Slovakia, funded by the Recovery and Resilience Plan of the Slovak Republic.

# Characterizing microbial diversity and community differences between early and late flowering inocula

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Microbial communities influence plant phenotypes by shaping processes such as nutrient acquisition and stress response. These changes in the costs and benefits of different strategies of resource allocation are likely to impact life history traits including growth rate, reproductive timing, and overall fitness. This has led to the concept of microbiome-dependent ontogeny timing (MiDOT), where host species rely on microbial cues to regulate developmental transitions like flowering time. MiDOT includes both accelerated and delayed developmental events in response to microbial communities, highlighting relationships between hosts and their microbial consortia. However, the specific microbial taxa and mechanisms influencing developmental transitions remain poorly understood, leading us to investigate the diversity and composition of microbial communities associated with early and late flowering phenotypes in Arabidopsis thaliana (A. thaliana). We used community selection to enrich for microbial communities promoting early or late flowering phenotypes; rhizosphere inocula were collected from diverse environments and used to inoculate A. thaliana seedlings to assess the effects of microbial communities on flowering time. Using life-history models we can compare the effects of microbial communities on resource allocation and optimal flowering time. In addition, preliminary characterization of microbial communities using 16S rRNA sequencing revealed distinct differences in microbial diversity and composition between early and late flowering groups. Early flowering inocula were associated with lower alpha diversity and dominated by taxa such as Stenotrophomonas and Pantoea agglomerans, which are known to promote growth and suppress pathogens. In contrast, late flowering communities exhibited higher diversity, including taxa (e.g. Penicillium), suggesting broader microbial interactions influencing delayed phenology. This study demonstrates distinct microbial community compositions are linked to early and late flowering phenotypes in A. thaliana, emphasizing the critical role of microbiota in regulating plant developmental timing.

**KEYWORDS:** MiDOT; *Arabidopsis thaliana*; Early flowering; Late flowering

### Microbiome modelling: from genomes to function

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Today, the relation between most diseases such as inflammatory bowel disease, obesity, diabetes, and neurodegenerative disorders, and the links between axes such as gut-brain, gut-cardiovascular, gut-lung, and gut-liver has started to be enlightened; thus, gut microbiota has become one of the most researched areas in recent years due to its significant impact on human health. This extensive research on gut microbiota has created large datasets that could not be analyzed without the help of bioinformatic tools. These datasets have led to the development of various subfields to analyze genomic and functional data. A wide range of software tools and algorithms have been developed to analyze microbial communities. For taxonomic profiling and diversity analysis of metagenomic sequencing data, commonly used tools include QIIME2 and R packages such as vegan and phyloseq. Assembly and annotation of sequencing reads can be performed using platforms like MEGAHIT and Prokka. In metatranscriptomic studies, tools such as SOAPdenovo-Trans are used to assess gene expression. Additionally, metabolomic and proteomic data obtained via LC/GC-MS are analyzed using specific pipelines to identify and quantify microbial metabolites and proteins. This presentation represents subgroup of various bioinformatic tools that has been widely used in gut microbiota research and summarizes their roles across in data processing. The aim is to provide a concise overview of computational approaches in microbiome studies.

**KEYWORDS**: Gut microbiota; Metagenomics; Bioinformatic tools; Microbial community analysis; Data visualization; Multi-omics integration

## Tomato rhizosphere comparison in standard and modified condition with AI aid

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Tomato (Solanum lycopersicum) is one of the world's most economically important crops, facing significant challenges from soil-borne pathogens. Understanding the rhizosphere microbiome is crucial for improving plant health, growth, and disease resistance to meet increasing global food demands. Our study analyzed 1,344 samples collected over two years. In the first year, we examined standard tomato growth conditions; in the second year, we applied various treatments to enhance productivity and plant health. Samples were analyzed using QIIME2 (v.amplicon-2024.10) with taxonomic classification performed using the Greengenes database (v.2022). To facilitate agricultural microbiome research, we developed Spectrum Database, a web platform for analyzing and visualizing 16S rhizosphere metagenomics data. This scalable MySQL-based system enables agronomists and biotechnologists to monitor agricultural activities through flexible data validation mechanisms. Our analysis revealed significant changes between standard and modified conditions across four tomato varieties. In Ciliegino variety, plants treated with TUSAL fungicide showed increased Shannon entropy during the multiplicative growth stage. Differential abundance analysis revealed elevated levels of Brevundimonas in treated plants compared to controls. This genus protects tomatoes from phytopathogen infections, indicating improved plant health. The increased alpha diversity in agrochemical-treated plants demonstrates enhanced species diversity and plant health. Agricultural partners confirmed increased production under modified conditions. This research demonstrates how microbiome analysis in agricultural contexts enhances our understanding of agrochemical effects. By improving crop cultivation methods and increasing production, these findings help address growing population demands. The integration of microbiome data with agricultural practices represents a promising approach for sustainable crop management and food security.

**KEYWORDS:** Microbiome; Tomato; AI; Rhizosphere; Agrochemicals

#### **ACKNOWLEDGEMENT**

"SPeCTRuM: Sustainable ProduCtion by Al-based Tomato's Rhizobiome Monitoring" (PSR Sicilia 2014/2020)

# From root to function: designing and modelling fungal consortia for poplar-based phytomanagement in contaminated soils

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Poplar (Populus spp.) represents a unique model for exploring root-associated microbial interactions due to its fast growth, clonal propagation, rich exudate profile, and well-documented symbioses with diverse fungi. In trace element-contaminated environments, poplars restructure their rhizosphere and endosphere microbiomes to mitigate abiotic stress, making them ideal candidates for phytoremediation and mycobiome engineering. In this study, we assembled synthetic fungal consortia using a bottom-up strategy, leveraging isolates obtained from legacy-contaminated sites. A screening platform was used to map multispecies compatibility, enabling the design of functionally coherent communities beyond binary antagonism tests. Each fungal strain was phenotypically profiled for spore production, stress tolerance, liquid culture potential, and plant-growth–promoting traits such as phosphate solubilization, IAA production, and siderophore activity. The compatibility-mapping platform used here enables identification of synergistic interactions and priority effects across complex fungal assemblages, providing an advance over traditional one-on-one screening methods. This modular system supports the functional deployment of fungal inoculants in the field and offers a framework for top-down approaches that integrate genomic and metagenomic data to model the links between microbial traits, community structure, and host responses. Moreover, the methodological framework is adaptable to other plant species and contaminated settings, paving the way for broader applications in phytomanagement strategies beyond poplar systems.

**KEYWORDS:** Bottom-up assembly; Functional screening; Microbial trait profiling; Mycobiome engineering; Phytoremediation

# Understanding plant and plant-associated microbiome responses to crop stress through in silico studies

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The current climate change is exacerbating many abiotic stresses, such as heat, nutrient deficiencies or drought, with strong negative effects on plant growth and productivity. Although most studies have focused on the aerial part of the plant, these environmental factors affect the root system, which is crucial for nutrient and water uptake, as well as for interactions with soil microbiota, and overall plant tolerance to stress. High temperatures are also known to affect plant-associated microbial communities and root phosphate (Pi) assimilation. To date, studies of the effects of heat stress on plants and plant-microbe interactions have used a constant temperature for both shoots and roots. However, this scenario does not accurately reflect natural soil thermodynamics, where temperature is buffered as a gradient. To address this, our group has developed a device, the Temperature Gradient Root Zone (TGRooZ), that mimics these soil conditions. In this study, we aim to better understand the responses of tomato roots and its rhizosphere to Pi deficiency and heat stress. We grew tomato plants under 36°C TGRooZ gradient and Pi starvation, and analyzed the changes in microbiota composition and in root transcriptome, by shotgun metagenomics and RNA sequencing, respectively. We observed changes in gene expression under both heat stress and Pi deficiency. Interestingly, the combination of stresses elicited a specific plant response that was significantly different from the individual stresses. With respect to the rhizosphere, preliminary tests showed that overall microbial diversity was not significantly affected by either gradient-induced heat stress or Pi deficiency. However, non-gradient heat treatment led to more uniform communities, with a significant reduction in the abundance of the most dominant species. Further analysis of full-length 16S and ITS amplicons revealed a variety of soil microorganisms to be explored and integrated with the results of the plant expression analysis using a multi-omics approach.

KEYWORDS: Plant-root; Abiotic stress; Metagenomics; 16S; ITS

#### **ACKNOWLEDGEMENT**

This work has been funded by grants: CEX2020-000999-S-20-4

# Adopting microbial ecologyp in the development of cultivation systems for complex microbial communities

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Beginning in September 2025, research group from our institute will initiate a multidisciplinary project aimed at applying microbial ecology principles to the design of cultivation systems for complex microbial communities. The central objective is to test the mass-transfer loop principle using a simplified, defined synthetic community. The project will systematically assess: (i) the effect of initial community richness and evenness on community stability in relation to inoculum size, (ii) the number and arrangement of connected vessels, and (iii) the rate of mass transfer to and from the regional pool vessel. A key task involves the development of metabolomics-based methods for characterizing the functional properties of microbial consortia. Community metabolomics will serve as a proxy for interspecies interactions and overall metabolic activity, and will be integrated with 16S rRNA and ITS1 amplicon sequencing data to correlate functional profiles with taxonomic structure. Beyond its scientific innovation, this project holds strong potential for climate change mitigation. The technology may support microbiome biobanking for biodiversity preservation, enhance environmental monitoring through metabolomics, and contribute to bioremediation strategies via maintenance of functional environmental consortia.

KEYWORDS: Climate change; Microbiome; Metabolomics; Bioremediation

# Metagenomic insights into soil microbiomes of natural and semi-natural habitats in Vojvodina: implication for carbon sequestration and nature-based solutions

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In a world facing global challenges such as climate change and soil degradation, understanding the role of the soil microbiome in carbon sequestration is essential for developing sustainable land-use strategies and preserving ecosystem services. This PhD research focuses on metagenomic analysis of soil microbiomes from natural and semi-natural habitats in Vojvodina (Serbia). The main objective is to investigate the taxonomic and functional diversity of microbial communities and their potential role in carbon sequestration. Special emphasis is placed on identifying microbial groups involved in the transformation of organic matter and the stabilization of organic carbon in soil. A total of 200 soil samples were collected during the spring/summer seasons in 2024 and 2025, and the ongoing analysis integrates shotgun sequencing data with physico-chemical soil properties to uncover relevant correlations. The expected outcomes will support soil health assessment and reveal opportunities for implementing Nature-based Solutions (NbS) in Vojvodina, with the overarching aim of conserving soil biodiversity and mitigating climate change. These findings will also inform regional land-use policies and foster stakeholder engagement in adopting NbS for enhanced climate resilience and sustainable soil management.

KEYWORDS: Soil microbiome; Metagenomics; Carbon sequestration; Nature-based solutions

#### **ACKNOWLEDGEMENT**

This PhD research is funded by the European Union's Horizon Europe Twinning project SONATA (Monitoring of nature infrastructure – Skill acquisition for Nature-based Solutions), under grant agreement No. 101159546.

## Genome insights into the phyllosphere colonization and biocontrol activity of *Bacillus velezensis* SS-38.4

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Soil isolated Bacillus velezensis SS-38.4, effectively colonizes the sugar beet phyllosphere and suppresses the highly virulent sugar beet pathogen Pseudomonas syringae pv. aptata P21, particularly when applied preventively. This finding prompted further investigation into the genomic basis of its biocontrol activity. We sequenced the SS-38.4 genome using PacBio SMRT technology, followed by de novo assembly with Canu v2 and annotation via the PGAP and RAST pipelines. Comparative genomic analysis was performed against 50 B. subtilis complex strains using TYGS for phylogeny, ANI for genomic similarity, and EDGAR for pan-genome analysis to refine its taxonomic classification. We further compared the SS-38.4 genome with that of P21 to identify traits that could explain SS-38.4's superior colonization of the phyllosphere and its biocontrol activity. Both genomes were screened for gene repertoires involved in nutrient and space competition, production of extracellular enzymes and antimicrobial compounds, hormone synthesis, nutrient solubilization, plant-growth promotion, quorum quenching, plant colonization, environmental stress adaptation, and virulence. SS-38.4 showed high genetic similarity to B. amyloliquefaciens GKT04, JP3042, HM618, and B. velezensis FZB42, particularly in genes associated with plant-beneficial and biocontrol traits. Genomic analysis revealed a robust arsenal of genes supporting nutrient acquisition, colonization, antimicrobial compound synthesis, and quorum quenching. Notably, SS-38.4 lacks pathogenicity-related genes, highlighting its safety as a phyllosphere biocontrol agent. Its biocontrol mechanism likely relies on superior motility, surfactin production, and biofilm formation, enabling it to effectively invade and establish in a non-native niche. This offensive strategy is further supported by iron depletion via siderophore (bacillibactin) production and antimicrobial compound synthesis. Therefore, SS-38.4's preventive success appears to result from a synergism of traits enabling rapid niche colonization, nutrient competition, and antimicrobial defence, offering a functional genomic basis for its role as a biocontrol agent in interaction with P21.

KEYWORDS: Biocontrol; Bacillus; Whole-genome sequencing; Comparative genomics; Colonization

# Engineering tomato-seed endophyte synthetic bacterial consortia for enhanced plant drought tolerance

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Drought tolerance is becoming increasingly vital in agriculture as climate change intensifies water scarcity, reducing crop yields and destabilizing farming systems. Endophytic bacteria offer a sustainable, eco-friendly solution to combat drought stress by improving nutrient uptake, osmotic regulation, and antioxidant activity—providing a viable alternative to chemical-dependent agriculture. These beneficial microbes colonize plant tissues and produce plant growth-promoting (PGP) compounds, such as phytohormones and ACC deaminase, which enhance root development and water retention under drought conditions. This study investigates the engineering and optimization of tomato-seed endophytic bacterial consortia, aiming to develop a novel bioinoculant that boosts drought tolerance in tomato, sugar beet and pepper via synergistic plant-microbe interactions. Two synthetic bacterial consortia, O1 and O3, were designed from a selection of 91 isolates based on their ability to promote tomato seed germination and seedling growth, combining strains with complementary drought-alleviating PGP traits. Under drought conditions, both O1 and O3 consortia had a positive effect on tomato, increasing fresh weight (89.6%) and shoot length (60.12%) while reducing H2O2 concentration (18.72%). Consortium O3 additionally elevated proline levels (60.05%)—a key osmoprotectant—suggesting its role in alleviating drought stress. In sugar beet and pepper, consortium O3 showed the superior beneficial effects, improving relative water content (RWC), fresh weight and shoot length, while lowering H2O2 levels. These results indicate enhanced drought tolerance in plants treated with O3. Future research will investigate the molecular mechanisms underlying drought tolerance in consortia-treated plants. Furthermore, we will employ WebgLV, a computational tool for modeling bacterial community dynamics, to validate existing synthetic consortia and develop novel formulations for plant treatment.

**KEYWORDS:** Endophytic bacteria; Synthetic bacterial consortia; Drought tolerance; Sustainable; Agriculture

# Enriching soil, empowering microbiomes: fermented organic manure as a key to soil health and metagenomic exploration

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Microbial communities play a critical role in maintaining ecological balance. The rise of genomic and metagenomics technologies has transformed our ability to explore microbial diversity in various environments. It facilitates the in-depth study of individual microbial genomes, uncovering their genetic potential and functional capabilities. This study investigates the microbial diversity enriched in soil following the use of fermented organic manure on apple cultivar Red Cap Valtod, revealing a wide array of beneficial microorganisms. Bacterial strains such as Pseudomonas spp., Bacillus spp., Phosphate solubilizing bacteria etc. were observed during the microbial analysis. Gram positive Actinomyces were also identified. Additionally, the study included the analysis of soil enzymatic activities such as dehydrogenase, urease, acid and alkaline phosphatases to better understand the enhanced biological activity. While the current work emphasizes culture-based identification, the integration of genomic and metagenomics approaches holds great promise for future research. Such tools can offer deeper insights into the taxonomic diversity, functional potential and ecological interactions of microbial communities in amended soils. These advanced techniques can help identify non-culturable organisms and provide a broader understanding of microbial interactions, functional genes, and their roles in promoting soil health and plant growth. Integrating such approaches would strengthen the current findings and contribute to a more holistic understanding of soil microbial ecology, ultimately supporting the development of sustainable and effective soil management practices.

**KEYWORDS**: Metagenome; Microbiome; Enzymes; Sustainability

#### **ACKNOWLEDGEMENTS**

I extend my heartfelt thanks to my major advisor, Dr. Rajesh Kaushal and the co-advisors Dr. Pramod Kumar and Dr. Upender Singh for their invaluable guidance, encouragement, and expertise throughout the study. Additionally, I would also like to acknowledge Indian Oil Corporation, New Delhi for their financial support, which made this study possible.

# Effects of three organic amendments practices on soil microbiome under rice-wheat-mungbean based cropping systems of 365 days of covering: A comparative analysis

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Conventional cropping system with fallow period and cropping system including cover crop have significant impact on soil microbiota. Soil amended differently also impact soil microbiome. Even the crops grown in the field are highly correlated with the microbial diversity. In the present study, under 365 day of soil covering along with three organic soil treatments we tried to identify the impacts on the bacterial and fungal diversity. Root rhizospheric soil of rice-wheat-mungbean cropping system under the three different treatments, BBEF-Biochar-Based Ecological Farming, CROF-Climate Resilient Organic Fertilizer, and LINF-Low Input Natural farming, was collected in year 2024 at the end of the trial, and analysed for metagenome profiling. 16S and ITS metagenomic analysis revealed significant differences in microbial diversity, structure, and community composition across both bacterial and fungal domains. For bacterial communities (16S), BBEF soil showed a Shannon index of ~5.2 and Simpson's index of ~0.91, with key genera including Bacillus, Pseudomonas, and Streptomyces accounting for over 45% of the relative abundance. In fungal profiling, BBEF also showed dominating alpha diversity (Shannon ~4.9, Simpson ~0.88, Chao1 ~120), with dominant beneficial genera like Trichoderma, Mortierella, and Aspergillus comprising ~40% of total reads. Whereas, CROF treatment soil showed comparatively moderate diversity in both bacterial (Shannon ~4.3) and fungal (Shannon ~4.1) domains, showing a community structure dominated by fewer functional taxa. LINF, the low-input treatment, showed the lowest diversity scores across both domains (bacterial Shannon ~3.6; fungal Shannon ~3.5), indicating a limited and uneven microbial environment. Beta diversity (PCoA) and dendrogram clustering highlighted distinct microbial community structuring in BBEF, with greater intra-treatment similarity and separation from CROF and LINF. Core microbiome analysis confirmed that BBEF retained 45% of dominant bacterial genera and 38% of fungal taxa across replicates, compared to just 24-32% in other treatments. At the phylum level, BBEF enriched Gram-positive groups like Actinobacteria and Firmicutes, linked to organic matter turnover. CROF favored Gram-negative phyla such as Proteobacteria and Bacteroidetes, associated with nutrient cycling, while LINF showed higher abundance of Acidobacteria and Chloroflexi, typical of low-input systems. Gram-positive genera like Bacillus were more prevalent under BBEF, whereas Pseudomonas and Burkholderia (Gram-negative) were enriched in CROF and LINF. These results demonstrate that input type and intensity distinctly shape microbial composition and ecological function. In conclusion, BBEF was identified as the most effective treatment in enriching both bacterial and fungal microbiomes, supporting functional taxa involved in nutrient cycling, plant growth, and disease resistance and thus making it a promising approach for improved soil health, sustainable and resilient rice-wheat-mungbean cropping system production.

KEYWORDS: Cover cropping; Organic amendments; 16S; ITS; Metagenomics; Soil-microbiome

### Endophytic and rhizospheric microbial interventions to boost Capsicum annuum productivity under climate and soil stress

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Capsicum annuum L. (chilli) is crucial for its pungency and nutritional content (vitamin C, protein, antioxidants) and has medicinal benefits, but yields are threatened by climate change and poor cultivation, which disrupt soil microbiomes. Beneficial rhizospheric and endophytic bacteria can mitigate these stresses and improve plant resilience. Bacterial isolates from the chilli rhizosphere and endosphere (fruit, flower, leaf, stem, root) total 154 were screened for plant growth–promoting (PGP) traits. Functional assays identified catalase activity in 117 isolates, amylase in 44, protease in 62; 60 solubilized zinc, 32 phosphates, and 15 each solubilized potassium and silicate. Production of indole-3-acetic acid (IAA) was found in 91 isolates, ammonia in 113, and siderophores in 99. Among endophytes, AzmCh-11 (Pseudomonas sp.) and AzmCh-15 (Pantoea agglomerans) showed strong antagonism against key pathogens (Fusarium udum, F. oxysporum, Alternaria sp., Macrophomina phaseolina). 27 strains were identified and tested in sterilized soil; seven significantly enhanced plant growth metrics. Notably, AzmCh-11 inoculation produced a 15.0-cm shoot with shoot fresh/dry weights of 3.10/1.99 g and root fresh/dry of 1.79/1.13 g, significantly above controls. AzmCh-15 gave 13.5-cm shoots and yielded the highest shoot fresh/dry (3.79/2.24 g) and root fresh/dry (2.60/1.35 g) weights. The endophyte AzmChl-56 induced the greatest chlorophyll accumulation. These results demonstrate that AzmCh-11 and AzmCh-15 significantly promote chilli growth under controlled conditions, outperforming uninoculated controls (p≤0.05) in biomass and physiological parameters. In conclusion, strains AzmCh-11 and AzmCh-15 are promising candidates for microbial consortia to sustainably enhance Capsicum annuum productivity. By improving growth and biotic stress resilience, these phytomicrobiome members could help increase chilli yield across diverse agro-climatic zones.

KEYWORD: Chilli; Endophytes; Plant growth-promoting; Nutritional content

# Exploring the bacterial, fungal and viral microbiota of chestnut blight-induced bark cankers by traditional and metagenomic approaches

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Cryphonectria parasitica is a phytopathogenic ascomycete, the causative agent of the chestnut blight disease. In nature, the disease progression is mitigated by viruses that reduce the virulence of the fungus towards its host. Among these, the interaction between C. parasitica and Cryphonectria hypovirus 1 (CHV1) has been studied extensively. However, the role of other microbes, which could potentially serve as biocontrol agents of C. parasitica, has been underexplored. Therefore, in our current project, we aim to investigate the complex microbial communities of chestnut bark cankers caused by C. parasitica infection. To characterise and model the bacterial and fungal communities of chestnut bark cankers, we will employ high-throughput sequencing using 16S ribosomal DNA and ITS metagenomic analyses, respectively. Apart from characterising the microbial communities co-inhabiting the chestnut bark cankers alongside C. parasitica, fungal isolates of C. parasitica will be screened for the presence of novel viruses by shotgun metagenomic analysis. We will complement the results of metagenomic analyses with traditional isolation, culturing and characterisation of bacteria and fungi isolated from the chestnut bark canker samples. The isolated bacteria and fungi will be used in downstream interaction experiments with C. parasitica. Identified microbial species will be tested for their biocontrol potential against C. parasitica, while the functional microbial community analyses will enable us to identify specific differences in microbial profiles between healthy and infected chestnuts. These results will contribute to the understanding of the chestnut blight disease and will potentially provide the identification of additional interaction partners and the expansion of our knowledge of the tripartite pathosystem of chestnut, chestnut blight fungus, and CHV1.

**KEYWORDS:** *Cryphonectria parasitica*; High-throughput sequencing; Microbial interactions; Plant pathology

#### **ACKNOWLEDGEMENT**

Croatian Science Foundation grant IPCH-2023-10-1986 Diversity and interactions of the chestnut bark canker mycobiome (DivelnBiome)



# ATTENDEES & POSTER PRESENTERS

### PP<sub>1</sub>

# Characterizing the microbiota in a pork processing facility: a basis for exploring pathogen stress responses in industrial environments

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Contamination during pork processing is a critical issue for food safety, as surfaces and equipment can harbour a variety of microorganisms, including spoilage microorganisms and occasional pathogens. These microbial communities can influence product quality, shelf life, and may pose health risks. Understanding the composition and behaviour of these communities is essential to guide sanitation practices and control strategies. Although culture-based techniques are still helpful for estimating microbial loads, our capacity to evaluate microbial diversity and recognize dominant taxa has greatly increased thanks to sequencing technologies, especially 16S rRNA gene profiling. Recent research has emphasized the need to connect these microbial profiles with functional responses to environmental stress, particularly under conditions commonly found in food processing, such as low temperatures and oxidative environments. A total of 60 samples were collected across 13 stages of pork chop production, including 40 surface swabs and 20 meat samples. Microbial counts ranged up to 1.5×10⁴ CFU/cm² on surfaces and 1×10⁵ CFU/g in meat. Pseudomonas spp., Enterobacteriaceae, and lactic acid bacteria were commonly isolated. Also, Listeria monocytogenes was detected in one surface sample. DNA was extracted from a subset of samples and the V3-V4 region of the bacterial 16S rRNA gene was sequenced. Proteobacteria were the most dominant phylum, followed by Firmicutes and Bacteroidota. Genera such as Pseudomonas, Acinetobacter, and Psychrobacter were most prevalent. The data reveal a diverse and persistent microbiota throughout the production line. The presence of genera associated with spoilage, along with occasional detection of pathogens, suggests the need for targeted interventions and monitoring. This study provides a microbiological baseline for future research on pathogen adaptation. It lays the groundwork for investigating the transcriptional responses of species such as Listeria monocytogenes under conditions that mimic those found in real-world food processing environments.

KEYWORDS: Food safety; Microbiota; 16S rRNA gene sequencing; L. monocytogenes

**ACKNOWLEDGEMENT** 

Fondecyt 1241084

### Impact of long-term heavy metal pollution on plant microbiomes

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Heavy metal pollution is a major global issue endangering biodiversity and environmental health. These chemicals with relatively high densities are introduced to the environment by several industrial activities such as mining, smelting, and agrochemical use. Heavy metals are non-degradable and bioaccumulative, especially in northern ecosystems where microbial activities are reduced during prolonged winters. Metal deposition causes impairment to health of environment and organisms. The impact of metal pollution on plant microbiomes remains elusive. Plant microbiomes are essential for plant health promoting nutrition, stress tolerance and disease tolerance. In this study, I investigate the effects of copper and nickel on lingonberry and pine in a long-term experimental field site in Northern Finland. The treatments simulate pollution loads near mining smelters in the Kola Peninsula. Bacterial culturing techniques were used to analyse the effect of treatments and bacterial isolates were identified by Sanger sequencing. Two genera Frigoribacterium and Frondihabitans were enriched in the treated samples. Using targeted amplicon sequencing, the bacterial community in lingonberry leaves, lingonberry roots and pine leaves was characterized. Eventhough, there were no significant difference in the overall community of metal-treated plants compared to the controls; certain bacterial taxa (including Frigoribacterium and Frondihabitans) were found to be specifically enriched in the treated samples. Both these taxa have been identified in other studies as responding towards metal zinc but their exact role in plant-microbe interactions especially in tolerating copper and nickel is yet to be investigated. Further investigations will be carried out to understand the role of these bacteria towards heavy metal tolerance in plants. This information helps to develop microbe-mediated phytoremediation strategies to mitigate heavy metals from the environment. With the increasing mining activities, we need to be prepared with remediation strategies now than eliminating them once the damage is irreversible.

**KEYWORDS:** Plant microbes; Heavy metals; Northern ecosystems; Bioremediation

#### **ACKNOWLEDGEMENT**

I would like to thank the Sakari Alhopuro Foundation for the providing grant for this research.

## Global epistasis as a tool for predicting final pH value in fermented soy beverage

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In biotechnology, even modest functional improvements can determine a technology's viability. Therefore, simply knowing if a microbial community can perform a function is insufficient; we must quantitatively identify the optimal consortia. This study explores the application of global epistasis modelling to predict fermentation outcomes in soy-based beverages using combinatorial starter cultures. A diverse library of 33 microbial strains (including *Lactobacillus, Streptococcus, Enterococcus*, and *Lactococcus* species) was used to construct 308 of possible 284273 unique combinations (including all single strain combinations and combinations of up to 5 strains) for fermenting soy drink. Commercial soy drink was used for fermentation. Strains were prepared in MRS or LM17 broth, and soy drink was inoculated with 3% of starter culture mix. Measurements of pH values were done at the beginning of fermentation and after 15h. A global epistasis model was trained on this dataset to predict post-fermentation pH change (ΔpH), sensory attributes, and texture properties. For validation, 9 representative combinations (spanning 2-, 3-, and 4-strain consortia) were experimentally tested. The model achieved high predictive accuracy for ΔpH, with a normalized root mean square deviation (RMSD) of 0.04 across validation samples. Key results include: Minimal ΔpH errors (e.g., 0.33% for a 3-strain consortium).

Robust performance across strain complexities (2–4 members).

Additional validation of sensory/texture predictions showed higher normalized RMSD (0.27 / 0.57 respectively).

These findings demonstrate that global epistasis modelling effectively captures microbial interactions in fermented soy products, enabling rational design of starter cultures with desired functional properties. The framework offers a scalable tool for optimizing fermentation processes in plant-based dairy alternatives.

KEYWORDS: Global epistasis; Starter culture; Microbial consortia; Prediction modelling

## Microbiome of chicken breast cuts with wooden breast myopathy and its succession over refrigerated storage

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Wooden breast (WB) is a prevalent quality defect in broiler chicken breast meat, characterized by muscle hardening and, in some cases, the presence of a serous exudate. This condition is associated with increased pH and reduced water-holding capacity, factors known to promote bacterial growth and raise concerns about the shelf-life of affected meat. This study aimed to identify bacterial communities involved in the spoilage of chicken breast cuts with WB and without (N) using 16S rRNA gene amplicon sequencing. Cubes (~25 g) from several WB and N breasts were vacuum-packaged and stored at 3.9 ± 0.8 °C for 17 days. On days 1, 8, 14, and 17, three replicates were diluted 1:10 in peptone saline solution (1% peptone, 0.85% NaCl), homogenized (Smasher blender), and used for bacterial cell isolation. DNA was extracted using the DNeasy PowerFood Microbial Kit (Qiagen), and sequencing was carried out at the DNA Sequencing and Genomics Laboratory (supported by HiLIFE and Biocenter Finland funding), Institute of Biotechnology, University of Helsinki. Initially (day 1), Lactobacillus spp. and Limosilactobacillus spp. were present, but were later replaced by other genera during storage. Dominance of different genera in successive storage times was not consistently related with breast type (N or WB). Independent of myopathy, a mixed population of Carnobacterium spp., Hafnia spp., and Pseudomonas spp. was detected in the majority of samples at different abundances. In other replicates, Photobacterium spp. dominated (> 70% of abundance), consistent with its recent emergence as an important meat spoiler. Although it is generally associated with fish, it has been previously detected in high abundances in refrigerated chicken cuts. Yersinia spp. prevailed only in one replicate at 17 days. Other important genera included Lactoccoccus spp., which was present since day 14 and dominated over one replicate, Serratia spp., and Vagoccocus spp., all common chicken spoilers.

KEYWORDS: Chicken myopathies; Metabarcoding; Shelf-life

#### **ACKNOWLEDGEMENT**

This work was partially funded by the IRTA Sponsored Fellowship Program 2022 Smart Agrifood Systems. CERCA program and 2021 SGR 00468 and SGR 00461 are also acknowledged. Erasmus + program supported travel costs associated with this collaboration.

## Introduction to the microbiology of activated sludge: the master key for wastewater treatment plant efficiency

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Activated sludge is a dynamic and intricate microbial community that plays a pivotal role in the wastewater treatment process. Composed of microorganisms from different trophic levels, this community is fundamental in breaking down organic pollutants, transforming wastewater into cleaner, safer effluent. The efficiency of a wastewater treatment plant (WWTP) depends largely on the health and functionality of the activated sludge microbiome. Thus, understanding the microbiological processes that occur within this community is the key to optimizing plant performance and addressing common operational challenges. The ecology and physiology of microorganisms within activated sludge are directly influenced by a variety of environmental factors, including temperature, pH, nutrient availability, and the presence of toxic substances. The interactions between microorganisms and their surrounding environment determine the structure and function of the microbial community, which in turn impacts the overall efficiency of the treatment process. Key issues such as poor sedimentation, bulking, foaming, and rising sludge are often a direct result of imbalances in this microbial community. Therefore, a comprehensive understanding of the microbiology of activated sludge is essential to mitigate these issues and improve the overall performance of WWTPs. Effective monitoring and management of the activated sludge community allow for the identification of specific microorganisms that contribute to treatment success. By selecting and optimizing microbial populations with desirable metabolic pathways, wastewater treatment plants can achieve better removal of organic matter, improved settling characteristics, and reduced operational costs. In recent years, research has focused on the role of bacteria, fungi, and protozoa in the activation process, with particular attention to the role of biofilms and extracellular polysaccharide production. The development of strategies to enhance microbial activity and community structure holds significant promise for increasing the efficiency and sustainability of wastewater treatment operations.

KEYWORDS: Activated sludge biocenosis; Wastewater treatment; Selection of microorganisms

### PP<sub>6</sub>

# Effect of water oxygenation on the microbiota of compacted soils in broccoli cultivation

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Soil compaction is an increasing problem in Europe, with more than 29% of subsoils affected. Soil mechanical impedance is caused primarily by natural processes and by the use of heavy machinery in agricultural practices. Plant roots are strongly influenced by the physical properties of the soil. Soil compaction, along with changes in soil water potential, are major factors contributing to high mechanical impedance or excessive soil strength. These factors not only affect the plant root system—leading to changes in root morphology—but also impact the soil microbiota. In this study, the effect of two levels of soil compaction on broccoli cultivation and soil biology was evaluated. Broccoli plants were grown in open-bottom pots placed in direct contact with the soil and subjected to different compaction levels (0, 200, and 400 psi), with and without oxygenation applied through the irrigation water. This resulted in six treatments: 0, 0+O<sub>2</sub>, 200, 200+O<sub>2</sub>, 400, and 400+O<sub>2</sub>. Plant growth and final yield were measured, along with soil enzymatic activities and metabarcoding analyses to assess variations in fungal and bacterial populations. While urease and dehydrogenase activities showed no significant differences, an increase in phosphatase activity was observed in all compaction treatments. β-glucosidase activity increased with oxygenation at all compaction levels, indicating a promoted microbiome activity by oxygenation. Metabarcoding analysis revealed that bacterial alpha diversity was lowest in the 0+O<sub>2</sub> treatment and highest in the 400 treatment, where high compaction (400 psi) promoted anaerobic bacterial populations compared to the control (0) and 400+O₂ treatments. Both compaction and oxygenation induced changes in bacterial and fungal communities suggesting that oxygenation can mitigate the negative effects of high compaction and improve soil health.

**KEYWORDS:** Broccoli; Oxygenation; Soil biology; Soil compaction

#### **ACKNOWLEDGEMENT**

This study was funded by the European project "Horticultural Innovations in Soil- Friendly Practices to Ensure a Sustainable Future" (Hort2theFuture, Proposal ID 101157434), supported by the European Union under the Horizon Mission A Soil Deal for Europe programme.

# The challenge of inferring interactions from microbial metagenomic data

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Microorganisms do not exist in isolation, but in diverse communities where species interact through a range of ecological relationships. As these communities grow in size and complexity, the number and nature of interactions expand, giving rise to intricate ecological networks. Understanding the structure of these networks is essential to predict microbial community dynamics and to enable the design of stable consortia for synthetic ecology applications. Our objective was to evaluate current methods used to infer microbial interactions from cross-sectional data, identifying their strengths and weaknesses, and assessing their ability to recover true ecological interactions. However, these methods infer statistical associations rather than ecological links, often assuming symmetry and missing indirect or asymmetric interactions. To test their accuracy, we simulated microbial communities using stochastic Lotka-Volterra models, generating controlled scenarios with known interaction networks. We found that in complex communities (high interaction strength, connectance, and number of species), FlashWeave recovered true interactions better. In contrast, simpler systems were more accurately captured by the SparCC method. Since most methods infer symmetric associations, they have difficulties detecting prey-predator dynamics unless prey loss exceeds predator gain. All methods showed robustness to environmental noise, and we observed a link between beta diversity and prey-predator structure. This work provides a critical benchmark for current bioinformatics tools used to infer microbial interactions, offering practical guidance on their applicability and limitations, and contributing directly to the development of more robust computational methods in microbial community analysis, enabling better interpretation of complex ecological data.

**KEYWORDS:** Microbiomes; Compositional data analysis; Inference of interactions; Lotka-Volterra simulations; Benchmark

# Reconstruction and comparative analysis of methanothrix soehngenii metabolic model to understand its syntrophic role in anaerobic digestion

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Anaerobic digestion is a key biotechnology for renewable bioenergy generation from wastewater, addressing both energy demands and environmental sustainability. However, the process is hindered by the slow growth of anaerobic microorganisms and the complex interdependencies within microbial consortia. Methanothrix soehngenii, an acetoclastic methanogen, plays a central role in methane production, yet its metabolic capabilities and interactions within syntrophic networks remain underexplored. In this study, we aim to reconstruct a genome-scale metabolic model (GEM) for M. soehngenii to elucidate its functional potential in anaerobic environments. The model reconstruction is based on high-quality genome annotation, pathway mapping, and curated biochemical reactions. The reconstructed model will be compared to that of Methanosarcina barkeri, a versatile methanogen capable of utilizing a broader substrate range. By comparing these two methanogens, we aim to identify core and unique metabolic features that shape their ecological niches and syntrophic behavior. The integration of these models with metagenomic and environmental data will provide insight into the functional role of each species in methanogenic consortia, particularly under sulfate-stressed and electron transfer-limited conditions. The results are expected to clarify how conductive materials and redox conditions influence microbial cooperation, electron flow, and methane yield. This research contributes to the broader goal of improving anaerobic digestion efficiency through informed microbial community engineering. Understanding the metabolic interactions between key methanogens can support the design of resilient microbial consortia and the development of high-efficiency bioenergy systems.

**KEYWORDS**: *Methanothrix soehngenii*; Genome-scale metabolic model; Syntrophy; Anaerobic digestion; Microbial interaction

#### **ACKNOWLEDGEMENT**

This project is co-funded by Science Foundation Ireland (SFI) and the Sustainable Energy Authority of Ireland (SEAI).

## Microsatellite (SSR) analysis of Dolichos lablab (Lablab purpureus (L.) sweet) germplasm in Namibia

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Dolichos lablab (Lablab purpureus (L.) sweet) is a multipurpose drought tolerant protein-rich legume crop native to Africa and grown in warm temperate to tropical climates for its edible seeds and manure. Literature informs that Lablab purpureus holds significant benefits to subsistence farmers and offers a great promise for sustainable crop productivity, especially in marginalised areas. Its uses range from human consumption as a vegetable to improving soil fertility, and as forage. Not with standing Lablab purpureus crucial potential functions in Namibia, there is currently limited information regarding the plant's genetic diversity. This study followed a descriptive quantitative research approach where the data collected was used to describe the samples collected. The study aimed at evaluating the genetic diversity among 26 accessions of Lablab purpureus. The plant's genetic diversity was determined by PCR amplification of three SSR markers namely VM38, AGB8 and GATS911. Analysis was considered for SSR primers that displayed scoreable DNA bands. Python software (3.12.0) was used to analyse the sample size, allele counts per polymorphic locus, Shannon diversity index, and Polymorphism Information Content (PIC) statistics. The SSR markers had PIC values of 0.452663 for marker VM38, 0.473373 for marker AGB8 and 0.260355 for marker GATS911. The Shannon Index of diversity gave a value of 1.25 meaning low levels of genetic diversity among the accessions. The findings of this study demonstrate that SSR markers successfully assessed the genetic variability among genotypes of Lablab purpureus. It indicated they are of a narrow genetic diversity. These results are expected to benefit in the conservation of Lablab purpureus germplasm in Namibia and aid in their breeding efforts as well. The study was the first report of Lablab purpureus's genetic diversity in Namibia.

KEYWORDS: Genetic diversity; SSR; Microsatellite markers; Lablab purpureus; Namibia

#### **ACKNOWLEDGEMENT**

The laboratory research was supported by the Institutional Research and Publication Committee (IRPC) from the Namibia University of Science and Technology (NUST).

# Elucidating the microbial mechanisms behind seasonal N<sub>2</sub>O emissions from WWTPs through multi-meta-omics

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Nitrous oxide (N<sub>2</sub>O) is a highly potent greenhouse gas that makes up the biggest part of scope 1 greenhouse gas emissions from most wastewater treatment plants (WWTPs). N<sub>2</sub>O production in WWTPs has a primarily microbial origin, and has a strong seasonal dynamic where most N,O is produced and emitted during a few months of the year. Understanding the underlying seasonal dynamics of the microbial community producing N<sub>3</sub>O is the key to engineering, modelling, and operating WWTPs that minimize N<sub>2</sub>O emissions. Research has shown that seasonal N<sub>2</sub>O emissions are correlated to seasonal differences in the physiology of the nitrifying microbial community, on genetic, proteomic, and activity level. During spring, the ammonium oxidizing bacteria (AOB) population tends to oxidize ammonium (NH<sub>A</sub>+) faster than the nitrite oxidizing bacteria (NOB) population oxidizes nitrite (NO<sub>2</sub>), causing NO<sub>2</sub> accumulation and subsequent denitrification to  $N_2O$ . Furthermore, modelling studies have indicated that the morphology of AOB and NOB microcolonies in activated sludge flocs are different, resulting in varying oxygen-dependent nitrification rates over the seasons, likely influencing  $N_2O$  production. Additionally, while denitrification tends to be studied with complete denitrifiers, partial denitrifiers often make up the bulk of the denitrifying community in WWTPs. So the role of partial denitrifiers in N<sub>2</sub>O emissions still remains unclear. We aim to investigate the ecophysiology of nitrogen-converters by tracking the microbial communities of varying WWTPs, using metagenomics and metaproteomics. Connecting the metabolic function of community members on both DNA and protein level to genome-scale and microbial community modelling will allow us to quantify ecophysiological changes to nitrogen-converting communities in a way that is unique to our field of research. Additionally, we aim to elucidate the principles underlying microbial N<sub>2</sub>O production, and give handles to WWTP engineers to minimize N<sub>2</sub>O emissions and decrease the environmental footprint of wastewater treatment.

**KEYWORDS:** N<sub>2</sub>O; Nitrification; Metagenomics; Metaproteomics; Wastewater treatment

#### **ACKNOWLEDGEMENT**

We thank Nina Roothans for inspiring the current research project. We also thank Stichting Toegepast Onderzoek Waterbeheer, Haskoning, Waterschap Aa en Maas, Waternet, Waterschap Brabantse Delta, Hoogheemraadschap van Delfland, Waterschap De Dommel, Hoogheemraadschap Hollands Noorderkwartier, Waterbedrijf Limburg, Hoogheemraadschap van Schieland en de Krimpenerwaard, and Hoogheemraadschap De Stichtse Rijnlanden for their collaboration and funding.

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# From the roots to the goods: understanding the microbiome associated with mangroves to provide tools for conservation and re-establishment

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Scientific societies have recently called for immediate and tangible actions to safeguard our planet from the escalating climate crisis. We have numerous solutions available when it comes to nature-based approaches, particularly those involving microbes. Carbon sequestration is one of the available solutions, which can be enhanced by microorganisms in soils and oceans within blue carbon ecosystems. Mangroves capture carbon from the atmosphere for growth, storing it in living and dead plant biomass, as well as in their waterlogged soils. Over 1 million hectares of mangroves are financially viable for carbon finance, providing more than 30 million tons of CO2 equivalent in annual climate mitigation. The root of such potential lies in mangrove soil and roots, whose compartments are inhabited by diverse groups of microorganisms, which are the main players in blue carbon sequestration and other mangrove ecosystem services. The Brazilian projects Manguebits – intelligent restoration of impacted mangroves - and the LTER Semiarid Coast of Brazil study the taxonomic and functional composition of bacterial and fungal assemblages associated with the roots (endosphere, rhizosphere, and soil) of Avicennia spp. and Rhizophora mangle using amplicon sequencing, metagenomics, and culturomics. Our results on 16S and ITS sequencing have revealed taxonomically and functionally diverse microorganisms that participate in element cycling, organic matter decomposition and mineralization, and promote plant growth. A clear differentiation between the endosphere and the [rhizosphere + soil] compartments for Bacteria, Archaea, and Fungi has been shown. Those differences between root compartments are more prominent than temporal and spatial differences. The main abiotic parameters that influenced the microbial community were organic matter, moisture, silt-clay, phosphorus, and pyritic iron content. These findings have been used to guide the isolation of plant-growth-promoting bacteria, which are being utilized to develop re-establishment consortia for mangrove soil or sediment reconditioning and improved seedling production.

KEYWORDS: Microbial ecology; Biotechnology; Bacteria; Fungi; Amplicon sequencing

#### **ACKNOWLEDGEMENT**

This study is funded by the Brazilian Semiarid Coast Long-Term Ecological Research Program (PELD CSB) (N° 442337/2020-5 - CNPq/PEL-00177-0005.01.00/21-FUNCAP) and Manguebits project (N° 440724/2022-8 – CNPp), with scholarship offered by the Coordination for the Improvement of Higher Education Personnel- Brazil (CAPES) - Financing Code 001.

# Impact of phytoremediation and sewage sludge amendments on soil microbiome: a pot trial study

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This study investigated the impact of phytoremediation, plant growth-promoting rhizobacteria (PGPR), and sewage sludge on soil microbial community composition. Pot experiments were conducted using soils collected from the City of Subotica wastewater treatment plant, with 13 experimental treatments and one untreated control. Alpha diversity metrics indicated no significant differences associated with treatment or sampling time point (start vs. end). However, beta diversity analyses revealed pronounced shifts in community structure. PERMANOVA results showed that treatment accounted for 73.1% of the variance in microbiome composition ( $R^2 = 0.731$ , p < 0.001), while sampling time point explained an additional 31.6% ( $R^2 = 0.316$ , p = 0.001), indicating both factors significantly influenced community structure. Across all samples, the dominant bacterial phyla were Bacteroidota, Firmicutes (Bacillota), and Proteobacteria (Pseudomonadota), followed by Actinomycetota and Verrucomicrobiota. Relative abundance profiles revealed moderate shifts between control and sludge-amended soils, with minor differences between plant species (sorghum and hemp). Principal Coordinate Analysis (PCoA) showed clear clustering of microbial communities by treatment. Plant-based treatments, particularly when combined with sludge and PGPRs, formed distinct clusters, while control and untreated soil samples displayed higher similarity. Moreover, samples from the end of the experiment were spatially distinct from those collected at the start, reflecting a significant temporal shift in microbiome structure. These results suggest that combined phytoremediation strategies with PGPRs and sludge strongly influence the soil microbiome and may contribute to long-term changes in microbial composition.

KEYWORDS: Microbial diversity; Phytoremediation; Sewage sludge; Soil health

#### **ACKNOWLEDGEMENT**

This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 67696769, Natural based efficient solution for remediation and revitalization of contaminated locations using energy crops – ReNBES.

# AMicrobioM: Bioactives from the blue-harnessing seaweed-associated microbiomes

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Climate change presents a major threat to global food systems, driving the need for agricultural solutions that do not further harm ecosystems. One promising strategy lies in bioactive compounds derived from marine microbiomes, which offer potential for sustainable crop enhancement. Yet, marine environments are increasingly vulnerable to climate-related stress, biodiversity loss, and pollution—particularly from plastic waste—highlighting the urgency to explore and safeguard their microbial potential. Within this context, seaweeds stand out as complex marine holobionts whose microbiomes play key roles in their development, resilience, and chemical diversity. The AMicrobioM project investigates these microbial communities to uncover bioactive compounds that support sustainable agriculture, including biostimulants, defence elicitors, and antimicrobial agents against crop pathogens. To capture this microbial diversity, we have isolated hundreds of bacterial strains from seaweeds collected along Ireland's east and west coasts. Ongoing efforts, including 16S rRNA sequencing, continue to expand the AMicrobioM microbial biobank. In parallel, collaborative research with partner institutions explores marine microbiomes associated with plastic debris in the sea, broadening our understanding of their functional and biotechnological potential. Our approach combines both culture-dependent and culture-independent techniques. Microbiome diversity is assessed through 16S metabarcoding, while isolated strains are tested for plant defence elicitation, antimicrobial activity against phytopathogens, and plant growth promotion. By integrating experimental work with computational analyses, AMicrobioM seeks to bridge microbial ecology with applied biodiscovery. All strains and datasets generated through this work are being compiled into an open-access resource for the scientific community.

**KEYWORDS:** Seaweed microbiome; Plant biostimulant; Bioactive screening; Marine bacteria; Plastic-associated microbiomes

#### **ACKNOWLEDGEMENT**

This research is supported by the AMicrobioM project under the Research Ireland Frontiers for the Future programme and hosted at Technological University of the Shannon, Ireland.

## Optimization of the ex-situ biological biogas upgrading process

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Biological biogas upgrading plants based on hydrogenotrophic methanation faces several commercial challenges, including high operational costs. A key bottleneck addressed to improve the technology is the poor solubility of H<sub>2</sub> in water, which limits its mass transfer. This research addresses this bottleneck through the design and operation of a novel pilot-scale biogas upgrading system, using a static hydrodynamic cavitation device as a solubilization strategy, and evaluating both process performance and microbial community evolution through time. This research evaluates a novel hydrodynamic cavitation device in a 200L bubble column reactor operated at 55°C for 14 weeks. The experimental phase was divided into two main stages, S1 and S2, according to the composition of gas feed mixture (H<sub>2</sub> and CO<sub>2</sub> for S1 and a biogas mix and H, for S2), keeping the volumetric ration between H, and CO2 4:1 as suggested by the Sabatier reaction. During the S1 steady state, the reactor achieved stable conversion with a final CH4 content exceeding 95%. However, during S2, foaming events occurred and process efficiency declined, despite high CO₂ solubilization (up to 99%). To understand the related biological processes, the evolution of the microbial community structure was monitored throughout the reactor's operation via 16S rRNA gene amplicon sequencing. Sequencing revealed the community shifted from a Nitrososphaeraceae-dominated inoculum (>90% relative abundance) to one enriched in Methanobacteriaceae during the successful S1 phase. During S2 phase the relative abundance of Nitrosospheraceae rose again, coinciding with process inefficiency. Although the cavitation device did not directly impact performance, its operation is linked to the detrimental microbial community shift and overall process instability. A key objective of our future work is to develop a kinetic model, based on Specific Hydrogenotrophic Methanation Activity (SHMA) assays, to quantify these microbial dynamics.

KEYWORDS: Biogas; SHMA; 16S rRNA

# Bio(electro)hydrogenation of waste fermentation products into liquid fuels – a modelling-driven approach

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Anaerobic chain elongation has emerged as a promising biotechnological approach for converting waste-derived short-chain fatty acids into medium or long chain carboxylic acids. These products are valuable for sustainable aviation and transport fuels. Despite recent advancements in microbial engineering and substrate diversification, current systems face challenges in yield optimization, chain-length specificity, and electron donor efficiency. Addressing these limitations, this Ph.D. project focuses on constructing modelling framework that explores the bio(electro)hydrogenation of fermentation-derived intermediates as a pathway to produce valuable fuels. Our work integrates metabolic and bioenergetic modelling to simulate chain elongation dynamics under various donor/acceptor ratios, hydrogen partial pressures, and bio electrochemical conditions. By coupling electron-rich waste fermentation products (e.g., ethanol, lactate, H<sub>2</sub>) with advanced co- culture systems, we aim to optimize conditions for the selective biosynthesis of C<sub>6</sub>–C<sub>8</sub> acids and their subsequent upgrading into alcohols and fuel-range hydrocarbons. The project's focus on biokinetic modelling, waste valorization and microbial process aligns with the themes on metabolic pathway prediction, sustainable bio transformations, and microbiome-function integration. Through a data-driven strategy, this work contributes to the broader vision of circular biorefineries and positions chain elongation as a scalable platform for green fuel production.

**KEYWORDS:** Biokinetic modelling; Waste valorization; Metabolic pathway prediction

# Rapid identification of antibiotic resistance patterns in patients with urinary tract infection using MicroScan WalkAway40 Plus system

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Multi-drug resistance (MDR) and extensively drug-resistant (XDR) organisms pose significant challenges to healthcare systems worldwide. This study aims to analyze the prevalence, patterns, and associated factors of MDR and XDR UPEC at a tertiary care referral center in North India. A retrospective analysis was conducted on data collected from clinical samples obtained from OPD patients and admitted to the hospital in year 2021. The study included 200 urine samples from routine clinical laboratory. Microbiological cultures were performed using standard techniques, and antibiotic susceptibility testing was done using Microscan and carried out according to Clinical and Laboratory Standards Institute guidelines. The results revealed a high prevalence of MDR and XDR Uropathogenic E. coli in the study population. 73% of the tested isolates demonstrated resistance to multiple antimicrobial agents, highlighting the complexity of treatment options. Furthermore, 60% of the MDR isolates exhibited XDR profiles, indicating resistance to even higher classes of antimicrobials. The study identified several risk factors associated with the development of MDR and XDR organisms, including previous exposure to antibiotics, prolonged hospitalization, and healthcare-associated infections. Additionally, the emergence of MDR and XDR organisms was noted in various clinical settings, including intensive care units, surgical wards, and outpatient departments. This study emphasizes the urgent need for effective infection control measures, rational antibiotic prescribing practices, and antimicrobial stewardship programs to combat the spread of MDR and XDR organisms. Understanding the local prevalence, patterns, and associated factors of drug resistance is crucial for tailoring appropriate strategies to mitigate the impact of these organisms on patient outcomes and public health. In conclusion, the findings of this study underscore the alarming prevalence of MDR and XDR organisms at a tertiary care referral center in North India. The study highlights the importance of continuous surveillance, prompt detection, and targeted interventions to prevent the further dissemination of drug-resistant pathogens and preserve the effectiveness of antimicrobial agents.

KEYWORDS: Multi-drug resistance; Extensively drug-resistant; Risk factors; Tertiary care; North India

## Microbiome recruitment in afforestation

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Afforestation of former agricultural land is a key strategy to stop biodiversity loss and combat climate change. However, little is known about how soil microbiomes assemble around tree seedlings during afforestation, or how this process can be influenced to improve restoration outcomes. In this PhD project, I investigate how soil origin—forest versus agricultural—affects the microbial communities associated with young trees, how these communities influence early plant development, and whether soil inoculation can improve seedling health and establishment. The project integrates afforestation experiments with inoculation using soil from an ancient donor forest. Seedlings grown in forest and agricultural soils will be analyzed using amplicon sequencing (ITS for fungi and 16S for bacteria) and compared to tree health metrics. This approach will allow us to track microbiome recruitment over time, assess the legacy effects of land use, and test whether these can be modified through inoculation. Two afforestation experiments are ongoing. The first is an oak seeding experiment, where 1,500 acorns were planted in former agricultural soil with and without soil inoculation. This setup allows testing of inoculation effects from the earliest stages of plant development. The second is a 5-hectare afforestation experiment involving one-year-old oak and maple seedlings inoculated at planting but otherwise managed with standard forestry practices to support future implementation. While data collection is ongoing, this research aims to fill a critical gap in our understanding of belowground processes in afforestation. Ultimately, it may help inform restoration practices that consider microbial dynamics to enhance tree establishment and long-term ecosystem development or alternatively, reveal the limits of microbial intervention under real-world conditions.

**KEYWORDS:** Afforestation; Ecological restoration; Microbiome recruitment; Soil ecology

# Desert soil microbiomes of the Western Iraqi Desert: diversity, function, and biotechnological potential

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Desert ecosystems represent extreme environments where only highly specialized microbial life can persist. This study investigated the composition, functional capacity, and biotechnological potential of microbial communities inhabiting the arid soils of the western Iraqi desert, specifically in Anbar Governorate. Soil samples were collected from multiple locations characterized by high temperatures, low moisture, and limited organic content. High-throughput 16S rRNA amplicon sequencing and whole-metagenome shotgun sequencing were used to characterize taxonomic diversity and functional gene abundance. Bioinformatic analysis revealed a predominance of thermotolerant and halophilic taxa, including members of the phyla Actinobacteria, Firmicutes, and Deinococcus-Thermus. Functional annotation of metagenomic data indicated enrichment in genes associated with oxidative stress resistance, osmotic regulation, and exopolysaccharide biosynthesis. Several biosynthetic gene clusters were identified that are linked to novel antimicrobial and thermostable enzyme production. In addition, a subset of isolates demonstrated plant growth-promoting traits, including phosphate solubilization, siderophore production, and nitrogen fixation. Statistical correlation between microbial community structure and soil physicochemical properties highlighted salinity and moisture content as major drivers of community composition. Laboratory-scale assays confirmed the ability of selected isolates to tolerate extreme conditions and produce industrially relevant enzymes such as amylases and lipases. These findings demonstrate that the desert soil microbiome of western Iraq harbors unique, stress-adapted microbial taxa with significant ecological and biotechnological relevance. This study provides a foundation for future efforts to utilize indigenous extremophiles in applications such as arid-land agriculture, soil restoration, and industrial biocatalysis.

**KEYWORDS:** Desert microbiome; Arid soils; Anbar Governorate; Thermotolerant microorganisms; *Actinobacteria* 

## Biodiversity of algae and fungi in freshwater habitats of Serbia

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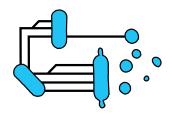
Freshwater ecosystems are of vital importance for humans and biodiversity, and their quality assessment is necessary for the promotion of sustainable water resources. However, comprehensive studies on algal and fungal communities in freshwater bodies in Serbia are limited. This research aimed to analyze the composition of algal and fungal communities of Danube, Sava and slow-flowing waters (SFW) in the territory of the Republic of Serbia. Water was sampled from 2018 to 2022 from 14 localities. Total algal and fungal communities were analyzed by using the Illumina MiSeq sequencing platform using 18S rDNA as barcode region. A comprehensive taxonomic classification was performed with DADA2 pipeline implemented in R studio. Alpha diversity was calculated with Phyloseq package and evaluated by Observed, Chao1, ACE, Shannon, and Simpson indices. Differential abundance analysis was conducted using the DESeq2 package. The distribution analysis at the phylum level revealed that the most prevalent representatives of the fungi were the phyla Ascomycota, Rozellomycota, Mucoromycota, Basidiomycota and Chytridiomycota. At the genus level, Candida, Mortierella and Saccobolus were the most abundant in the Danube and Sava rivers, while Glomus and Malassezia were the most abundant in the Danube, Sava and SFW. The most common representatives of algae at the phylum level were Chlorophyceae, Eustigmatophyceae and Trebouxiophyceae. Some of the enriched genera in the analysed water bodies were identified as Nannochloropsis, Choriocystis, Desmodesmus, Spumella, Navicula and Mantoniella etc. Based on the estimated alpha diversity there were no statistically significant differences in the algal and fungal communities between the Danube, the Sava and SFW. This study provides a first detailed insight into the composition of algal and fungal communities in various water bodies in Serbia that are considered economically or strategically important and provides a basis for future monitoring.

KEYWORDS: Unicellular eukaryotes; Metabarcoding; Freshwater ecosystems; 18S rRNA

#### **ACKNOWLEDGEMENT**

This work was supported by the Ministry of Science, Technology and Innovation of the Republic of Serbia [#Grant Nos. 451-03-135/2025-03/200178 and 451-03-136/2025-03/200178].

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From Genomic Analysis to Functional Models in Microbiomes and Synthetic Consortia

15 - 19 September Belgrade, Serbia









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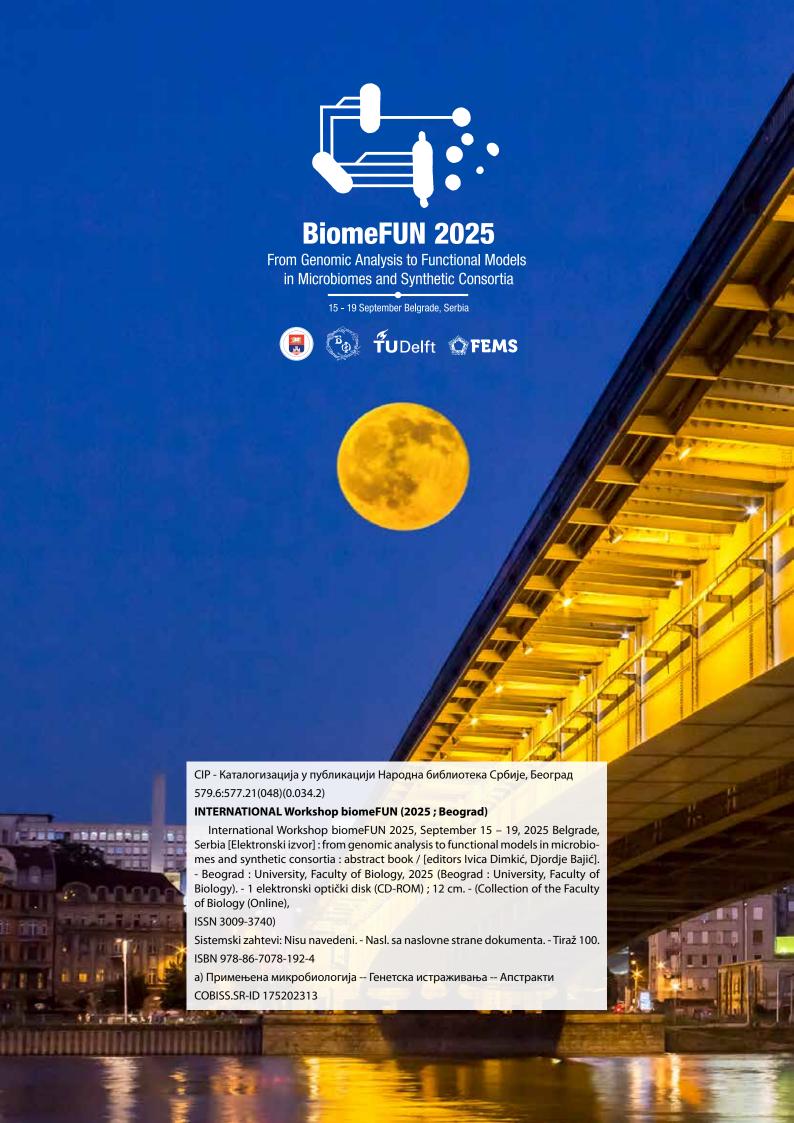
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