EcotoxicoMic 2026

5th International Conference on Microbial Ecotoxicology

12-14 October 2026

Thessaloniki, Greece



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Welcome to EcotoxicoMic 2026

Dear colleagues,

On behalf of the Organizing and the Scientific Committee, I am pleased to welcome you to EcotoxicoMic 2026. The series of EcotoxicoMic conferences was established in the last decade as a leading bi-annual international conference in microbial ecotoxicology. After the first four editions, organized in 2017 in Lyon (France), in 2020 in virtual format, in 2022 in Montpellier (France), and in 2025 in Gothenburg (Sweden) we are now happy to host the 5th EcotoxicoMic Conference in 2026 in Thessaloniki, Greece.

The overall aim of the conference is to provide an up-to-date state-of-the-art in the field of **microbial ecotoxicology** and pinpoint emerging issues and areas of research where future efforts are needed, considering all types of microorganisms including **prokaryotes and microeukaryotes.**

EcotoxicoMic 2026 will bring together scientists from various disciplines (microbial ecotoxicology, environmental microbiology, environmental chemistry, antimicrobial resistance, risk assessment, and big data), in a highly interactive environment.

The scientific programme covers presentations from invited speakers and presentations selected from submitted abstracts along with pitch presentations. There will be ample opportunities to present posters and there will be three poster corners where presenters will have the opportunity to present their posters and discuss with the audience.

EcotoxicoMic 2026 comes timely to highlight the central role of microorganisms in polluted environments as:

- Mediators of pollutant degradation and detoxification, and potential vehicles for ecosystem remediation;
- Ecotoxicological indicators revealing pollutant effects on biodiversity and ecosystem functioning, valuable for risk assessment;
- Carriers of antibiotic resistance genes, promoting dispersal across environmental compartments within the One Health framework.

This edition will also broaden its scope to explore new and exciting research areas:

- Interactions between microbiomes and nature-based or bio-based solutions;
- Pollutant–microbiome interactions in engineered environments;
- Impacts of climate change on pollutant–microbiome dynamics

We are pleased to welcome you to Thessaloniki for EcotoxicoMic 2026!

Professor Dimitrios Karpouzas, University of Thessaly, Greece
Chair of EcotoxicoMic 2026

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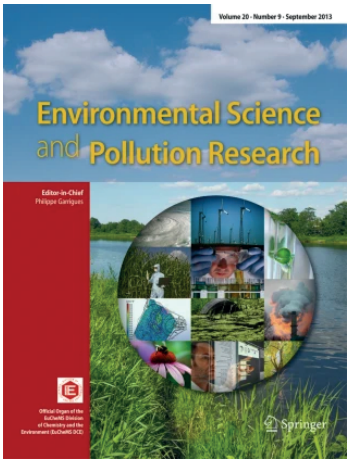
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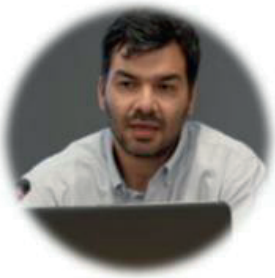
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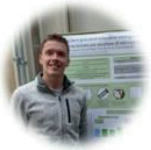
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Venue

The conference takes place at **Thessaloniki Concert Hall** which is located on the east side of Thessaloniki coastal area.

Address: 25 Martiou Street, Thessaloniki 54646, Greece

Conference Center website:https://www.tch.gr/



Conference dinner

The **conference dinner** will be held on **Tuesday** evening, 13th October at 20.30 at **Kreonidis**

"The Balcony" tavern which is located in the outskirts of the Acropolis of Thessaloniki (or upper city) overlooking the Thermaikos gulf.
Dinner tickets are required for this event and there are only 100 seats available.

Website: https://kreonidis.gr/



Program at a glance

Time	12 October 2026, Monday	Time	13 October 2026, Tuesday	Time	14 October 2026, Wednesday
		8.30-10.30	**Session 4 - Biodegradation of Pollutants**	8.30-10.00	**Session 6 - Microbes - Pollutants and Bioprocess Engineering**
		10.30-11.00	Coffee Break	10.00-10.30	Coffee Break
		11.00-13.00	**Session 4 - Biodegradation of Pollutants (continue)**	10.30-12.10	**Session 5 - Microbes - Pollutants under Climate Change Scenarios**
11.00-12.30	Registration - Opening	13.00-14.00	Lunch Break	12.10-13.30	Lunch Break
12.30-15.00	**Session 1 - Antimicrobial Resistance**	14.00-15.40	**Session 3 - Natural Toxins - Microbiota**	13.30-15.30	**Session 2 - Effects of Pollutants on Microbes**
15.00-15.30	Coffee break	15.40-16.00	Coffee Break	15.30-16.00	Coffee Break
15.30-17.00	**Session 1 - Antimicrobial Resistance (continue)**	16.00-17.00	**Round Table What is new in regulating pesticides toxicity on the soil microbiota**	16.00-18.00	**Session 2 - Effects of Pollutants on Microbes (continue)**
17.00-18.30	Poster Session - Welcome Reception	17.00-18.00	Poster Session	18.00-18.30	Closure of the Meeting - Awards
		20.30	Social Dinner		

Scientific programme

Time	12 October 2026, Monday
11.00-12.30	**Registration - Opening**
	Session 1 - Antimicrobial Resistance **(Chairs: Lise Barthelembs, Sotirios Vasileiadis, Ed Topp)**
12.30-13.10	**Plenary Lecture -** Uli Klümper - Predicting environmental antimicrobial resistance spread from ecological and evolutionary mechanisms
13.10-13.30	**Riva Valentina** - Deciphering the dynamics of antimicrobial resistance transfer through natural transformation in bacterial communities
13.30-13.50	**Vaccari Filippo** - AI-assisted review of microplastics mediated antimicrobial resistance gene exchange
13.50-14.10	**La Thu Phuong** - Exposure to pathogens and antibiotic resistance during community composting in urban areas: a case study of the Rennes metropolitan area
14.10-14.30	**Udikovic-Kolic Nikolina**- Microbial community structure and antibiotic resistance genes in Dinaric karst groundwater under contrasting hydrological conditions
14.30-14.50	**Baroudi Malek** - Micropollutant-driven restructuring of microbial diversity and resistome dynamics in receiving waters under effluent discharge
14.50-14.55	**Jendris Marija** - Seasonal dynamics of antibiotic resistance genes in Dinaric karst groundwater: a one-year monthly monitoring study
15.00-15.30	**Coffee Break** *Lab Supplies Scientific*
	Session 1 - Antimicrobial Resistance - continued **(Chairs: Francesca Mapelli, Kostas Kormas)**
15.30-15.50	**Lamprou Eleni** - Plastisphere-mediated microbial adaptation in contaminated agricultural soils
15.50-16.10	**Valmas Marios** - Persistence, dissipation and hazard traits of the pig-manure microbiome, resistome and mobilome in an agricultural soil
16.10-16.30	**Mejia Camacho Ana Luisa** - Limited persistence and ARG dissemination of a multidrug-resistant *Escherichia coli* in freshwater sediment under sub-inhibitory antibiotic exposure
16.30-16.50	**Gkoni Ioanna** - Sediment characteristics modulate the effects of antibiotics and coalescence on microbial diversity and resistome after soil erosion
16.50-16.55	**Agosti Maria Sofia** - Year long study of AMR and pathogens transfer in the Gurri river
16.55-17.00	**Martin Jeans -** Dynamics of bacterial resistance to antibiotics (BRA) in a small rural watershed in Ardèche (France) as influenced by the hydrological regime, soil use and human activities
17.00-18.30	**Poster Session** **Welcome Reception**

Scientific programme

Time	13 October 2026, Tuesday
	Session 4 - Biodegradation of Pollutants (Chairs: Ines Petric, Stefan Vuilleumier, Evangelos Topakas)
8.30-9.10	**Plenary Lecture - Djorde Bajic** -Emergent interactions shape the effects of metabolites on the assembly of microbial communities
9.10-9.30	**Bergese Francesco** - PFAS-driven restructuring of sediment fungal microbiota: selecting tolerant fungi for future bioremediation
9.30-9.50	**Nielsen Tue Kjærgaard** - Mobilization of genes encoding potential PFAS-degrading enzymes: positive genetic selection in cyanobacteria and potential for bioremediation
9.50-10.10	**Gallois Nicolas** - Unraveling the fate of phenanthrene in a microbe-poplar system: linking the active microbiome to metabolic pathways
10.10-10.30	**Spina Federica** - Unveiling the potential of fungi as bioremediation agents against CECs
10.30-11.00	**Coffee Break** ANTISEL
	Session 4 - Biodegradation of Pollutants - continued (Chairs: Katharine Thompson, Zisis Vryzas)
11.00-11.20	**Quinn Andrew** - Mapping reciprocal metabolic interactions between the honeybee gut microbiota and an agrochemical library
11.20-11.40	**Dhommee Roxane** - Microbial degradative capacities of *Rhodococcus* sp. as a strategy to reduce pesticide metamitron pollution in agricultural systems
11.40-12.00	**Krappmann David** - Surfactants in glyphosate-based herbicides may stimulate glyphosate-degradation in agricultural soil
12.00-12.20	**Khalfaoui-Hassani Bahia** - Response of marine bacteria to contaminants of emerging concern: resistance, biodegradation and surfactant-mediated interactions
12.20-12.40	**Saint-Picq Camille** - Revealing marine plastic degraders using stable isotope tracers
12.40-12.45	**Huđ Anastazija** - From pollution to remediation: tolerance to diverse pollutants and functional potential of marine sediment fungi
12.45-12.50	**Mion Sonia** - From pollution to solution: The hunt for nature's dehalogenases
12.50-12.55	**Perruchon Chiara** - Isolation and characterization of a *Piscinibacter* sp. bacterium able to degrade the nitrification inhibitor DMPP
12.55-13.00	**Cebron Aurélie** - Identification of bacteria and metabolic genes involve in lindane degradation using DNA-SIP and metagenomics
13.00-14.00	**Lunch Break (13.00-13.15 Ecotoxicomic Network Working Group "Opening the Steering Committee to International Members" C. Thiour-Mauprivez)**
	Session 3 - Natural Toxins - Microbiota (Chairs: E. Papadopoulou, C. Tassou, K. Brandt, E. Puglisi)
14.00-14.40	**Plenary Lecture - Paolina Garbeva** - The Good, the Bad and the Biodegradable: Rethinking plastics in agriculture

Scientific programme

14.40-15.00	**Charrier Fanny** - Biopesticide-induced responses in periphytic microbial communities: New insights from an untargeted meta-metabolomic approach
15.00-15.20	**Osipenko Maria** - Effects of pelargonic acid on soil microbial community abundance, diversity and function
15.20-15.25	**Vryzas Zisis** - Fit-for-purpose risk assessment for low-risk pesticides
15.25-15.30	**Moutzoureli Chrysovalantou** - Biological nitrification inhibitors: Curse or blessing for the soil microbiota
15.30-15.35	**Beeckman Fabian** - High-throughput Microtox screening of non-target effects of biological nitrification inhibitors
15.35-15.40	**Solve Jonathan** - Probing for bioavailable nitrification inhibitors in rhizosphere soil using a novel, high-throughput *Nitrosomonas europaea* solid-phase contact bioreporter assay
15.40-16.00	**Coffee Break**
16.00-17.00	**Round Table - What is new in regulation for assessing pesticides soil microbial toxicity (D. Karpouzas, C. Sweeney, F. Martin-Laurent, V. Ducrot, M. Arena)**
17.00-18.00	**Poster Session** Poster Corner 1 - Tracking antimicrobial resistance in aquatic environments Poster Corner 2 - Bioindicators for Environmental Assessment Poster Corner 3 - Advances in pollutants biodegradation and bioremediation
20.30	**Social Dinner** - Tavern Kreonidis

Scientific programme

Time	14 October 2026, Wednesday
	Session 6 - Microbes - Pollutants & Bioprocess Engineering (Chairs: C. Pateraki, S. Ntougias, P. Kougias)
8.30-9.10	**Plenary Lecture - Korneel Rabay** - New rules, new opportunities – novel bioprocesses for a more sustainable urban water cycle
9.10-9.30	**J.M.F. Martins** - Improving microbial tolerance to metal mixtures from printed circuit boards (PCB) by accelerated adaptive evolution in the laboratory
9.30-9.50	**Nikolaidou Charitini** - Chemical rectification of different soil textures by the utilization of optimized biochar-digestate mixtures
9.50-10.00	**Pateraki Chrysanthi** - Valorization of the Organic Fraction of Municipal Solid Waste, Poly(3-hydroxybutyrate), enzymatic degradation and upcycling for circular bio-based polymers
10.00-10.30	**Coffee Break**
	Session 5 - Microbes - Pollutants under Climate Change (Chairs: S. Genitsaris, M.Schmitt-Jansen, M. Omirou)
10.30-11.10	**Plenary Lecture - Josephine Leflaive** - Biofilm response to stressors is determined by their thermal and hydrological history
11.10-11.30	**Susset Louis** - Combined exposure to heat waves and antibiotics shows interacting effects on community tolerance and molecular responses in freshwater biofilms
11.30-11.50	**Pinheiro Costa Pimentel Mariana** - Freshwater periphyton meta-metabolome unravels environmental drivers of spatial microbial metabolism heterogeneity: a predictive approach
11.50 - 12.10	**Mathonat Frédéric** - Bacterial contribution to nitrogen processing in the atmosphere
12.10-13.30	**Lunch Break**
	Session 2 - Effects of Pollutants on Microbes (Chairs: C. Bonnineau, C. Sweeney, P. Karas)
13.30-14.10	**Plenary Lecture - Emillie Muller** - Degradation potential is not enough: Effective bioremediation involves microbial community adaptation to environmental conditions
14.10-14.15	**Papadopoulos Christos** - Understanding the mechanism of interspecies variation in the tolerance of *Rhizophagus* species to glyphosate
14.15-14.20	**Rolli Eleonora** - Sunflower cry-for-help in presence of petroleum hydrocarbons (PHCs) alters the root exudation chemistry, potentially affecting rhizocompetence of PHC-degrading bacteria
14.20-14.40	**Schmitt-Jansen Mechthild** - The Plastisphere: what drives the structure and functioning of plastic-colonizing microorganisms?

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Scientific programme

14.40-15.00	**Gréau Lilian** - Investigating multi-contaminant profiles across the European coastline and its impact on benthic biodiversity
15.00-15.20	**Kolovou Maria** - Using species sensitivity distributions to assess the toxicity of pesticides on nitrifiers and broader microbial groups: from single-species tests to soil amplicon sequencing
15.20-15.25	**Lasserre Claire** - Lake environment shapes macrophyte-associated microbial communities more than host species
15.25-15.30	**Duperron Sebastien** - How eutrophication shapes microbial communities in peri-urban lakes: insights from the COM2LIFE project
15.30-16.00	**Coffee Break**
	Session 2 - Effects of Pollutants on Microbes - continued (Chairs: N. Corcoll, S. Geisen, M. T. Marx)
16.00-16.20	**Dalkidis Dimitrios** - Synthetic nitrification inhibitors DMPP and allylthiourea use distinct mechanisms to inhibit an archaeal soil nitrifier
16.20-16.40	**Ljesevic Marija** - Microbial adaptation to PFAS stress: Proteomic response of environmental *Pseudomonas chlororaphis* exposed to PFOA
16.40-17.00	**Alavikakhki Zahrasadat** - Kinetic multi-level responses of periphyton to pharmaceuticals reveal early sub-threshold effects
17.00-17.20	**Griffero Luciana** - Using microbial indicators to predict ecotoxicological risk from emerging contaminants in South Atlantic coastal lagoons
17.20-17.40	**Chapeau Florian** - Ecotoxicology of Graphene Oxide in Aquatic Organisms: From Microbiota Disruption to Biological Transformation
17.40-18.00	**Hampel Justyna** - Beyond sediment contaminant burdens: eDNA biodiversity reveals ecologically relevant exposure to environmental contaminants across the Baltic Sea
18.00-18.30	**Closure of the Meeting - Awards**

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Posters

Poster No.	Presenter		Title
P0-1	Ana	Puljko	Environmental and clinical *Stenotrophomonas maltophilia* isolates from Croatia and their potential role in antibiotic resistance dissemination
P0-2	Vanessa	Moreira	Evidence for upgrading wastewater regulation: antimicrobial resistance persistence after secondary treatment
P0-3	Hongo Koffi	Anderson	Metagenomic characterization of antimicrobial resistance genes in lake Kassembie, Cote d'Ivoire (West Africa)
P0-4	Lluis	Bertrans-Tubau	Nature-based solutions to reduce antimicrobial resistance spread from urban wastewater treatment plants
P0-5	Anita	Solem	Wastewater-seeded plastic biofilms as reservoirs of antibiotic resistance in marine environments
P0-6	Stavroula	Makri	A new whole genome sequencing analysis tool for risk assessment of microbial pesticides
P0-7	My Dung	Jusselme	Advanced wastewater treatment: efficiency on antimicrobial resistance
P0-8	Sonja	Fagervold	Aquatic biofilms as possible reservoirs for antibiotic resistance genes; a case study of the Tech river (France)
P0-9	Chloen	Lhomme	Tiered assessment of the toxicity of veterinary medicines on the soil microbiota: from in vitro to soil assays
P0-10	Oriane	Della-Negra	Biotic and abiotic drivers of antimicrobial resistance selection and mitigation in soils amended with livestock effluents
P0-11	Lena	Brouwir	Fate and removal of antibiotics and antibiotic resistance genes in a rural wastewater treatment plant: a microbial perspective of nature-based versus advanced technologies
P0-12	Marina	Héry	Tracking antimicrobial resistance transfer in a Karst aquifer under flood conditions: An ecological approach to health
P0-13	Elisa	Catao	Unveiling antimicrobial resistance in marine coastal biofilms using metagenomics

Posters

Poster No.	Presenter		Title
P0-14	Benjamin	Alric	A new framework for ecosystem assessment through the integration of eDNA inventories with graph theory and niche modeling
P0-15	Esteban	Nieto	Alteration of prey-predator interactions due to pesticide pollution: its impacts on the nitrogen cycle
P0-16	Betty	Chaumet	Biofilm biochemical composition shapes the toxicity of pesticide mixtures
P0-17	Tobias	Servulo	Chemical contamination gradients drive spatio-temporal dynamics of diatom communities in marine biofilin in a highly anthropized Mediterranean bay
P0-18	Agnès	Richaume	Direct vs digestate-mediated contamination of soil with LDPE: Evaluation of microplastic and additives toxicity in a multi species-context and functional effects on key microbial processes
P0-19	Ines	Petrić	When pollutants collide: microbial life under multi-stressor pressure
P0-20	Audrey	Vauloup	Ecotoxicological impact of PFAS on microbial communities and plants during phytoextraction assays
P0-21	Laís	Conceição Menezes da Silva	Environmental and land-use drivers of fungal and bacterial diversity in ponds
P0-22	Alessandra	Narciso	Environmental compatibility of lunar regolith using *Aliivibrio fischeri* as a sensitive model organism and a natural soil microbial community as an adaptable biological entity
P0-23	Kleinjan	Hetty	Evaluation of biological quality elements using periphyton genomics:from community profiling to novel biological indices for in field assessment
P0-24	Olwen	Simenel	Evolution of energetic metabolism and arsenic resistance genes in microbiome of mine residues during phytostabilization
P0-25	Lena	Brouwir	A periphyton-based genomic bioindicator of surface water quality: insights from a field data
P0-26	Gilles	Quabron	MICROSENSE : integrating microbial bioindicators and functional biomarkers for pesticides water quality assessment

Posters

Poster No.	Presenter		Title
P0-27	Vincent	Tardy	Using PICT approach to diagnose chemical pressure in aquatic ecosystems: towards an operational framework
P0-28	Elena	Gorokhova	From microbiome shifts to functional evidence: quantitative indicators for environmental assessment of chemical stressors
P0-29	Jean-François	Briand	From the Atlantic to the Mediterranean: how environmental factors, colonisation kinetics and polymer type shape diatom diversity in the plastisphere
P0-30	Satti Sadia	Mehmood	Impact of plastic mulch film (polyethylene) on rhizosphere microbial community structure and functions
P0-31	Désoeuvre	Angélique	Linking taxonomic and functional microbial signatures to trace arsenic sources during flood events in a mining-impacted watershed
P0-32	Saleem	Fatima	Bacterial Community Composition and Structure in Urban Ponds and Their Potential for Organic Contaminant Degradation
P0-33	Maiwenn	Le Meur	Influence of cyanobacterial blooms and associated bioactive metabolites on fish gut microbiota
P0-34	Maëlann	Roger	Influence of early-life contaminant exposure on the bacteriome structure and the response to a viral infection in oyster
P0-35	Lin	Zi	Linking community-level metabolic shifts to pesticide modes of action in aquatic periphyton: a high-throughput metabolomics approach
P0-36	Olivia F	Sieniawski	Low concentrations of 'inert' surfactants inhibit nitrifying microorganisms
P0-37	Elisa	Catao	Metagenomic and genome-resolved Insights of marine biofilms resistome across contrasted coastal environments
P0-38	Ivana	Babić	Microbial eDNA signatures in the Adriatic: linking sediment signatures and sea urchin gut health to plastic pollution
P0-39	Clemence	Thiour Mauprivez	Microplastic and herbicide degradation by the soil bacterium *Cupriavidus necator* JMP134
P0-40	Catherine	Badel	New approaches in genomics of microbial ecotoxicology

Posters

Poster No.	Presenter		Title
P0-41	Asadulla	Galib	Periphyton based effect directed analysis to link the chemical exposome to functional impairment of microbial ecosystems
P0-42	Thibaut	Ponchon	Pesticides modify bacterial-fungal interactions during leaf-litter decomposition in streams
P0-43	Abiola	Akinwale	Pharmaceuticals in waste water treatment plants: characterizing the microbial diversities and the biodegradation under ketoprofen and oxazepam pressure
P0-44	Mathilde	Vincent	Pollution footprints in paradise: impacts of hydrocarbons, PCBs, and metals on a polynesian atoll soil microbiomes
P0-45	Milena	Frelih	Potential impact of nanomaterials on aquatic microbial communities
P0-46	Evangelia S.	Papadopoulou	A tiered approach combining nitrifier single-species tests and OECD 216 to assess pesticide effects on soil microorganisms
P0-47	David	Hartnell	Beyond growth inhibition: lipidomic indicators of chemical stress in microalgae using high resolution mass spectrometry.
P0-48	Julian	Schumacher	Co-stressor dynamics reprogram arsenic detoxification under salt stress in *Chlamydomonas reinhardtii*
P0-49	Stathis	Lagos	Combined effects of microplastics and organic pollutants on soil microbial functions and plant growth in agricultural soil
P0-50	Giulia	Cheloni	Contaminants impact on diatom resting spores
P0-51	Amélie	Polrot	Impact of glyphosate and its transformation product, AMPA, on soil microbial communities
P0-52	Anniet	Laverman	Impact of metolachlor and metolachlor ESA on microbial and denitrifying activity in soils and river sediments in an agricultural watershed in Brittany (France)
P0-53	Anniet	Laverman	Impact of Quaternary Ammonium Compounds (QACs) on the environmental denitrifier *P. veronii*
P0-54	Lauris	Evariste	Integrated assessment of nano-formulated and conventional copper-based pesticides on microbiota across soil, aquatic organisms and mice within a One Health framework

Posters

Poster No.	Presenter		Title
P0-55	Vanessa	Moreira	Integrating DNA metabarcoding into diatom-based regulatory frameworks:bridging biodiversity analysis and water quality assessment
P0-56	Conrad	Marshall	Interpreting toxicant-induced microbiome shifts in *Daphnia magna* requires baseline characterization
P0-57	Marina	Héry	Karst microbiome dynamics as tracers of water circulation and contamination pathways in complex hydrosystems
P0-58	Francesca	Arici	Native bacteria mitigate diuron and copper toxicity to microalgae isolated from a chronically contaminated coastal lagoon
P0-59	Christopher	Sweeney	OECD 216: a case study exploring reliability, sensitivity, and test design for soil microbial pesticide risk assessment
P0-60	Stephane	Pesce	PICT approach applied to pharmaceuticals: influence of mixture effects on periphyton tolerance acquisition
P0-61	Sotirios	Vasileiadis	Updated *mcrA* reference databases for methanogen community profiling with amplicon sequencing
P0-62	Chiara	De Carolis	Response of a soil microbiome-plant system to drought stress under different inputs of organic amendment
P0-63	Paraskevi	Amanatidou	Assessment of environmental factors regulating BNI activity in *Trigonella foenum-graecum* using soil-relevant ammonia-oxidizing microorganisms
P0-64	Agapi	Doulgeraki	Chemical profiling and antimicrobial activity of birch bark and propolis extracts obtained using sustainable solvents
P0-65	Panagiotis	Karas	Determination of the background levels of microorganisms used as plant protection products in EU soils using shotgun metagenomics and q-PCR approaches
P0-66	Fotios	Bekris	Exogenous application of dsRNA induces soil-dependent changes on fungal but not on bacterial and protists communities
P0-67	Agapi	Doulgeraki	Impact of biological agents and biopesticides derived from tomato by-products on human health and environmental risk assessment

Posters

Poster No.	Presenter		Title
P0-68	Despoina	Zoumpourtikoudi	Microbial metabolites used as biopesticides
P0-69	Annika Grubbe	Eising	Screening novel winter wheat breeding lines for biological nitrification inhibition using a soil-based phenotyping pipeline
P0-70	Aymé	Spor	Ecological engineering of herbicide- and antibiotic-degrading microbial consortia using fragmentation and coalescence approaches
P0-71	Lazaros	Pantelidis	Detoxification of effluent in hybrid nano-innovative NBS solutions
P0-72	Orlando	Riquelme	From agricultural waste to functional nanomaterials: ecotoxicity and bactericidal activity of green synthesized copper oxide and magnetite nanoparticles using grapevine cane extracts
P0-73	Abdulsamie	Hanano	Genome-based analysis of *Bacillus velezensis* Bm3 isolated from a vulnerable dioxin-contaminated site in central Syria reveals mechanisms of dioxin degradation
P0-74	Nolhan	Berceaux	Identification and large-scale functional characterization of phytochelatin synthases across the fungal kingdom
P0-75	Ahmad	Shaban	Mechanism-first AI framework for discovering FAcDs for PFAS bioremediation
P0-76	Enrico	Bocconetti	Bacterial defluorination from fluoroacetate to short-chain PFAS
P0-77	Francesca	Mapelli	Rhizosphere microbiome engineering and root exudate metabolites as strategies to enhance the bioremediation of petroleum hydrocarbons in polluted soils
P0-78	Aneta K.	Urbanek	Adaptive Laboratory Evolution (ALE) towards microbial degradation of polyethylene terephthalate (PET)
P0-79	Johan	Vandewalle	Assessing the potential of dichloromethane degradation in environmental samples through the absolute DNA quantification of two biomarker loci
P0-80	Sana	Romdhane	Bioaugmentation using 2,4D-degrading bacterial biocomposites: Efficiency, stability and unintended ecological impacts

Posters

Poster No.	Presenter		Title
P0-81	Luigi	Bazzana	Bioaugmentation with a synthetic community mitigates petroleum hydrocarbon- induced stress in sunflower by shaping the rhizosphere microbiome
P0-82	Abiola	Akinwale	Biodegradation of atrazine : insights from consortia and isolated strains in full-scale wastewater treatment plants
P0-83	Eleni	Tsiami	Exploring bacterial N-acetylation of harmful halogenated anilines: Implications for ecotoxicology and environmental health
P0-84	Konstantina	Rousidou	Functional metagenomic discovery and characterization of a pyrethroid-hydrolyzing esterase from a biobed system
P0-85	Aurélie	Cebron	Identification of bacteria and metabolic genes involved in lindane degradation using DNA-SIP and metagenomics
P0-86	Mateusz	Szczepańczyk	Improvement of polycaprolactone (PCL) microbial degradation by Adaptive Laboratory Evolution (ALE)
P0-87	Chiara	Perruchon	Isolation and characterization of a *Piscinibacter* sp. bacterium able to degrade the nitrification inhibitor DMPP
P0-88	Sonia	Gaaied	Isolation of PFAS-degrading bacteria and assessment of microbial diversity in contaminated soil in North Italy
P0-89	Venetios	Michelioudakis	Novel HRMS approaches for identifying transformation products of the antiparasitic toltrazuril, combined with predictive tools to assess their environmental fate.
P0-90	Fadwa	Khalfallah	Who recovers, Who fails? Microbial responses to drought along an urban land use gradient
P0-91	Michalis	Omirou	DNA-based assessment of tetracycline-driven restructuring of root-associated and bulk soil bacterial communities in a Mediterranean oregano agroecosystem
P0-92	Jennifer	Harris	Evaluating nitrate transformation in aquifers using microbiological, molecular and isotopic tools for enhanced management of key groundwater ecosystem services.

Posters

Poster No.	Presenter		Title
P0-93	Magdalena	Hizak	From microbes to drought resilience: integrating SynComs and climate-risk modelling in agriculture
P0-94	Nicolas	Gallois	Unveiling the microbial filtering capacity of sandy beaches facing climate change and chemical pollution
P0-95	Hamza	Benrahma	Application of bacterial bioluminescence (Microtox assay) as a method for evaluating the toxicity of pure compounds and complex effluents along the Moroccan Atlantic coast
P0-96	Branka	Lončarević	Soil microbial respiration and ecotoxicity reveal the environmental compatibility of modified levan biopolymers
P0-97	Marta	Sena Velez	Black poplar root mucilage mediates plant-bacteria communication and contributes to trace metal stabilization in soil
P0-98	Sofie	Bollen	Experimental evaluation of predicted PFAS defluorinating enzymes in *Microcystis aeruginosa*
P0-99	Thomas	Casuccio	Influence of hydrological connectivity and surface inputs on groundwater microbial communities across contrasted French aquifers
P0-100	Eva	Hojgaard Jensen	Nothing is forever: Analogue enrichment on acetate yields a microbial consortium that defluorinates the ultra-short-chain PFAS trifluoroacetate
P0-101	Cécile	Lelong	Understanding the effects of perfluoroalkyl substances (PFASs) on bacteria
P0-102	Mathieu	Delitte	**I**ntegrated multi-level analytical framework for the quality assessment of microbial biocontrol formulations: a proof of concept developed within the BAC-Id project

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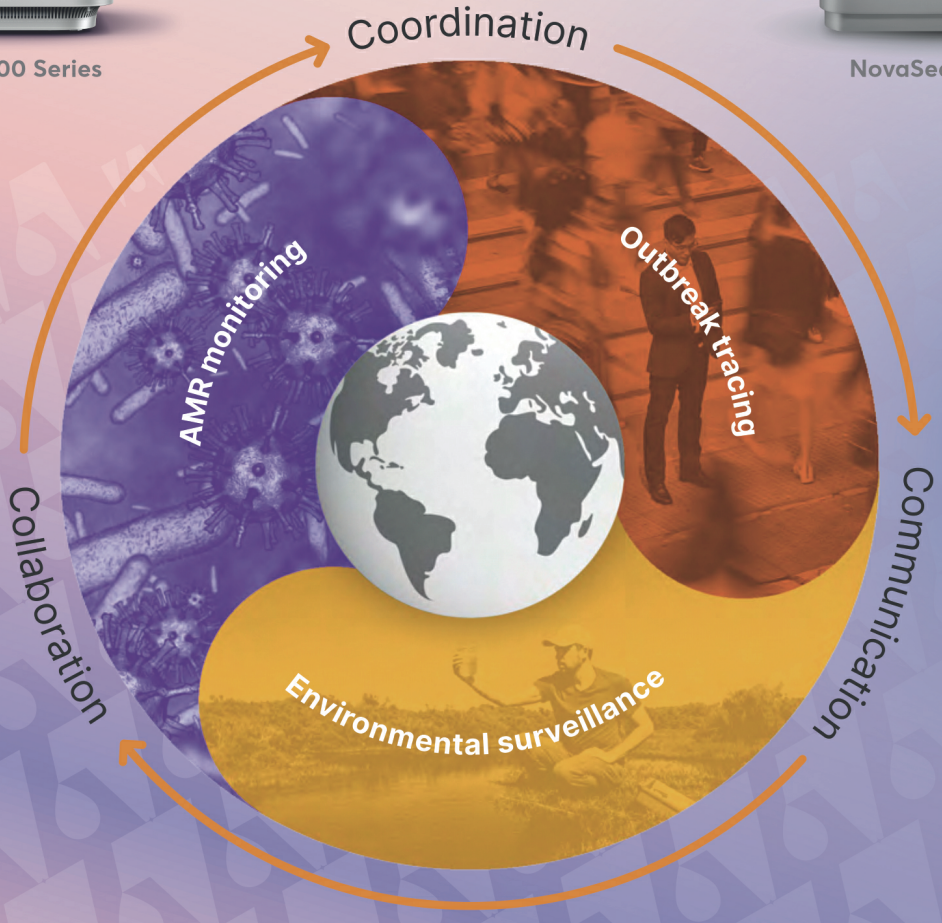
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Session 1

Antimicrobial Resistance

Invited speaker

Predicting environmental antimicrobial resistance spread from ecological and evolutionary mechanisms

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The environmental spread of antimicrobial resistance (AMR) is shaped not only by the presence of resistant bacteria and resistance genes, but also by ecological and evolutionary mechanisms that determine their persistence, transfer, and establishment. Across interconnected One Health compartments, these mechanisms are influenced by microbial community composition, biodiversity, host range, interspecies interactions, chemical stressors, nutrient availability, temperature, and hydrological connectivity. A central challenge is to identify which processes are sufficiently consistent and measurable to support prediction of environmental AMR trajectories.

This presentation examines how mechanistic understanding can improve prediction of three major components of environmental AMR spread: invasion by antibiotic-resistant bacteria, resistance gene transfer, and selection under environmental stress. Particular emphasis is placed on microbial communities as natural barriers to dissemination. Standardized invasion experiments using freshwater biofilms collected from 40 river sites across seven European countries showed that persistence of an introduced antibiotic-resistant Escherichia coli was strongly structured by community properties. Higher Shannon diversity and greater mean phylogenetic distance from the invader predicted faster bacterial loss, while biodiversity loss during transfer and acclimatization increased susceptibility to invasion. These findings show that invasion outcomes can be predicted from measurable indicators of niche saturation, phylogenetic structure, and community stability.

Predictability also extends to resistance gene dissemination. Experiments with conjugative and mobilizable resistance plasmids showed that plasmid co-residence systematically reshaped their incorporation into extracellular vesicles. Although the conjugative plasmid dominated vesicle cargo when present alone, dual carriage reproducibly enriched the mobilizable plasmid and depleted the conjugative plasmid relative to intracellular abundance across two environmental bacterial hosts.

Together, these examples show that environmental AMR spread follows identifiable and partially predictable ecological and evolutionary rules. Integrating biodiversity, phylogenetic structure, community stability, plasmid interactions, mobility, and selection into surveillance and risk assessment could improve forecasts of AMR establishment and dissemination and support targeted ecosystem-based mitigation strategies.

Acknowledgments: This work was supported by the JPIAMR TEXAS and, the JPIAMR SEARCHER project as well as by the ANTIVERSA project (BiodivERsa2018-A-452) here funded by the German Bundesministerium für Forschung, Technologie & Raumfahrt under grant numbers 01KI2401 & 01KI2404A & 01LC1904A.

Keywords: antimicrobial resistance; microbial invasion; horizontal gene transfer; plasmids; extracellular vesicles; microbial biodiversity; ecological predictability; One Health; environmental risk assessment

Deciphering the dynamics of antimicrobial resistance transfer through natural transformation in bacterial communities

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Agri-food ecosystems are key hotspots for antimicrobial resistance diffusion within the One Health perspective, as antibiotic-resistant bacteria and resistance genes (ARGs) can enter these environments via wastewater treatment plant (WWTP) effluents discharged into surface waters and reused for irrigation. Natural transformation, a mechanism of Horizontal Gene Transfer (HGT) driving ARGs spread, has been mainly studied in single bacterial populations using a limited number of model strains. Therefore, the role of multi-species interactions in modulating natural transformation in complex microbial communities remains poorly understood. Here, we investigated the effect of environmental bacterial strains on the transformation frequency of the model strain Acinetobacter baylyi BD413, using the plasmids pSEVA431-Gfp and pZR80(gfp) as extracellular DNA. Environmental isolates were obtained from surface waters collected downstream a WWTP and selected based on their relevance in the One Health framework. In particular, the selection focused on bacterial genera able to interact with multiple hosts, to act as opportunistic pathogens and took into account the antibiotic resistance profiles. Natural transformation assays revealed a consistent decrease of one order of magnitude in the transformation frequency of A. baylyi in the presence of Acinetobacter, Klebsiella and Escherichia environmental isolates, regardless of the plasmids used. These strains neither acquired the plasmids nor exhibited DNase activity under the tested conditions, suggesting that alternative mechanisms could be involved. We therefore hypothesized a “dilution effect” driven by the presence of additional non target bacterial species in the adopted in vitro system. This hypothesis was supported by the reversion of the phenomenon using increasing quantity of plasmids or decreasing concentration of the environmental strains. Overall, the data suggests that it is pivotal to integrate the effects of multi-species interactions to better estimate HGT occurrence into the environment.

Keywords: Microbiome, Multi-species interactions, Acinetobacter baylyi, One Health, Horizontal Gene Transfer

Micropollutant-Driven Restructuring of microbial diversity and Resistome Dynamics in receiving waters under Effluence Discharge

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Despite wastewater treatment processes, many micropollutants are not completely removed and can be discharged into aquatic environments, where they may contribute to antimicrobial resistance (AMR). Understanding how these chemical mixtures influence environmental resistomes has become a major concern within a One Health framework. In this study, we investigated how wastewater-derived micropollutants affect microbial diversity and resistome dynamics in receiving waters, using the Seine River as a case study.

Laboratory microcosms were established using surface water of the Seine River and exposed to extracted-micropollutants from wastewater at concentration of 40, 200, and 280 µL per 400 mL. Microbial and resistome dynamics were monitored at different time points 0 day, 7 days, and 14 days using shotgun metagenomics sequencing.

Taxonomic analyses revealed restructuring of microbial communities, notably marked by expansion of *Acinetobacter johnsonii* across exposed conditions and the biotic control, suggesting microcosm-driven shifts independently of micropollutant exposure. In parallel, taxa associated with xenobiotic degradation, including *Sphingobium*, *Sphingomonas*, *Sphingopyxis*, and *Pseudomonas*, showed stronger enrichment under micropollutant exposure.

Consistent with these microbial shifts, exposed conditions displayed enrichment of multidrug efflux-associated ARG families, particularly resistance-nodulation-cell division (RND) and major facilitator superfamily (MFS) transporters, alongside depletion of tetracycline- and glycopeptide-associated resistance determinants. The intermediate contamination scenario showed the strongest selective enrichment profile, notably involving the plasmid-associated fluoroquinolone efflux gene *QepA4*.

Several enriched ARGs were additionally associated with insertion sequences and plasmid- like contigs. In particular, the multidrug efflux-associated gene *mdsB* shifted from association with insertion sequences at J7 to a plasmid-associated contig at J14, suggesting potential mobilization of resistance-associated genetic elements under micropollutant exposure.

These results suggest that wastewater-derived micropollutants may contribute to microbial and resistome restructuring in receiving waters and influence AMR dynamics under effluent discharge conditions.

Keywords: Antimicrobial resistance, Micropollutants, Shotgun metagenomics, Wastewater,Aquatic microbiomes

Sediment characteristics modulate the effects of antibiotics and coalescence on microbial diversity and resistome after soil erosion

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Headwater streams are strongly influenced by terrestrial inputs such as erosion, and contaminants from point and diffuse sources (e.g. pharmaceuticals, organic matter (OM), and soil microorganisms). However, the consequences of such inputs on exposed benthic microbial communities remain poorly defined. We investigated the effects of combined exposure to antibiotics (sulfamethazine or ofloxacin) and soil samples on river sediment bacterial diversity, antimicrobial resistance (AMR), and antibiotic degradation capacity (antibiotrophy). We hypothesized that antibiotics facilitate the establishment of bacteria derived from a soil previously exposed to such chemicals, promoting AMR and antibiotrophy, along with the selective adaptation of pathogens in the post-coalescence community.A 27-day microcosm experiment was conducted using two river sediments with contrasting OM content (High-OM: 12.9%; Low-OM: 1.9%). A full factorial design crossed three antibiotic treatments (no antibiotic, sulfamethazine, ofloxacin) with three soil treatments (no soil, sterile soil, non-sterile soil). Responses were evaluated by assessing bacterial diversity (16S rRNA gene metabarcoding), abundances of AMR-related genes (qPCR targeting sul1, dfrA, blaOXA-20, and intI1 genes) and selected pathogens (qPCR targeting Clostridium perfringens, Klebsiella pneumoniae, and Aeromonas caviae), and sulfamethazine mineralizing capacities (radiorespirometry measurements).

Findings revealed that microbial responses depend on the initial sediment properties. Effects occurred mainly in Low-OM sediment, where bacterial abundance was lower than in High-OM sediment. In the Low-OM sediment, bacterial beta diversity was strongly influenced by soil addition. Antibiotics increased AMR-related gene abundances, an effect amplified by soil addition. While no initial antibiotrophy was observed in the sediments, this capacity emerged only in sulfamethazine-treated Low-OM samples and was suppressed by soil addition. These results demonstrate that antibiotic exposure and microbial coalescence following soil erosion can interact to reshape river sediment microbial diversity, resistome and antibiotic biodegradation capacities. Importantly, microbial communities in Low-OM sediments were more vulnerable to these stressors than those in high-OM sediments.

Keywords: Antimicrobial resistance (AMR); Antibiotrophy; Pharmaceutical pollution; Bacterial communities; Soil-sediment continuum

AI-assisted review of microplastics mediated antimicrobial resistance gene exchange

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Microplastics (MPs) are recognized as potential hotspots for microbial colonization, biofilm formation, and gene exchange. Their persistence, high surface-area-to-volume ratio, and capacity to interact with microorganisms, antibiotics, and extracellular genetic material raise concern about their possible role in the environmental dissemination of antimicrobial resistance genes (ARGs). However, the available literature is highly heterogeneous, with studies differing in polymer type, particle size, concentration, microbial system, exposure conditions, endpoints, and analytical methods. This complexity limits direct comparison and calls for reproducible approaches to structure the evidence. We present an AI-assisted review workflow to identify, screen, extract, and harmonize experimental evidence on microplastic-mediated antimicrobial resistance and horizontal gene transfer. A broad Scopus search was designed to capture studies linking microplastics with antibiotic resistance, antimicrobial resistance, gene transfer, transduction and horizontal-transfer-related processes. After the search and initial filtering, 1100 records were retrieved. Abstracts were processed using an AI-assisted pre-screening script, with relevance scoring optimized by reviewing abstracts around an inclusion threshold and refining the screening prompt to prioritize experimental studies addressing microplastic effects on ARGs, antimicrobial resistance, or horizontal gene transfer. Selected articles were analyzed using Elicit, an AI-based literature review platform that extracts information from scientific papers according to user-defined prompt columns. The prompts assessed study acceptability, extracted structured experimental information, and classified whether a microplastic-associated horizontal gene transfer event was reported, absent, or inconclusive. Extracted variables included plastic polymer, particle size, concentration, plastic background, antimicrobial exposure, ARGs, microbial species or community, exposure time, analytical method, result class, directional result codes, effect percentage, and source notes. Outputs were parsed into a standardized spreadsheet, assigned unique identifiers, and manually refined to verify relevance and exclude studies lacking appropriate microplastic-negative controls. Data analysis is ongoing, and preliminary evidence suggests that microplastics can favour ARG persistence, antimicrobial-resistant bacterial aggregation, and horizontal gene transfer under specific experimental conditions. This workflow provides a semi-automated framework for converting heterogeneous literature into structured evidence supporting environmental antimicrobial resistance risk assessment.

Keywords: Microplastics; Antimicrobial resistance; Horizontal gene transfer; AI-assisted review

Plastisphere-mediated microbial adaptation in contaminated agricultural soils

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Plastics are emerging contaminants in agricultural soils where they interact with co-occurring pollutants such as pesticides and antibiotics. Plastic fragments offer surfaces for microbial colonization, forming a distinct ecological niche known as plastisphere, and at the same time support sorption of pesticides/antibiotics on their hydrophobic surfaces. We hypothesized that these tripartite interactions (plastics–pesticides/antibiotics–microbiota) make plastisphere a hotspot of microbial adaptation reflected in enhanced dynamics of pesticide catabolic traits and antibiotic resistance genes (ARGs) in the plastisphere compared to soil. To test this hypothesis, a soil microcosm experiment was conducted. LDPE-based and PBAT- based macroplastics were incorporated in soil and subjected to repeated applications (every 30 days) of biodegradable pesticides (iprodione, oxamyl) and antibiotics (sulfamethoxazole, tetracycline) with contrasting hydrophobicity. Bulk soil and plastisphere samples were collected after the first, third, and fifth treatment cycles, for DNA extraction and downstream qPCR analysis of pesticide-degrading genes and ARGs, as well as shotgun metagenomics. qPCR data suggested a clear enrichment of plastisphere microbiota to ARGs like tetM and pesticide- degrading genes like cehA (oxamyl hydrolase) regardless of chemicals application, while oxamyl application boosted cehA in the plastisphere compared to soil. Metagenomic analyses of taxonomic composition and functional potential revealed clear plastisphere-associated responses compared to soil and across different treatments. Under control conditions, plastisphere communities were dominated by the taxon Amycolatopsis reported as polymer degrader. The application of oxamyl further shifted plastisphere microbial communities favoring bacteria carrying pesticide-degrading capacities like Sphingomonas, Variovorax, Lysobacter, Rhodococcus. Whereas antibiotic favored members Candidatus Saccharibacteria characterized by symbiotic lifestyles and reduced genomes. Functional analyses revealed conserved core metabolic functions in theplastisphere compared to soil. Furthermore, the analysis of plasmidome and virome, combined with metagenome-assembled genome (MAG) reconstruction and functional annotation, isongoing.

Acknowledgements: This work was funded by the EU funded Horizon 2020 project MINAGRIS (Project Number: 101000407)

Keywords: plastisphere, metagenomics, microbial adaptation, co-contamination

Persistence, dissipation and hazard traits of the pig-manure microbiome, resistome and mobilome in an agricultural soil

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Manuring is a sustainable agriculture practice leading to pathogen and antibiotic microbial resistance dispersal with implications for One Health. Here, we model pig manure microbial inputs in a soil microcosm approach. Manure was conditioned with tetracycline and/or sulfamethoxazole at realistic concentrations, mixed with soil at 2 % (w/w) and incubated at 25˚C. Additionally, soil/manure mixtures were incubated at various temperature succession regimes of 25 and 37 oC, mimicking realistic seasonal changes. Total prokaryotic diversity was analysed while, the 16S rRNA gene, intI1, and an array of antibiotic resistance genes (ARGs) were quantified via qPCR. Shotgun sequencing was performed for key samples, for complete pathobiome, resistome and mobilome analysis, also contributing to study-specific ARG selection in the aforementioned qPCR quantification/modelling. Finally, we long-read-sequenced complete class 1 integron amplicons to assess their load. Manure taxa comprised ~20% of the manure-amended soil community, with taxa dissipating immediately (e.g. Pseudomonas), or after 5 days (e.g. Streptococcus), following exponential decay, whereas clostridia (among others) persisted beyond 25 days. Manure-enriched soil taxa were dominated by Streptomycota. Manure-associated ermB, tetM, tetO, sul1 and intI1, decayed exponentially, with all but ermB persisting beyond 25 days. Antibiotic application favoured manure taxa/ARGs according to DT50 values. Along temperature successions, most ARG abundances dropped upon temperature increase to 37˚C after 20 days at 25˚C except for genes like tetM and tetO that increased. Manure-introduced ARG locus analysis depicted more hazardous flanking regions compared to those found in corresponding soil variants. Similarly, more ARG-hazardous cassette content was observed in manure-derived integrons and plasmids, showing dissipating trends. ARG-poorer soil-derived genetic elements were enriched by manure, probably through nutrient-mediated host growth. Overall, we delineate manure-associated hazardous microbiome entities and mechanisms, useful for informed risk assessment studies and regulatory action.

Acknowledgment: Marios Valmas was funded by the Hellenic Foundation for Research and Innovation (HFRI).

Keywords: microbial coalescence; mobile genetic elements; antimicrobial resistance;risk assessment; One Health

Limited persistence and ARG dissemination of a multidrug-resistant *Escherichia coli* in freshwater sediment under sub-inhibitory antibiotic Exposure

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Sediments in aquatic ecosystems are reservoirs of multidrug resistance (MDR) bacteria and plasmid-borne antibiotic resistance genes (ARGs). However, the extent and mechanisms of their mobilization remain poorly understood. This study examines the invasion capacity and persistence of a MDR plasmid-carrying Escherichia coli in freshwater sediment, its ability to disseminate ARGs into native microbial communities, and whether sub-inhibitory antibiotic concentrations modulate these processes.

Two E. coli CV601 strains were used: a plasmid-free strain and a MDR transconjugant carrying a plasmid from wild-type E. coli, conferring resistance to trimethoprim (dfrA14), beta-lactams (blaCTX-M-15), and fluoroquinolones (qnrS1). Growth rates and biofilm formation assays were used to characterize potential fitness differences between strains associated with MDR plasmid carriage. A microcosm experiment using sediment and water from Lake Vänern, Sweden, was spiked with each E. coli strain (6000 cells/mL) and incubated for 10 days under static conditions, with and without an antibiotic mixture of trimethoprim (10 µg/L), cefotaxime (4 µg/L), and ciprofloxacin (1 µg/L). MDR E. coli was detected in the sediment only at day one of the experiment, regardless of antibiotic exposure. However, plasmid-borne ARGs were detected in sediment, but declined over time, suggesting no horizontal gene transfer between MDR E. coli and native bacterial communities. Bacterial community composition and diversity remained stable across all treatments. No growth differences were observed between strains. Therefore, ongoing genomic comparisons aim to identify the mechanisms driving E. coli persistence within sediment. Overall, our study suggests that MDR E. coli has limited survival capability and ability to transfer resistance genes in sediment under sub-inhibitory antibiotic concentrations. Still, the influx of MDR E. coli contributes to the occurrence of ARGs in sediments, but not necessarily because of its transmission to the native bacterial communities.

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Keywords: Multidrug-resistance, E.coli, Antibiotic resistancegene, sediment,microbial communities

Exposure to pathogens and antibiotic resistance during community composting in urban areas: a case study of the Rennes metropolitan area

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Community composting, expanding rapidly in urban areas, plays a key role in the circular economy by recycling food waste (FW). However, its effectiveness in eliminating pathogens and antibiotic resistance vectors remains underdocumented, while compost use in community gardens may pose health risks, potentially compromising food chain safety.

This study assessed the sanitization performance of five shared composting sites managed by residents in the Rennes Mıtropole area. Each site included four composting stages (C1 to C4), from youngest to most mature. Samples were collected three times over 18 months from FW and compost, then analyzed for *Escherichia coli* (a treatment efficiency indicator) and two emerging opportunistic pathogens, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The study also examined i) the genetic diversity and antibiotic resistance of *P. aeruginosa* and *A. baumannii* isolates, and ii) the presence and abundance of antibiotic resistance genes (integrons and genes conferring resistance to aminoglycosides, sulfonamides, and beta-lactams) in total DNA extracted from FW and compost samples.

Results showed *E. coli* concentrations increased between FW and the input composter (C1), then gradually decreased through to mature compost (C4), with reductions ranging from 2 to >4 $\log_{10}$, indicating overall effective sanitization. *P. aeruginosa*, undetected in FW, was frequently found in composts, including at the most advanced maturation stage, with the highest concentrations in C1, suggesting input composter conditions may promote its growth. *A. baumannii* was detected in 8% of samples, present in both FW and composts across maturation stages.

Genetic diversity analysis revealed a wide variety of strains common across sites and persistent over time. While all *P. aeruginosa* isolates (n=165) were antibiotic-susceptible, some *A. baumannii* strains (n=3/62), though carbapenem-susceptible, exhibited multidrug-resistant profiles associated with plasmid-borne genes harboring numerous integrases and insertion sequences, potentially facilitating horizontal gene transfer. The simultaneous presence of opportunistic pathogens and most screened resistance genes in the bacterial community of FW and certain compost samples raises concerns about resident exposure to antibiotic-resistant pathogens, particularly during fresh waste handling. Although isolated pathogens lacked clinically relevant resistance, composting creates environments where opportunistic pathogens and antibiotic resistance genes co-exist, potentially fostering the emergence of multidrug-resistant clones.

Microbial community structure and antibiotic resistance genes in Dinaric karst groundwater under contrasting hydrological conditions

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Karst aquifers represent a critical yet vulnerable drinking water resource, characterised by rapid infiltration and limited natural filtration. Despite their importance, integrated studies combining microbial community profiling and resistome characterisation in karst groundwater remain scarce. This study investigated microbial community composition and antibiotic resistance gene (ARG) profiles of five karst groundwater sources in the Dinaric karst region of coastal Croatia, sampled under contrasting hydrological conditions (wet and dry period).

Microbial community analysis by 16S rRNA amplicon sequencing revealed that hydrological conditions were the primary driver of community composition, with TOC identified as the key physicochemical predictor. Site-specific faecal pressure, reflected in ammonium and E. coli levels, further differentiated sampling sites regardless of hydrological conditions. Resistome profiling by high-throughput qPCR (96 gene targets) revealed distinct ARG profiles across sites and sampling periods, with clinically relevant resistance genes detected at multiple locations. Spearman correlation analysis linked dominant bacterial taxa to specific ARG classes, with mobile genetic elements suggesting horizontal gene transfer as a dissemination mechanism.

These findings demonstrate that hydrological conditions and site-specific anthropogenic pressures jointly shape both microbial community structure and resistome composition in karst groundwater, with implications for drinking water safety and One Health surveillance. .

Acknowledgements: This work was supported by the Croatian Science Foundation under project IP-2024-05-7387(ARES–Antimicrobial Resistance Surveillance via analyses of groundwaters).

Keywords: karst groundwater, antibiotic resistance genes, microbial community, resistome, One Health

Year long study of AMR and pathogens transfer in the Gurri river

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Antimicrobial resistance (AMR) is recognised as a major global health threat, and growing evidence shows that natural environments act as reservoirs of antibiotic resistance determinants. Rivers, in particular, can function as hotspots for AMR due to the convergence of urban, agricultural, and wastewater inputs, yet studies that jointly assess seasonal dynamics, hydrological variability, land use gradients, and differences between water and biofilm matrices remain limited.

This study presents a year-long investigation of antibiotic resistance, pathogenic bacteria, and chemical contaminants in the Gurri River (Spain), a system currently being evaluated as a potential alternative drinking water source during severe drought. The project combines chemical and microbiological approaches to characterise pollution sources and potential AMR drivers. Passive samplers (POCIS) were deployed during two seasonal campaigns to obtain time-integrated profiles of pharmaceuticals, pesticides, and other emerging contaminants along the river continuum. In parallel, qPCR assays were used to quantify antibiotic resistance genes (ARGs), microbial source tracking (MST) markers, and key Gram-negative pathogens, including Pseudomonas aeruginosa, Aeromonas caviae, and Klebsiella pneumoniae, in both water and periphytic biofilms. Broader microbial community structure and resistome profiles are being further explored through metagenomic sequencing.

qPCR analyses revealed clear spatial and seasonal patterns in the abundance of ARGs and pathogenic bacteria. Pristine upstream sites showed the lowest gene copy numbers for all targets, indicating minimal anthropogenic influence. Urban and wastewater-impacted sites exhibited the highest concentrations, particularly for the human faecal marker and the pathogens K. pneumoniae and P. aeruginosa in both matrices. A. caviae and ruminant markers were consistently low or undetectable, possibly reflecting limited inputs during the sampling windows or faster environmental decay of these indicators. Seasonal comparisons showed higher gene abundances in summer, likely driven by reduced flow and enhanced microbial persistence.

Funding: support is provided by the EuropeanUnion through the Marie Skłodowska-Curie Actionsprogramme.

Keywords: Antimicrobial resistance, Freshwater rivers, Peryphyton, Emerging Contaminants

Dynamics of bacterial resistance to antibiotics (BRA) in a small rural watershed in Ardèche (France) as influenced by the hydrological regime, soil use and human activities.

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The widespread presence of pharmaceuticals, particularly antibiotics, in the environment promotes the emergence and dissemination of antibiotic-resistant bacteria (ARBs) and the spread of antibiotic resistance genes (ARGs) across differ-ent ecosystem compartments. While this dynamic is well-documented for urban context, it remains poorly understood in rural environments, especially the role of certain ecosystem compartments as reservoirs of resistance. In rural areas, land use and agricultural practices contribute to the dissemination of antibiotics, resistance co-selectors (such as bio-cides and metals), and microorganisms or their genes into soils and surface waters. This study aims to (i) assess the prevalence of ARGs and ARBs in the soils and surface waters of a small rural catchment subject to various land uses, potential contamination, and an intermittent Mediterranean hydrological regime, and (ii) identify the sources and se-lection pressures controlling their spatio-temporal dynamics. The results reveal a systematic presence of ARGs and ARBs at all monitored sites, with concentrations varying depending on the site, the markers analysed, and the season. Certain markers, such as sul genes (resistance to sulfonamides), were detected everywhere, while others, such as blaCTX-M (resistance to recent beta-lactam antibiotics), which pose a greater risk, were never observed, likely due to the different historical use patterns of these antibiotics. The prevalence of ARGs and ARBs in surface waters is clearly influenced by domestic discharges (livestock farming, wastewater treatment plants, campsites, hospitals), especially during low-flow periods. The detection of ARGs is often linked to high geochemical concentrations (major ions, trace elements), which are specific to certain activities such as intensive livestock farming and wastewater treatment. During periods of rainfall or flooding, certain water flows are activated, mobilizing water bodies or sediments enriched with ARGs and ARBs from various sources distributed throughout the catchment. In conclusion, soils and waters are contaminated to varying degrees, reflecting the differential distribution and influence of antibiotic resistance gene sources. At the temporal scale, contamination varies according to the seasonality of human activities (agricultural, tourism-related) within the catch-ment.

Keywords: Bacteria, antibiotic resistance genes, genetic markers, surface waters, soil use, river flow

Seasonal dynamics of antibiotic resistance genes in Dinaric karst groundwater: a one-year monthly monitoring study

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Karst aquifers are highly vulnerable to surface-derived contamination due to rapid infiltration and limited natural filtration, yet their resistome dynamics over annual cycles remain poorly understood. This study monitors the seasonal dynamics of antibiotic resistance genes (ARGs) in five karst groundwater sources in the Dinaric karst region of the Kvarner area, Croatia over a one-year period with monthly sampling frequency.

A targeted 24-gene HT-qPCR panel was designed based on prior resistome profiling of the same sampling sites (wet and dry season, 2024), selecting genes of high abundance, WHO priority resistance classes and seasonal markers. Efflux pump-encoding genes were excluded to focus on acquired and clinically relevant resistance determinants. The panel covers beta-lactam, aminoglycoside, quinolone, sulphonamide, trimethoprim, tetracycline and vancomycin resistance gene classes, as well as mobile genetic elements as indicators of horizontal gene transfer potential.

Monthly sampling across five sites with contrasting anthropogenic pressures revealed distinct temporal patterns in ARG abundance, with notable variation linked to precipitation events and hydrological conditions. Critical periods of elevated ARG abundance were identified, with site-specific resistance profiles reflecting differences in land use and contamination sources. These findings contribute to the characterisation of resistome dynamics in karst groundwater systems and provide data relevant for drinking water safety management and One Health surveillance.

Acknowledgements: This work was supported by the Croatian Science Foundation under project IP-2024-05-7387 (ARES – Antimicrobial REsistance Surveillance via analyses of groundwaters).

Keywords: karst groundwater, antibiotic resistance genes, seasonal dynamics, HT-qPCR, One Health

Dynamics of abundance and diversity of extended-spectrum β-lactamase (ESBL)- producing bacteria in aquatic ecosystems of Foumbot, an agricultural area of Cameroon (Central Africa)

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Antimicrobial resistance is a major public health challenges of the 21st century with a rapid increase in strains producing extended-spectrum β-lactamases (ESBL). This study examined the abundance and diversity of ESBL-producing bacteria in the aquatic ecosystems of Foumbot, an agricultural region. Sixteen sampling points were chosen to collect wastewater, surface, well and spring water. ESBL-producing bacteria were isolated on Chromagar-ESBL culture medium using filtration membrane method and identified using API 20E systems while physicochemical parameters were measured using appropriate techniques. The bacterial diversity was assessed by calculating specific richness, Shannon and Weaver diversity index, Sorensen similitude index and Pielou equitability index. Statistical analysis were performed using PAST software 16.0. Results showed that abundance mean values varied from one sampling point to the other. They fluctuated from 20x104 to 13x105 CFU/100mL in wastewater, 18x102 to 13x103 CFU/100mL in surface water, 141 to 80x102 CFU/100mL in well water and 14 to 366 CFU/100mL in spring water. 37 ESBL-producing species were isolated with 22 belonging to the Enterobacteriaceae family (Cedecea lapagei, Citrobacter braakiii, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter gergoviae, Escherichia coli, Klebsiela oxytoca, Klebsiella pneumonia, Rahnella aquatilis, Raoultella ornithinolytica, Salmonella choleraesuis, Salmonella paratyphi A, Salmonella spp, Serratia ficaria, Serratia liquefaciens, Serratia marcescens, Serratia odorifera, Serratia phymuthyca, Serratia rubidaea, Shigella spp, and Yersinia pestis). Surface water was the most diversified ecosystem whereas groundwater was the least. A high equitability (H'>0.7) was recorded at almost all the sampling points and a high similarity (S>0.7) of bacteria community was noted between surface, well and spring water. The bacterial abundance and species richness were significantly influenced by factors such as pH, nitrites, ammonia nitrogen (affecting only the abundance), phosphates, phosphorus, color, and turbidity. This study revealed the health risk for humans and aquatic environment of Foumbot.

Keywords: Water, ESBL-producing Bacteria, Abundance, Diversity, Health

Nature-based solutions to reduce antimicrobial resistance spread from urban wastewater treatment plants

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Urban wastewater treatment plants (UWWTPs) must urgently improve their capacity to limit the spread of antimicrobial resistance (AMR). However, under the current European Union Directive (2024/3019), small facilities (< 10,000 population equivalent) are not required to implement advanced treatments capable of reducing AMR dissemination. Developing alternative and cost-effective treatment strategies is therefore essential to improve wastewater management in these systems. In this research, a nature-based solution (NBS) combining biofilms and planktonic microbial communities was evaluated as an alternative approach to improve the removal of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), which play a key role in horizontal gene transfer. The results showed a substantial reduction of ARGs conferring resistance to widely used antibiotic families, including aminocoumarins, β-lactams, fosfomycin, and quinolones. Additionally, significant decreases were observed in several MGEs (e.g., integron integrases, insertion sequences, mobilization relaxases, and transposons). These results indicate that the proposed NBS can effectively reduce genetic determinants associated with AMR, thereby improving treated water quality in small UWWTPs. Implementing such strategies could contribute to the One Health framework by limiting the potential spread of AMR into aquatic environments. Rethinking treatment approaches for small UWWTPs may therefore support evidence-based decision-making by policymakers in the medium term. Lluís Bertrans-Tubau thanks ICRA and BETA colleagues who contributed to the experimental work but are not co-authors. This research was funded by the Spanish Ministry of Science and Innovation (PID2021-127326OB-100) and the Spanish Ministry of Science and Innovation, the State Investigation Agency, and the Regional Development of European Funding (FRENAWASTE Project: PID2022-1385630A-100). Lorenzo Proia was supported by a Ramón y Cajal contract '[RYC 2020-029829-I]'. Lluís Bertrans-Tubau received funding from the European Social Funding Plus (FSE+) and the Generalitat de Catalunya (grant number 2022_FI_B200027). Sergio Martínez-Campos was supported by the Investigo Programme funded by the European Union's Next Generation funds.

Keywords:Antimicrobial resistance, biofilms, urban wastewater treatment plants, nature-based solution

Evidence for upgrading wastewater regulation:antimicrobial resistance persistence after secondary treatment

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The World Health Organization has listed antimicrobial resistance (AMR) among the top ten global health threats. Antibiotic residues, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) enter the environment via multiple pathways and, even at sub-inhibitory concentrations, promote resistance selection and horizontal gene transfer (HGT). Wastewater treatment plants (WWTPs) are central to these processes but are not optimized to remove trace antibiotics or ARGs.

The Aartselaar WWTP (Belgium) was selected for this pilot study to investigate antibiotics, AMR markers, and removal efficiency of secondary treatment. Triplicate WWTP influent and secondary treatment effluent samples were filtered, and processed for DNA extraction, followed by SmartChip qPCR targeting 248 ARGs across 10 antibiotic groups.

The lowest absolute ARG removal efficiency was observed for quinolones (86%) and the highest for Multidrug Resistance (MDR; 99.8%). A significant reduction in absolute ARG abundance from influent to effluent ($p<0.05$) suggests removal via biomass reduction, particularly through sludge processes. Despite this, ARG relative abundance increased significantly ($p<0.05$) in the effluent for quinolones, aminoglycosides, vancomycin, beta-lactam, and phenicol. While MDR, sulfonamide, tetracycline, and trimethoprim decreased, and macrolide-lincosamide-streptogramin B remained stable. This indicates that total bacterial biomass decreased more than the ARGs, resulting in an increased relative abundance of ARGs within the microbial community.

Additionally, the detection of unique ARGs in the effluent suggests that the activated sludge system provides a dynamic microbial environment that facilitates HGT. The significant decrease in total ARG abundance, including complete removal of 37 out of 248 ARGs, indicates that the WWTP can function as a sink. Conversely, the apparent selection of two specific ARGs and the increase in relative abundance for most ARG groups highlight its role as a potential source of AMR. A follow-up study will evaluate the implementation of ozonation and activated carbon to improve removal of residual antibiotics and co-selective Agents.

Keywords: Next Generation Environmental Risk Assessment; water quality directives; resistome dynamics; contaminant mixtures; environmental reservoirs

Wastewater-Seeded Plastic Biofilms as Reservoirs of Antibiotic Resistance in Marine Environments

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Vast amounts of plastic debris float in our oceans and plastics are increasingly recognized as a potential vector for antimicrobial resistance. Simultaneously, antibiotic resistant bacteria, resistance genes, selective agents and other contaminants enter the oceans via wastewater effluents. Biofilms formed on plastics in the ocean may be influenced by wastewater effluents and could thereby contribute to spread of resistance genes and resistant bacteria from wastewater, throughout marine environments. In this study, lab-based biofilm experiments were performed to investigate the effect of wastewater and different plastic materials on bacterial biofilm communities. Polystyrene, polyethylene and polyvinyl chloride microplastics, as well as glass and wood, were exposed to wastewater effluents from three different wastewater treatment plants. Biofilms were allowed to form on the materials and were subsequently analysed for the presence of resistance genes, resistant bacteria and potential pathogens. Bacterial biofilm communities were characterised using 16S rRNA gene sequencing together with culture-dependent analyses, while the resistomes were analysed by probe-based sequencing of 2700 target genes. Susceptibility to 10 antibiotics was assessed using broth dilution assays. Bacterial community compositions differed substantially in biofilms formed in the presence and absence of wastewater effluents. Furthermore, a higher number of antibiotic resistance genes and higher relative abundances of clinically relevant resistance genes were detected in biofilms formed in the presence of wastewater effluents compared to seawater-only controls. Faecal indicator bacteria were only detected in biofilms exposed to wastewater effluents. Additionally, both faecal and environmental indicator bacteria displayed material preferences, although less clear. Bacterial isolates from plastic-associated biofilms showed decreased susceptibility to several antibiotics, including critically important antibiotics. This shows that plastic biofilms may contribute to the spread of antibiotic-resistant bacteria and antibiotic resistance genes, and that wastewater effluents substantially affect these processes.

Keywords: Antibiotic resistance, microplastic, biofilm, wastewater

Tiered assessment of the toxicity of veterinary medicines on the soil microbiota: from *in vitro* to soil assays

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Soil pollution from veterinary pharmaceuticals is a major problem for One Health. Therefore, it is important to study the fate of those pharmaceuticals and assess associated ecological risks. We aimed to evaluate the toxicity of veterinary pharmaceuticals on soil microorganisms at different experimental scales. As a first step we screened nine antibiotics or anthelmintics (sulfamethazine, ofloxacin, neomycin, tetracycline, trimethoprim, tilmicosin, moxidectin, toltrazuril, and florfenicol) for their toxicity (EC_{50}) to strains from two key functional microbial groups: ammonia-oxidizing microorganisms (AOM (ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)), and arbuscular mycorrhizal fungi (AMF) using single species tests, and spore germination test respectively. None of the tested compounds showed toxicity to AMF, while toltrazuril and tetracycline showed toxicity to AOM. These compounds were selected and tested in a soil microcosm assay alongside albendazole, which served as positive control. Two soils, an acidic (Ampelies pH 4) and an alkaline (Chalastra pH 7.9), were treated with five different concentrations per compound. The dissipation of each compound was initially determined. For albendazole, different patterns of dissipation were observed, with DT_{50} increasing with the applied concentrations of albendazole and higher DT_{50} values were observed in the alkaline compared to the acidic soil (DT_{50} 24.8 and 4.6 days respectively at the highest rate of 24.3 mg/kg). For tetracycline, no dose-dependent dissipation pattern was observed in the alkaline soil (DT_{50} 6.5 days at 1,63 mg/kg rate and 6.7 days at 132,3 mg/ kg rate). Effects on the functioning and diversity of key soil microbial groups are determined via q- PCR and amplicon sequencing. The latter combined with Species Sensitivity Distribution and Principal Response Curve will allow derivation of ecotoxicological thresholds for risk assessment.

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Keywords:Soil, veterinary pharmaceuticals, dissipation, qPCR

Environmental and clinical Stenotrophomonas maltophilia isolates from Croatia and their potential role in antibiotic resistance dissemination

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Stenotrophomonas maltophilia is an environmental, gram-negative bacterium mostly characterized by the intrinsic multidrug resistance (MDR). In recent years, it has been recognized as a clinically relevant opportunistic pathogen. These bacteria have been found in a wide range of environments, as well as in hospital settings. In this study, environmental S. maltophilia isolated from groundwater used as drinking water and clinical isolates were investigated and compared to assess the potential link between environmental reservoirs and human infections. Environmental bacteria were isolated from groundwater collected in the Rijeka region, Croatia, while 18 clinical isolates were collected from different departments of Rijeka and Split hospitals. S. maltophilia isolates were confirmed with MALDI-TOF. Antibiotic susceptibility profile was made using the disk diffusion method. The genetic diversity of the environment and clinical isolates was assessed using ERIC-PCR fingerprinting. Additionally, six environmental and six hospital isolates were selected according to their ERIC PCR clustering for whole genome sequencing (WGS). It was confirmed that 93 isolates from groundwater were S. maltophilia, and they were susceptible to four different antibiotics, which are routinely used for antibiotic susceptibility testing in hospitals. WGS analysis revealed that environmental and hospital isolates harbored resistance to beta-lactams (blaL gene) and aminoglycosides (aph(3')-IIc or aac(6')-Iz). Additionally, in one clinical isolate, multidrug resistance efflux pumps associated with Pseudomonas aeruginosa were found, and a highly promiscuous IncP plasmid. Additionally, four novel sequence types were confirmed in our clinical and environmental samples. Despite antibiotic resistance being primarily intrinsic in all isolates, detection of novel sequence type and the presence of broad-host range plasmid and multidrug efflux pumps in a clinical isolate, suggest a potential role in the spread of antibiotic resistance.

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Keywords: Stenotrophomonas maltophilia, groundwater, hospital, multidrug resistance, multidrug efflux pumps

Aquatic biofilms as possible reservoirs for antibiotic resistance genes; a case study of the Tech river (France)

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A global rise in antibiotic resistance (AR) poses a significant public health threat. The main vectors of AR are antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs). ARB and ARGs are present in the environment, particularly in anthropized systems. Urban rivers are one such system. Rivers receive numerous inputs that carry not only antibiotics, but also other pollutants potentially adding to the selective pressure of ARG.

Aquatic biofilms, aggregates of microorganisms attached to substrates, plays a central role in aquatic ecosystems. Biofilms can concentrate pollutants and they have high cell densities, providing micro-niches where horizontal gene transfer is facilitated. Thus, aquatic biofilms are “hot spots” for the dissemination of ARB and ARG.

The objective of this project is to characterize the coupling between pollutant levels and the abundance of ABR and ARG in biofilms and in sediments, and then to estimate their export to the coastal environment. The Tech River, a coastal river flowing into the Mediterranean Sea, constitutes an interesting case study. Along its watershed, the juxtaposition of point-source pressures (wastewater treatment plant outlets, discharge points) and diffuse pressures (agricultural and urban) generates gradients of chemical and microbiological pollution.

Three samplings were conducted in March, April, and May 2026 at four different locations along the river. Chemical analysis of sediments and biofilms yielded similar results regarding heavy metals, Zn being the element detected at the highest concentration in every sample. Four PAHs included in the priority substances of the DCE list were detected in both matrices.

Bacterial strains isolated from both sediment and biofilms were phylogenetically diverse. Antibiotic susceptibility and biofilm formation testing of the bacterial strains is currently underway. DNA was extracted from each environmental sample and will be used for bacterial community analysis using nanopore sequencing. In addition, ARGs will be analyzed after the last sampling by qPCR.

Keywords: antibiotic resistance, aquatic biofilms, pollutants

Fate and Removal of Antibiotics and Antibiotic Resistance Genes in a Rural Wastewater Treatment Plant: A Microbial Perspective of Nature- Based Versus Advanced Technologies

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Micropollutants (MP), antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARG) pose a growing threat to our environment and human health, resulting in increasingly stringent regulations on wastewater management, particularly in the revised Urban Wastewater Treatment Directive (UWWTD, 2024/3019/EU). Within this context, the performance of a vertical-flow constructed wetland (CW) as a natural solution for removing MPs and ARB and ARGs from treated urban wastewater was evaluated in comparison to advanced technologies. The study was part of the CoMinGreat project (2021–2023; Interreg V-A Greater Region program, IP-2-03-207).

During a one-year campaign, monthly samples were collected from the inlet and outlet of a wastewater treatment plant (WWTP; 13000 EH, activated sludge) and after quaternary treatment with vertical-flow constructed wetlands (CW), granular activated carbon (GAC), and ozonation combined with GAC (O3+GAC). Antibiotics and/or their metabolites (sulfamethoxazole, SMX, a N-acetyl sulfamethoxazole, N-SMX; Clarithromycin, CLA) were quantified (LC-MS/MS), as well as ARGs and integrons (qPCR, metagenomics) known to be involved in conferring antibiotic resistance (blaAmpC, ermB, sul1, tetW, intI1). Bacterial communities were characterized with metabarcoding and plate counting methods.

In general, in this study, the quaternary treatments reduced the amounts of ARGs (blaAmpC, ermB, sul1, and tetW) and intI1 more effectively than the wastewater treatment plant alone. The addition of the CW allowed for adequate and reliable removal of all ARGs and intI1, with low temporal variability compared to O3+CAG and CAG. Only minor effects of ARG release were observed in the CW. Although O3+CAG achieved a similar average removal as CW, its inability to remove intI1 highlighted the advantage of CW.

This study highlights the potential of constructed wetlands, showing that they can match advanced quaternary treatments in terms of ATB and ARG removal efficiency and operational reliability, offering a sustainable and cost-effective means to reduce the spread of ATBs and ARGs via wastewater.

Keywords: constructed wetlands, quaternary treatment, antimicrobial resistance, antibiotics, sustainable treatment technologies

Port eco-exposome influence on bioaccumulation, metabolome and microbiome diversity and function in coastal fish across development

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Port infrastructures, by reducing habitat complexity, represent a major threat to juvenile fish in these nursery areas. Ecological restoration is widely implemented to mitigate these impacts. However, organisms inhabiting restored structures are often exposed to chronic, low-level anthropogenic pollution. This study aims to evaluate how the port eco-exposome influences metal accumulation, microbiota diversity and function, and the juvenile fish metabolome.

Research was conducted at two contrasting nurseries, an artificially restored port and a natural area. The eco-exposome was characterized by metal contamination and bacterial communities. In parallel, analyses were performed on Diplodus vulgaris juveniles from the 2023 and 2024 cohorts throughout their development.

Port waters contained higher levels of heavy metals and urban-associated microbial communities, along with a greater abundance of pollution-related genes compared to the natural coastal site. These patterns were influenced by seasonal variation and climatic events. Juvenile fish from the port accumulated more metals and displayed distinct metabolic and microbiota profiles compared to those from the natural nursery across development, whereas no significant differences were observed in the abundance of resistance-associated genes. Instead, a decrease in these genes at both sites was observed in the later fish developemental stage. Metabarcoding analyses revealed taxa enriched in the port-exposed juvenile microbiota, known for their antibacterial activity and ability to degrade complex compounds. In parallel, metabolomic analyses revealed expression changes in stress-associated metabolites, including amino acids and lysophosphatidylcholines, in port-exposed juveniles at intermediate and late developmental stages.

Overall, these findings highlight the need for a holistic framework including both environmental biogeochemical characterisation and multi-omics approaches to better assess the exposome impact on marine organisms.

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Keywords: Port eco-exposome, Juvenile fish, metal accumulation, microbiota, metabolome

Metagenomic characterization of antimicrobial resistance genes in lake Kassembié, Côte d'Ivoire (West Africa)

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Freshwater ecosystems are recognized as key reservoirs for antimicrobial resistance genes (ARGs), yet environmental AMR surveillance in West Africa remains largely absent from the scientific literature.

We report the first metagenomic characterization of the resistome of lake Kassembié, Côte d'Ivoire. Contigs from water and sediment samples, assembled with MEGAHIT, were screened against the Comprehensive Antibiotic Resistance Database (CARD) using RGI v6, retaining only Perfect and Strict hits.

Fifty-five unique ARGs were identified. The water column harboured a markedly richer resistome (52 ARGs; 84% of total diversity) than sediment (10 ARGs), with only 7 shared genes. Dominant classes included glycopeptides (n=36), fluoroquinolones (n=31), and biocide/antiseptic resistance genes (n=17). Carbapenem-resistance determinants (OXA-420, OXA-821, ACT-111) and multiple vancomycin-resistance cluster variants were detected, highlighting the presence of last-resort antibiotic resistance.

These results establish lake Kassembié as a significant AMR reservoir and call for enhanced freshwater surveillance across West Africa within a One Health framework.

Keywords: antimicrobial resistance genes; resistome; freshwater metagenomics; West Africa; One Health

Advanced wastewater treatment: efficiency on antimicrobial resistance

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Antimicrobial resistance (AMR) in wastewater and effluents of wastewater treatment plants (WWTPs) is a major global health and environmental challenge. According to the World Health Organization (WHO), antimicrobial-resistant pathogens cause more than 700,000 deaths annually, and a number projected to reach 10 million by 2050. In response, the upcoming DERU2 regulation will impose stricter requirements for AMR management and monotoring.

Several advanced processes including chlorination, oxydation by peracids, UV disinfection have been investigated to limite AMR dissemination. However, under real operating conditions, the reduction in AMR is often limited, generally achieving only 0-3 log reductions in antibiotic resistance genes (ARGs). In some cases, treated effluents have even shown increased availability of genetic material such as plasmids and ARGs. The discharge of insufficiently treated wastewater containing antibiotic resistant bacteria (ARB) and ARGs therefore contributes to the spread of AMR, threatening both human health and ecosystem. Effective treatment strategies and optimize operational conditions are urgently needed to preserve water quality and reduce these the risks.

Our work addresses these challenges by evaluating a disinfection process based on performic acid (PFA). PFA has recently attracted growing interest due to its strong antimicrobial activity, low-cost and greater environmental compatibility compared with conventional disinfectants. Laboratory experiments demonstrated that increasing PFA concentration and contact time significantly improves the reduction of microbial abundance and ARGs. These results highlight the potential of PFA to effectively reduce both microbial contamination and AMR, even at low concentrations and short exposure times. Nevertheless, further studies are required before large-scale implementation in WWTPs to confirm its effectiveness and evaluate its environmental impacts.

Keywords: antimicrobial resistance; metagenomic; wastewater treatment plan; advanced treatment

Tracking antimicrobial resistance transfer in a Karst aquifer under flood conditions: An ecological approach to health

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Karst aquifers, a major source of drinking water worldwide, are hydrodynamically complex systems. They combine diffuse and concentrated recharge, rapid conduit flow, and slower matrix circulation, generating strong spatial and temporal variability in water sources and hydrochemical properties and high vulnerability to surface-derived anthropic contamination. The Lez aquifer, located in a Mediterranean context and used for drinking water supply in the Montpellier area, is representative of such systems. Understanding karst functioning is essential for sustainable water resource management. It is traditionally based on geochemical tracers, which may not fully resolve the complexity of hydrodynamic behaviour and mixing in heterogeneous systems. Groundwater microbiomes remain largely underexplored compared to surface waters. Yet microorganisms are tightly linked to environmental conditions, and bacterial community composition can provide a biological signature of water masses. Microbial diversity therefore represents a complementary tracer to investigate water origin, mixing processes, flow pathways and contamination sources within karst, from recharge zones to springs. This study, conducted within the PEPR K3 project (ANR-22-PEXO-0012), aims to (i) characterize the karst microbiome and its spatio-temporal dynamics, (ii) improve understanding of karst hydrosystem functioning by integrating microbial and geochemical tracers, and (iii) assess potential health risks related to anthropic contamination.

Water samples were collected under contrasting hydrological conditions, including flood events, across multiple compartments of the Lez aquifer (temporary streams, infiltration zones, boreholes, and springs). Bacterial diversity was analyzed using 16S rRNA gene metabarcoding, complemented by qPCR targeting fecal indicators and antibiotic resistance genes, and culture-based analysis of resistant bacteria. Microbial source tracking and indicator species analyses were used to link bacterial signatures to hydrological conditions and water sources. Preliminary results reveal strong spatial and temporal variability in bacterial communities linked to hydrological dynamics, highlighting the value of integrating microbial and geochemical approaches to improve karst water resource characterization and management. .

Keywords: Antimicrobial resistance, flood events, karst

Biotic and abiotic drivers of antimicrobial resistance selection and mitigation in soils amended with livestock effluents

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Antimicrobial resistance (AMR) is recognized as a global One Health threat linking human, animal and environmental heaths. Livestock effluents are reservoirs of antibiotic-resistant bacteria and resistance genes introduced into agricultural soils, as fertilizer, through land application. However, the mechanisms controlling AMR dissemination in soils remain poorly understood, particularly regarding the combined effects of soil properties, exogenous microbial communities, organic matter and contaminants inputs and soil biodiversity. Among soil organisms, earthworms are key ecosystem engineers influencing soil structure, organic matter redistribution and microbial dynamics through bioturbation, which may affect AMR dissemination in soils.

To investigate the influence of these components on AMR dynamics, soil microcosms were established using two contrasting agricultural soils, amended (FB) or not (MIN) with cow manure-digestate, with or without oxytetracycline (OTC) and the endogeic earthworm *Allolobophora chlorotica*. Earthworm bioturbation was monitored over 90 days using luminophore tracers. Results showed significant effects of soil type and OTC on earthworm activity. Surface bioturbation was higher in FB than in MIN soil and decreased in the presence of OTC. Conversely, vertical bioturbation increased under OTC exposure, suggesting stress-induced avoidance behavior and deeper burrowing. To disentangle the roles of digestate organic matter, microbial communities and antibiotics in AMR dissemination, these digestate fractions were separated, recombined and used for soil microcosms. Preliminary results showed that digestate addition stimulated soil respiration, while OTC inhibited microbial activity. Interestingly, the inhibitory effect of OTC was reversed when digestate-derived microbial communities were introduced, suggesting a buffering effect mediated by resistant or tolerant microorganisms and/or antibiotic biotransformation.

These preliminary results indicate that interactions between soil biodiversity, exogenous microbiota, organic matter and antibiotics influence soil microbial functions. Ongoing 16S/ITS sequencing, high-throughput qPCR, antibiotic monitoring and gene transfer experiments will clarify the driving mechanisms and their role in AMR dissemination.

This work, part of the ATBR-SOL project, was funded by the PNR-EST funding (n°ANSES-24-EST-186).

Keywords: antimicrobial resistance, livestock, soil, microbial communities, earthworm

Unveiling antimicrobial resistance in marine coastal biofilms using metagenomics

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Marine biofilms formed on artificial substrates provide a time-integrated view of microbial dynamics and environmental contamination, contrasting with transient planktonic water samples traditionally used for coastal monitoring. By bioaccumulating contaminants and promoting horizontal gene transfer, biofilms may act as reservoirs for metal and antibiotic resistance genes (MRGs and ARGs) in anthropogenically impacted coastal environments. While oceanic dilution generally keeps marine antibiotic concentrations low, harbour-associated metal pollution is hypothesized to drive MRG and ARG co-selection. To investigate whether local pollution profiles select distinct marine biofilms resistomes, DNA was sequenced from biofilms developed on submerged plates for one month across five harbours. Shotgun metagenomics generated over 100 million sequences per sample. MRGs and ARGs were annotated by aligning both the contigs >1 kb and the metagenome-assembled genomes (MAGs; <15% contamination; >50% completeness) with DIAMOND against CARD, SARG, PanRes, BacMet databases. A threshold of 70% identity and minimum 100 bp coverage led to very few canonical ARGs in the total dataset: 21 distinct ARGs in the contigs, and 4 in the MAGs. The *rpo*B and *qnr*S genes, conferring resistance to rifampin and quinolone, respectively, were the most abundant. From the 21, 9 ARGs were classified as resistance-nodulation- cell division antibiotic efflux pump, which are known to confer cross-resistance to metallic biocides. Therefore, further analyses will investigate the co-selection of ARGs and MRGs in these metagenomes, considering the 83 identified MRGs. The low detection rate of known ARGs suggests that standard databases may underrepresent environmental resistance diversity. This is coherent with the literature, where studies show high sequence diversity in ARGs, clade- specific and related to the sampled environment. Ongoing analyses will integrate the prevalence of mobile genetic elements in marine biofilm metagenomes. These results propose a specialized coastal resistome possibly driven by metal-induced cross-resistance and unexplored ARG diversity.

Keywords: Marine Biofilms; Resistome; Metagenomes; Antibiotic Resistance Genes; Genetic variability

A new whole genome sequencing analysis tool for risk assessment of microbial pesticides

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An innovative risk assessment (RA) framework is needed to support the safe market introduction of microbial pesticides, including bacteria, fungi, phages, and microbiome-based products. Although these solutions provide clear environmental, they are currently subject to the same rigorous regulatory requirements as synthetic pesticides. This has slowed their market uptake in Europe compared to regions such as the United States, India, and China. To address these challenges, the EU-funded project RATION developed a dedicated RA framework tailored to microbial substances with potential low-risk profiles. A central objective of the project is to integrate genomics into the existing RA process for microbial pesticides, enabling the evaluation of key issues such as virulence and pathogenicity, antimicrobial resistance (AMR), gene transfer potential, and the production of toxic secondary metabolites. By optimizing regulatory assessments and eliminating redundant testing requirements, the project ensures a scientifically robust yet efficient pathway for microbial pesticide evaluation. Currently, whole genome sequencing (WGS) analysis for microbial pesticides is conducted using the EFSA tool MOPS, a closed system accessible only to regulatory authorities and subject to several limitations. In this context, RATION has developed an intuitive, fully automated, and openly accessible pipeline that integrates state-of-the-art bioinformatics tools across the entire WGS workflow. Designed to ensure scientific robustness and usability, the platform enables comprehensive screening of virulence factors to assess potential pathogenicity, detection of antimicrobial resistance (AMR) and its transferability, identification of toxic secondary metabolite biosynthesis pathways and the detection of mycotoxin-related biosynthetic pathways. By linking advanced scientific methods with practical regulatory needs, this tool supports stakeholders in navigating microbial pesticide risk assessment, with the goal of accelerating approval processes, fostering sustainable agriculture, and promoting the broader adoption of microbial low-risk products across Europe.

Keywords: Risk assessment (RA), Antimicrobial resistance (AMR), Low-risk Pesticides (LRPs), Whole-genome sequencing (WGS), Microbial pesticides

Integrated multi-level analytical framework for the quality assessment of microbial biocontrol formulations: a proof of concept developed within the BAC-Id project

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The increasing use of microbial biocontrol products raises important challenges for verifying microbial identity, purity, viability and safety. Although microorganisms are becoming key components of sustainable crop protection, their quality assessment still relies largely on methodologies developed for chemical plant protection products. As a result, no comprehensive analytical framework is currently available to evaluate the biological integrity of formulated microbial products.
Within the BAC-Id project, we developed a proof-of-concept workflow combining complementary microbiological, genomic and chemical approaches to assess critical quality attributes, including strain identity, purity, viable cell concentration, genetic safety and formulation integrity.
The workflow was applied to nine commercial microbial biocontrol products, representing 20% of the 45 formulations currently available on the Belgian market. It combines culture-based viable counts, MALDI-TOF MS, long-read rrn metabarcoding (16S–ITS–23S), Oxford Nanopore whole-genome sequencing to confirm strain identity and screen for virulence factors, antimicrobial resistance genes and mobile genetic elements, and UHPLC-MS/MS fingerprinting of formulation-specific bioactive compounds, including Cry protein-derived peptide markers in *Bacillus thuringiensis* products.
Application of the workflow revealed substantial discrepancies between product labels and analytical observations. Only three formulations fully matched their declared specifications regarding microbial identity, viable cell concentration and absence of microbial contaminants. These discrepancies could only be resolved by combining complementary analytical approaches, highlighting the limitations of single-method quality control for complex microbial formulations.
This proof of concept demonstrates that integrated microbiological, genomic and chemical analyses provide a robust quality assessment framework for microbial plant protection products. Developed in close collaboration with Belgian regulatory authorities, this approach supports product development, industrial quality control and regulatory evaluation, contributing to safer, more reproducible and more reliable microbial biocontrol solutions.

Keywords: Quality assessment, Microbial formulations, Integrated analytical framework

Session 2

Effects of Pollutants on Microbes

Invited speaker

Degradation potential is not enough: Effective bioremediation involves microbial community adaptation to environmental conditions

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Organic contaminants alter microbial community composition and functioning, and may also serve as carbon and energy sources for some microorganisms. The ability of microorganisms to grow with organic pollutants has prompted research into the mechanisms and genetic determinants of biodegradation and enabled the identification of biomarker genes for monitoring natural attenuation. However, the effects of contaminant exposure and prevailing environmental conditions on biomarkers and biodegradation efficiency of microbial communities remain difficult to disentangle *in situ*.
This issue was investigated in water-sediment microcosms of different size and complexity, using biodegradation of the toxic solvent dichloromethane (DCM, CH_2Cl_2), a common groundwater contaminant, as a model. Key functional genes of bacterial *dcm* and *mec* pathways may serve as biomarkers of DCM transformation. Previous work showed that water-level fluctuations affect both composition of microbial communities harbouring these genes and DCM degradation efficiency (Prieto *et al.*, 2021). We then evaluated experimentally the effect of environmental variables on DCM degradation efficiency. Water content was the primary control on DCM degradation, with oxygen availability a secondary factor. Marked differences in degradation were also observed for microbial communities compared to a reference DCM-degrading strain. Along with modelling work (Prieto *et al.*, 2023), these results suggest that DCM-degrading populations within complex microbial communities occupy different ecological niches with distinct requirements for maintaining or expressing the DCM degradation function.
Ongoing investigations aim to characterise these ecological niches and to quantify the relative effect of environmental parameters on biodegradation of DCM. This knowledge should support bioremediation strategies that account not only for biodegradation potential, but also for microbial community composition and compatibility with site-specific environmental conditions.

Acknowledgments: This study was supported by the CNRS EC2CO Program, with an ED414 University of Strasbourg PhD grant from the Ministry of Higher Education and Research to CLS.

Keywords: Dichloromethane contamination, Disturbance, Ecological niches, Oxygenation, Water content

Ecotoxicology of Graphene Oxide in Aquatic Organisms: From Microbiota Disruption to Biological Transformation.

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Carbon-based nanomaterials, such as graphene oxide (GO), are attracting increasing interest due to their remarkable physicochemical properties and their wide range of industrial, biomedical and environmental applications. However, their growing production and use increase the likelihood of their release into aquatic ecosystems, where their fate, bioavailability and biological effects remain insufficiently understood. Ecotoxicological assessment of these materials is particularly challenging, as their toxicity depends not only on chemical composition, but also on surface properties, aggregation state and interactions with biological compartments.

In this context, this work aims to better understand the mechanisms underlying GO toxicity in aquatic organisms by integrating its effects on the host, associated microbiota and the fate of the nanomaterial after interaction with biological interfaces. Particular attention is given to amphibians, which are relevant sentinel organisms in aquatic environments. Their skin and digestive tract represent two major interfaces with the environment and host microbial communities involved in essential functions such as digestion, metabolic homeostasis, immunity and protection against pathogens.

Using realistic exposure scenarios, including both direct exposure through the water column and trophic exposure, our results show that GO can disrupt skin and intestinal microbiota, suggesting an alteration of the amphibian holobiont. These microbial changes are associated with broader physiological and metabolic adjustments in the host. In parallel, an original part of this work demonstrates that the digestive tract, and likely the intestinal microbiota, can modify GO properties during its passage through amphibian larvae. This transformation, probably driven by digestive physicochemical conditions and interactions with biological components of the gut, influences the bioavailability and toxicity of the nanomaterial.

Overall, these findings highlight the need to consider nanomaterials not as inert and static contaminants, but as dynamic objects whose toxic properties evolve through interactions with living organisms.

Keywords: Nanomaterials, Amphibians, Microbiota, Biotransformation.

Kinetic multi-level responses of periphyton to pharmaceuticals reveal early sub- threshold effects

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Pharmaceutical contaminants in freshwater ecosystems may pose significant ecological risks, particularly for periphyton—a water bioindicator that underpins primary production and nutrient cycling. This study aims to identify early metabolic biomarkers of periphyton response to pharmaceuticals and link them to subsequent physiological and structure changes. Additionally, we assess whether human modes of action (MoA) can reliably predict ecological effects across microbial compartments.

Twelve pharmaceuticals from different therapeutic classes (antibiotics, non-steroidal anti-inflammatory drugs, cardiovascular, and anticancer compounds) were tested using an eight-point concentration gradient in microcosm kinetic experiments (4 h to 7 days). Physiological endpoints included photosynthetic efficiency (PSII) and extracellular enzyme activities (β-glucosidase and leucine aminopeptidase, LAP). Structural endpoints were assessed after 7 days via chlorophyll a content and phototrophic community composition. Based on these results, six compounds were selected for untargeted metabolomics (LC-HRMS/MS, ZenoTOF 8600) to detect early molecular responses below physiological and structural thresholds.

Kinetic screening revealed diverse response patterns that frequently diverged from predictions based on human MoA. For autotrophic function (PSII), different response profiles were observed: immediate inhibition without recovery (e.g., erythromycin), transient inhibition followed by recovery (e.g., atenolol), and progressive inhibition over time (e.g., 5-fluorouracil). In heterotrophic assays, diclofenac and paracetamol induced stronger LAP inhibition than antibiotic, while ofloxacin suppressed β-glucosidase activity and gabapentin triggered compensatory stimulation. Notably, structural shifts sometimes were observed at lower concentrations than physiological changes; for instance, the β-blocker atenolol selectively reduced cyanobacterial abundance at concentrations where no measurable physiological effects were yet detected.

Preliminary metabolomics analyses identified candidate molecular biomarkers occurring prior to detectable physiological and structural alterations, providing early fingerprints of chemical stress. Overall, this multi- level approach shows that critical ecological changes occur at sub-lethal concentrations and often deviate from expectations based on human MoA, supporting the integration of molecular, physiological, and structural endpoints in ecotoxicological risk Assessment.

Keywords: biofilm, antibiotic, photosynthesis, beta glucosidase, leucine aminopeptidase, metametabolomics

oral presentation

The Plastisphere: what drives the structure and functioning of plastic-colonizing microorganisms?

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Over the last decades trillions of plastic particles entered the aquatic environment, which are heavily overgrown with biofilms, termed the plastisphere. While there is evidence that these communities built a new entity in the environment, less is known on the structural-functional relationships of plastic-colonizing microbial communities. As recent developments in metagenomics enable a better understanding of the structure and functional potential of microbial communities, we applied metagenomics to understand the principles of colonization and adaptation of the plastisphere in comparison to plankton communities. The contribution summarizes results from experimental and field studies. In a first study, we address the question of the relevance of plastic additives for colonisation of microorganisms. Different PE-material with known mixtures of additives (flame retardants, plasticizers and UV and heat stabilizers) as well as a PE consumer product were exposed in fresh-water microcosms for several weeks. The study revealed that the additive mixture rather than the polymer type defines the structural composition and functional potential of microbial communities of the plastisphere. In a further study, environmental plastic debris was collected from the open-ocean North Atlantic and North Pacific garbage patches to address the question if general principles in adaption of the plastisphere structure and functioning exists in these ultra-oligotrophic environments. This study revealed that the plastisphere is characterized by larger genome sizes and a higher functional potential in comparison to the streamlined sympatric planktonic community. We found more gene copies for pathways coping with the harsh environmental conditions of the ocean surface, such as nutrient deficiency and high light exposure, compared to plankton, even though they live in the same environment. These results will be discussed in the context of colonisation and survival strategies of the plastisphere microbial communities in the environment.

Keywords: Metagenomics, plastic additives

Microbial adaptation to PFAS stress: Proteomic response of environmental Pseudomonas chlororaphis exposed to PFOA

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Perfluorooctanoic acid (PFOA) is a persistent PFAS contaminant that can exert strong selective pressure on microorganisms in polluted environments, with potential consequences for microbial functions, ecosystem processes and remediation potential. This study investigated the physiological and proteomic response of Pseudomonas chlororaphis subsp. aurantiaca PF-16, isolated from a PFAS-contaminated environment, under high PFOA exposure. Bacterial growth, respiration, PFOA concentration, selected enzyme activities and secondary metabolites related to redox and iron homeostasis were monitored, while comparative LC–MS/MS-based proteomics was used to identify molecular mechanisms of adaptation.

Exposure to PFOA caused reduced growth and a prolonged lag phase, indicating strong physiological stress followed by adaptation. Proteomic analysis revealed extensive remodeling of outer membrane structures, including changes in porins, lipoproteins and proteins involved in membrane permeability and lipid metabolism. PFOA exposure also altered redox homeostasis, iron- and sulfur-related pathways, and the abundance of Fe-, Fe-S- and heme-containing proteins, accompanied by changes in antioxidant enzyme activities. Central metabolic pathways were reprogrammed, with changes in respiration, the tricarboxylic acid cycle, glyoxylate and methylcitrate-associated pathways, carbohydrate metabolism and lipid catabolism. In addition, proteins related to stress response, motility, chemotaxis and virulence-associated traits were affected.

These results demonstrate that PFOA acts not only as a persistent chemical contaminant but also as a strong ecological stressor capable of reshaping bacterial physiology and functional potential. The identified proteomic signatures provide insight into pollutant–microbiome interactions under PFAS pressure and may contribute to the development of microbial indicators of PFAS impact, as well as to the selection of resilient microorganisms for future bioremediation strategies.

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Keywords: PFOA, Proteomic response, Microbial adaptations

Investigating multi-contaminant profiles across the European coastline and its impact on benthic biodiversity

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The chemical exposome associated with human activities encompasses a wide range of organic and inorganic compounds, including both legacy pollutants and contaminants of emerging concern (CECs). The Traversing European Coastlines (TREC) expedition is a pan-European initiative aiming to comprehensively investigate coastal ecosystems and their responses to environmental change and pollution pressures.

This study explores how contamination gradients influence benthic microbial biodiversity and functional potential across European coastal sediments. A total of sixty-one sites along the North-East Atlantic and Mediterranean coasts were surveyed. A broad spectrum of contaminants was monitored and analyzed, including: (i) legacy organic pollutants and CECs, such as Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ethers (PBDEs), Per- and Polyfluoroalkyl Substances (PFAS), and Organophosphate Esters (OPEs); (ii) trace metals and rare-earth elements (REEs); and (iii) 64 unregulated CECs recently prioritized by Yulikayani *et al.* (2025). Microbial diversity and predicted functional profiles were assessed using 16S and 18S rRNA metabarcoding and subsequently linked to sediment contamination.

Sites were categorized based on their contamination levels and types, reflecting surrounding anthropogenic pressures (i.e., estuaries, industrial zones, ports, and mining areas). Some locations exhibited contamination profiles dominated by PAHs, likely due to their proximity to refineries and port activities, while others were characterized by elevated PFAS levels, probably linked to nearby industrial sources. In addition, several hotspots of metal contamination were identified, likely associated with mining or industrial activities. We hypothesized that benthic microbial diversity and functional structure would vary along these contamination gradients. Multivariate analyses revealed associations between site-specific contamination profiles and the presence and relative abundance of particular taxa and functional pathways.

This large-scale assessment provides a unique baseline for understanding how complex contamination gradients shape benthic microbial communities and offers a framework for integrating chemical and biodiversity monitoring in European coastal ecosystems.

Keywords: contaminant; monitoring; biodiversity; CECs; metabarcoding

Using species sensitivity distributions to assess the toxicity of pesticides on nitrifiers and broader microbial groups: from single- species tests to soil amplicon sequencing

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Pesticides are widely used in modern agriculture, but their effects on soil microorganisms remain insufficiently addressed by outdated ecotoxicological tests. Recent studies by our group highlight ammonia-oxidizing microorganisms (AOM) as sensitive indicators of chemical stress in soils. Building upon prior single-species tests with AOM, we investigated the effects of pyraclostrobin, glyphosate and metsulfuron-methyl on nitrification. The more consistent impact of metsulfuron-methyl on nitrification led us to focus on its effects on the abundance and diversity of AOM and broader microbial groups such as total prokaryotes and fungi. An integrated approach combining amplicon sequencing with species sensitivity distributions (SSDs) was applied to derive thresholds relevant for risk assessment. Metsulfuron-methyl showed negative effects on AOM and prokaryotes, while fungi were more tolerant. SSDs based on single-species AOM data and soil microcosm amplicon sequencing yielded hazard concentration 5% (HC_5) values of 0.001 and 0.06 mg kg^{-1}, respectively, verifying the conservative nature of the former assays. SSDs constructed from amplicon sequencing provided HC_5 values of 0.042 and 0.1 mg kg^{-1} for prokaryotes and fungi, respectively. Calculated predicted no-effect concentrations (PNEC), indicated unacceptable risk for AOM only when data derived from single-species tests were used.

We propose the use of SSDs either as a Tier I stand-alone approach or integrated into an updated tiered risk assessment complementing the OECD 216 soil test (Tier II).

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Keywords: between 3 to 5 Soil microbiome; ammonia-oxidizing microorganisms (AOM); pesticide ecotoxicity; species sensitivity distributions (SSDs); amplicon sequencing

Synthetic nitrification inhibitors DMPP and allylthiourea use distinct mechanisms to inhibit an archaeal soil nitrifier

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Microbial ammonia oxidation, the first and rate-limiting step of nitrification catalyzed by ammonia monooxygenase (AMO), plays a central role in soil nitrogen cycling. This process is particularly relevant in agricultural soils, where nitrifiers compete with crops for ammonia-based fertilizers and contribute to environmental pollution and climate change through reactive nitrogen losses. To mitigate these effects, synthetic nitrification inhibitors are widely used over the last 30-40 years alongside fertilizers to reduce the activity of the main drivers of this process, namely ammonia-oxidizing archaea (AOA) and bacteria (AOB). However, the physiological responses of ammonia-oxidizing microorganisms to nitrification inhibitors at cellular level remain insufficiently understood. In this study, we investigated the responses of the AOA Nitrososphaera viennensis to the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and allylthiourea (ATU), using a combination of transcriptomics and proteomics, physiological assays, and relief experiments. Our results challenge the prevailing view that both compounds act primarily through copper chelation and instead reveal distinct cellular responses. Both inhibitors affected ammonia oxidation and induced broader changes in respiratory activity, energy metabolism, and stress-response pathways. However, their effects diverged markedly: DMPP exhibited characteristics of competitive inhibition of AMO, as its effects were partially alleviated by additional ammonia and accompanied by activation of urea acquisition pathways, whereas ATU behaved as a non-competitive inhibitor inducing a more general quiescent state. This work provides new mechanistic insight into how nitrification inhibitors interact with AOA and establishes an experimental framework for dentifying the modes of action of other nitrification inhibitors in future studies.

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Keywords: nitrification, Nitrososphaera viennensis, nitrification inhibitors, omics, microbial responses

Using microbial indicators to predict ecotoxicological risk from emerging contaminants in South Atlantic coastal lagoons

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Emerging contaminants are increasingly detected in aquatic ecosystems and represent a growing environmental concern due to their ecological effects and complex mixture dynamics. Assessing their impact remains challenging because conventional monitoring often fails to capture subtle biological responses to low contaminant concentrations and multiple stressors. Microbial communities provide a promising alternative, as they respond to environmental change and integrate signals from diverse environmental pressures.

This study aimed to identify microbial indicators associated with emerging contaminant pressure in coastal lagoons o the South Atlantic. Environmental condition was characterized using a water quality index (AQI) based on ecotoxicological risk, integrating the occurrence and concentrations of multiple emerging contaminants to classify sampling sites into environmental status categories. Bacterial community composition was characterized through high-throughput sequencing of the 16S rRNA gene. Indicator species analysis was applied to identify microbial taxa and combinations of taxa associated with each environmental category, allowing the detection of microbial signatures linked to different levels of contamination risk.
Robust indicator sets across impact categories were identified. In most categories, indicators comprised widely distributed and ecologically versatile lineages. Rather than reflecting completely distinct communities, AQI categories captured shifts in the relative contribution of tolerant or responsive microbial groups along the impact gradient.

To assess predictive performance, indicator sets were incorporated into a machine learning classification framework validated by cross-validation. The model showed high overall discrimination capacity (accuracy = 92.7%; Kappa = 0.87). Importantly, microbial indicators successfully predicted ecotoxicological risk categorization even when emerging contaminants were not the primary drivers structuring microbial communities. This highlights the sensitivity of microbial assemblages to environmental stressors and their potential as integrative indicators of contamination.

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Keywords:Microbial indicators, Emerging contaminants, Ecotoxicological risk assessment, Machine learning

Beyond sediment contaminant burdens: eDNA biodiversity reveals ecologically relevant exposure to environmental contaminants across the Baltic Sea

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The Baltic Sea has experienced long-term accumulation of anthropogenic contaminants, including metals and organic pollutants. It is also characterized by pronounced environmental gradients and ecologically distinct basins, making it challenging to disentangle the effects of contamination from natural variability on biodiversity. Here, we assessed how contamination influences both microbial and eukaryotic sediment communities across six Baltic Sea basins. Environmental DNA was characterized using amplicon sequencing targeting the 16S rRNA (bacteria) and COI (eukaryotes) marker genes. Chemical contaminants (heavy metals, PCBs,PAHs) were summarized using principal component analysis, and their relationship with biodiversity and community composition was evaluated alongside environmental variables and geographic structure using linear mixed models and distance-based redundancy analysis.Bacterial community composition was significantly associated with contamination gradients after accounting for spatial and environmental structure; however, this signal overlapped with strong basin-level patterns. PCBs were the primary contributors to the main contamination gradient. In contrast, eukaryotic biodiversity showed a consistent, negative response to contamination. Species richness and Shannon diversity declined with increasingcontamination, and this relationship remained significant after accounting for environmental and geographic variation, with metals emerging as the primary drivers of biodiversity loss. We show that both microbial and eukaryotic communities respond to contamination, albeit in different ways. Bacterial community composition reflects multiple interacting gradients, while eukaryotic diversity provides a clearer signal of contaminant stress. Together, these findings highlight the value of integrating multiple biodiversity metrics and statistical models to better understand ecological impacts in complex marine systems. This work was supported by the Biodiversa+, the European Biodiversity Partnership.

Keywords: Baltic Sea; contaminants; biodiversity; eDNA; amplicon sequencing

Lake environment shapes macrophyte-associated microbial communities more than host species

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Lakes provide essential ecological services, yet they are increasingly under pressure from global change, particularly through climate shifts and intensifying multi-contamination. While these ecosystems rely on robust biodiversity, the accumulation of pollutants threatens to disrupt functional stability across trophic levels, from primary producers, aquatic macrophytes, to their associated microbiomes forming holobionts. The latter constitute key drivers in nutrient cycling and water quality. However, the relative influence of host identity versus environmental pressure in shaping these microbiomes remains unclear,especially in contaminated settings. Hence, we characterised the composition, diversity and predicted functional potential of microbial communities (16S rRNA gene metabarcoding) associated with six aquatic macrophyte species sampled according to a multi-contamination gradient in Lake Bourget (France). Main results showed that community structure (Beta diversity) was reshaped by the environment while microbial richness remained stable across host species and sites. Notably, environmental conditions exerted a stronger influence than host identity, microbial communities shifted markedly between sites with high and low contamination levels. While host species within the same site shared similar microbiomes, a same species exhibited distinct microbial signatures among sites, confirming a dominant environmental filtering effect. Bacterial co-occurrence network analyses further revealed shifts at sub- community levels (modules) between sites. Despite changes in network module structure, functional redundancy across sites suggests that shifts in species composition do not affect key microbial functions. Overall, these findings demonstrate that multi-contamination overrides host species effects in shaping macrophyte-associated microbiomes. This supports that holobiont community assembly in freshwater ecosystems is largely governed by environmental filtering rather than host specificity. This highlights the need for additional experiments to clarify how holobiont dynamics shape lake responses to global change, and to refine the use of macrophyte-microbiome associations in water quality management through the development of engineered microbial inoculum.

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Keywords: Microbiome, Macrophytes, Pollution gradient, Lake ecosystems

Understanding the mechanism of interspecies variation in the tolerance of Rhizophagus species to glyphosate

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Pesticides are major environmental pollutants affecting humans, terrestrial and aquatic organisms, and beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF). Glyphosate, the most widely used herbicide, targets the EPSPS enzyme in plants. While previous studies have reported effects of glyphosate on AMF spore germination and sporulation — attributed to either direct toxicity or indirect effects via the host plant — its precise mechanism of toxicity remains elusive. In our previous work, two *Rhizophagus* species (*R. irregularis* – sensitive, *R. clarus* - tolerant) showed differential responses to glyphosate at spore germination.

To explore the mechanism for this differential response, we performed *in silico* structural prediction and molecular docking of AMF EPSPS-glyphosate, complemented by assays evaluating effects at asymbiotic and symbiotic stages. We assessed germ tube elongation and spore nuclear abundance by targeting the single-copy MAT locus via ddPCR. At the symbiotic stage, root colonization and plant phosphorus acquisition in *Lotus japonicus* were evaluated using the gnotobiotic AMF-sandwich test, while extraradical mycelium (ERM) spatial development was assessed through time-lapse high-throughput microscopy.

In silico analysis ruled out a target-site mechanism. Glyphosate reduced nuclear abundance and inhibited germ tube elongation in *R. irregularis*, whereas *R. clarus* exhibited increased nuclear abundance and formed spore-like structures at low glyphosate concentrations that lacked germination capacity. Root colonization by *R. irregularis* decreased in a dose-dependent manner, while *R. clarus* colonization remained unaffected, with a concurrent increase in phosphorus uptake. ERM development was equally impaired in both species.

These contrasting interspecific responses, confined to the spore stage and not extending to ERM, suggest that differential glyphosate penetration — possibly linked to cell wall thickness differences — rather than target-site resistance, underlies the observed effects. Ongoing experiments using ^{13}C-labeled glyphosate and NanoSIMS aim to verify this hypothesis.

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Keywords:Arbuscular mycorrhizal fungi (AMF), glyphosate, nuclear abundance, spore-like structures (SLS), toxicity

How eutrophication shapes microbial communities in peri-urban lakes: insights from the COM2LIFE project

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The eutrophication of peri-urban lakes, a natural process amplified by human activity, raises global health ("One Health") concerns due to the interdependence of ecosystem, animal, and human health. Anthropogenic pressures, through their influence on eutrophication, are expected to modulate microbial diversity across freshwater ecosystems. Although microbial community composition and spatiotemporal structure are anticipated to vary, their core metabolic functions may remain conserved owing to substantial functional redundancy. The COM2LIFE project tests these hypotheses by examining the effects of eutrophication on microbial communities in lakes of the Île-de-France region, the most densely populated area in France.

Microbial taxa and gene content were monitored over 18 months in 9 lakes spanning a gradient from oligotrophic to highly eutrophic conditions. Analyses combined marker-gene and (meta)genome sequencing of dominant microorganisms with characterization of lake physicochemical parameters. This integrative approach enabled comparisons of taxa and their associated functions across seasons and lakes and the identification of key factors shaping microbial dynamics at the regional scale.

Eutrophication level strongly influenced the composition of both prokaryotic and eukaryotic communities in the water column, while its effect on prokaryotic functional profiles was weaker but still significant. In the most eutrophic lakes, community composition showed greater intra-season variability and appeared to drift over time, suggesting instability at this temporal scale. The influence of eutrophication exceeded that of seasonal variation—an unexpected result given the physicochemical similarities and geographic proximity of the lakes—and highlighted the unique dynamics of each system.

In addition, the gut microbiota of a native and an invasive fish species were characterized in all lakes. Microbiota alterations increased with eutrophication, confirming a direct effect on fish holobionts and underscoring links between environmental conditions and fish health. The invasive species appeared more resilient than the native species.

Overall, the findings demonstrate that Greater Paris constitutes a valuable "natural laboratory" for investigating peri-urban lake ecology, with more than 240 lakes sharing major environmental characteristics yet displaying substantial variation in trophic status.

Keywords: Paris, bacteria, microbiota, fish, eukaryotes

Sunflower cry-for-help in presence of petroleum hydrocarbons (PHCs) alters the root exudation chemistry, potentially affectingrhizo competence of PHC-degrading bacteria

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To mitigate phytotoxic effects in polluted soils, plants tune root chemistry releasing a “cry-for-help” to recruit microbial communities, able to degrade the pollutants and to sustain plant growth.

Although root exudation is a crucial mechanism to cope with soil pollution, the shift it induces in root exudation patterns is poorly characterized. In this study, the sunflower “cry-for-help” response to PHCs was investigated through untargeted metabolomics: i) in vitro, challenging plants with xylene and ii) in a historically PHC-polluted soil under outdoor conditions. The two experimental designs significantly impacted the detected exudation patterns: a more complex profile was displayed in the soil setup, impacting the abundances of key secondary metabolites like coumarins, terpenes and flavonoids. DistLM analysis performed on the metabolome and 16S rRNA amplicon sequencing datasets highlighted that coumarins and pyrimidines significantly correlated with the structure of rhizosphere microbial communities in the polluted soil, potentially influencing the recruitment of PHC degrading populations. Indeed, supplementation of selected exudates, provided as pure compounds, impacted the rhizocompetence of PHC-degrading strains. Two metabolites, whose abundance increased under PHC stress, stimulated swimming motility and biofilm formation, important features for microbe establishment in the rhizosphere. On the other hand, it was observed that a root metabolite, less exudated under PHC stress, showed growth inhibition activity on selected strains, suggesting a preservation strategy by the plant to protect and maintain degrading microorganisms in the rhizosphere.

Unravelling the plant “cry-for-help” to PHCs is essential to improve bioremediation effectiveness, by identifying regulators of key events for the recruitment of degrading microorganisms.

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Keywords: root exudates, cry-for-help, rhizoremediation, holobiont, rhizocompetence

Implementing arbuscular mycorrhiza fungi in an environmental risk assessment – case study with benomyl considering effects on spore germination and symbiosis in natural soil

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous in terrestrial ecosystems and form symbioses with most plants, thereby can enhance nutrient uptake, pest tolerance, and yield. Despite their ecological importance, AMF have been recognized as non-target soil organisms relevant for environment risk assessment (ERA) of plant protection products (PPP) within the EFSA soil opinion (2017). The current OECD nitrogen transformation test (OECD Test Guideline (TG) 216), used to assess PPP effects on soil microorganisms, does not address impacts on AMF.

To close this gap, the UBA project MICROSOIL proposes to include a spore germination test (ISO 10832) as a Tier 1 screening method. However, this method does not capture the symbiosis between AMF and host plants. Therefore, a symbiosis-test-method was developed within the UBA project ERAMYC for assessing the effects of PPP on the symbiotic phase of AMFs. In our study, we observed the effect of a PPP in artificial and natural soil on AMF using both methods.

Preliminary experiments using *Funneliformis mosseae* confirmed sufficient spore germination in artificial soil and the reference soil RefeSol 02A, which were selected as substrates for all further test systems. The fungicide Benomyl was used as a reference substance and showed a clear dose–response relationship on spore germination in two independent experiments. In the spore germination tests an EC_{50} of 4.5 mg/kg dry weight (dw) soil in artificial substrate and of 3.23 mg/kg dw soil in natural soil were determined. The ISO 10832 states that the EC_{50} should be between 1 and 10 mg/kg dw soil and therefore the results show that the test worked well in the natural soil.

In the subsequent Tier 2 symbiosis test, *Allium ampeloprasum* (leek) serves as the host plant with *F. mosseae* as the fungal partner. After a 56-day exposure, endpoints include intraradical colonization, extraradical mycelium production, and plant biomass. In parallel, the OECD TG 216 test is conducted to compare AMF sensitivity to the standard soil microbial assay used in ERA. The experiments on the symbiosis of AMF were initiated in September/October 2025. The tests will be terminated and evaluated in February 2026, the results will be presented on the poster.

Results from all tests will be analyzed to evaluate sensitivity, ecological relevance, and potential regulatory applicability.

Influence of Early-life Contaminant Exposure on the Bacteriome Structure and the Response to a Viral Infection in Oyster

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Coastal ecosystems are increasingly exposed to complex contaminant mixtures derived from anthropogenic activities. Such exposures can disrupt microbial communities that underpin ecosystem processes and host health. However, the consequences of chemical stressors for host-associated microbiome assembly and functioning remain insufficiently characterised. The Pacific oyster (Magallana gigas), a keystone species sustaining coastal ecosystem functioning and global aquaculture, provides a relevant model system. Aquaculture production is recurrently threatened by Ostreid herpesvirus 1 (OsHV-1), which causes mass mortalities in juveniles. This context offers an opportunity to investigate how contaminant exposure may alter early microbiome assembly and influence host resilience to disease. We hypothesised that early-life exposure to a realistic contaminant mixture would (i) durably modify bacteriome diversity and assembly dynamics and (ii) affect susceptibility to viral infection later in development.

To test these hypotheses, six oyster families contrasting in resistance to OsHV- 1 were exposed for ten days post- hatching to an environmentally realistic mixture of eighteen contaminants (pesticides and formulation-related metals). Bacterial communities were characterised at larval and juvenile stages using full- length 16S rRNA sequencing (Oxford Nanopore Technology).

Exposure reduced fertilisation success in most families and significantly affected bacteriome composition, with marked influences of developmental stage, host genotype, and contamination on microbiome structure. However, despite sustained microbiome alterations, early- life exposure did not significantly change survival after viral challenge.

These findings demonstrate contaminant-induced restructuring of host-associated microbial diversity, consistent with symbiotoxic effects. Although no immediate change in disease mortality was observed, altered microbiome assembly may have functional consequences under more complex environmental conditions. Incorporating microbiome diversity and functioning into ecotoxicological assessments will improve understanding of holobiont vulnerability to multiple stressors.

Keywords: microbiome ; oyster; pesticides; symbiotoxicity ; OsHV-1 ; ecotoxicology

MICROSENSE : Integrating Microbial Bioindicators and Functional Biomarkers for pesticides Water Quality Assessment

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Traditional chemical monitoring of water matrices fails to capture the full ecological impact of pesticide contamination. The MICROSENSE project addresses this gap by leveraging microbial community responses in wastewater as indirect indicators of phytopharmaceutical product (PPP) pollution, enabling detection of both current and historical contamination events.

Bioreactors containing urban wastewater were chronically exposed to five relevant PPPs (two fungicides, three herbicides). Sequential sampling enabled tracking of bacterial community shifts via 16S rRNA metabarcoding and functional gene characterization through metagenomics.

Two robust water-phase bioindicators were validated: Dokdonella immobilis, whose population systematically increases in the presence of all tested PPPs, and Pseudoxanthomonas dokdonensis, specifically responsive to the herbicide S-metolachlor. Functional biomarker genes associated with membrane stress responses (K00021, K01641, K04708, K16436) and benzene ring cleavage (cmtC_K1062) were identified in response to triazole fungicides and S-metolachlor, enabling molecular-level contamination fingerprinting.

These laboratory-validated bioindicators and biomarkers are being translated into rapid, field-deployable proof-of-concept kits integrating LAMP-qPCR for functional gene detection and Cell-SELEX-optimized aptasensors for whole-cell indicator detection, offering a competitive advantage over conventional chemical methods through dramatically reduced analysis times.

Keywords: Micropollutants, Bioindicator, biomarker, Metagenomics

Biofilm biochemical composition shapes the toxicity of pesticide mixtures

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Pesticides include diverse chemical classes with varying properties and modes of action and are frequently present as mixtures in aquatic environments. These mixtures can affect biofilms, structured microbial communities that support primary production and nutrient cycling in aquatic systems. Mixture toxicity cannot be predicted from water concentrations alone, because responses depend on both chemical properties, such as hydrophobicity, and the composition of the biofilm. This study investigates how the biochemical composition of biofilms influences the impact of pesticide mixtures. Lab-grown diatom–cyanobacteria biofilms were established at controlled taxonomic ratios (4:1, 2:1, and 1:2). Biofilms were characterized using pigments, lipids, and polysaccharides, then exposed for 24 h to three binary pesticide mixtures, including mixtures of two Photosystem II-inhibiting herbicides and mixtures combining a PSII inhibitor with a fungicide. Mixtures combining two PSII inhibitors consistently induced the strongest photosynthetic inhibition, whereas mixtures including the fungicide resulted in weaker effects. Toxicity also depended strongly on biofilm composition. Diatom-dominated biofilms, characterised by higher chlorophylls and lipids content, exhibited the highest sensitivity, while cyanobacteria-rich biofilms, enriched in polysaccharides and phycocyanin, showed greater tolerance. These patterns reflect coupled taxonomic and biochemical differences, which cannot be disentangled in this study. Correlation analyses showed that chlorophyll a was strongly positively associated with toxicity, consistent with its role as a proxy for the abundance and functional importance of PSII targets, with lipids as a secondary parameter, while polysaccharides and phycocyanin were negatively associated with inhibition. Because biofilm composition in natural systems is dynamic, these findings may help explain why pesticide toxicity differs among field biofilms. They also suggest that biochemical indicators, such as pigments and extracellular polymeric substances-related components, could improve future ecotoxicological assessments.

Potential Impact of Nanomaterials on Aquatic Microbial Communities

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Nanomaterials are increasingly released into the environment through anthropogenic activities, including industrial processes, consumer products, and wastewater discharge. Once in aquatic systems, these materials may interact with microbial communities that play a central role in biogeochemical cycling, organic matter degradation, and overall ecosystem functioning. Despite their ecological importance, the responses of complex microbial communities to nanoparticle exposure remain poorly understood.

This study aims to investigate the effects of zinc oxide (ZnO) and iron oxide (Fe_2O_3) nanoparticles on natural aquatic microbial communities, with a focus on potential changes in community composition under environmentally relevant exposure conditions.

To address this, controlled laboratory microcosm experiments were conducted using natural microbial communities obtained from freshwater collected from the River Spree in Berlin. These communities were exposed to varying, environmentally relevant, concentrations of nanoparticles over a period of four weeks. Community composition was analyzed using 16S rRNA amplicon sequencing, with subsequent functional analyses being planned. Nanoparticle stock solutions were characterized by transmission electron microscopy (TEM) to determine particle size and morphology. Both stock solutions and exposure media were analyzed using total reflection X-ray fluorescence (TXRF) and inductively coupled plasma mass spectrometry (ICP-MS) to quantify elemental composition and nanoparticle concentrations over the course of the experiment. These analyses confirmed successful introduction of nanoparticles into the exposures systems, showing a significant increase in zinc and iron concentrations compared to the control condition. We will present initial results of the microcosm experiments, comparing diversity and taxonomic composition of the microbial communities in response to the different treatments.

This work contributes to the development of a methodological baseline by identifying key parameters for assessing the impact of nanomaterials on aquatic microbial communities, emphasizing the importance of community-level approaches for environmental risk assessment.

Keywords: microbial communities, anthropogenic influence, nanoparticles

Physicochemical stress responses of a toxic cyanobacterium isolated from a summer bloom in the Macta Ramsar wetland (Algeria)

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Toxic cyanobacterial blooms are an increasing threat to aquatic ecosystems and public health, particularly in wetlands exposed to environmental fluctuations. This study focuses on the isolation and characterization of a cyanobacterial strain from a summer bloom in the Macta Ramsar wetland (western Algeria). The strain was isolated and identified using 16S rRNA gene analysis, followed by sequencing for phylogenetic characterization. Screening for toxin- related genes confirmed its toxic potential. In vitro experiments were conducted to assess the physiological response of this cyanobacterium to various physicochemical parameters, including pH, salinity, nutrient availability (nitrate and phosphate depletion) and light intensity. The strain exhibited tolerance to a wide range of environmental conditions, highlighting its ability to persist under fluctuating physicochemical stressors. These findings emphasize the ecological risk associated with the persistence and proliferation of toxic cyanobacteria in wetland ecosystems and support their use as indicators of environmental disturbances.

Acknowledgments:This work was conducted at the University of Oran 1 Ahmed Ben Bella. The authors gratefully acknowledge all members of the *AquaBior* laboratory for their support. No specific funding was received for this study.

Keywords: Cyanobacteria, 16S rRNA, Cyanotoxins, Wetland, Western Algeria

Environmental and land-use drivers of fungal and bacterial diversity in ponds

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Fungi and bacteria play a central role in the dynamics of lentic environments, contributing to organic matter decomposition, nutrient cycling, and the maintenance of ecosystem functioning. Changes in land use surrounding these systems may alter the structure of the aquatic microbiome through increased inputs of sediments, nutrients, and anthropogenic contaminants such as pesticides. This study aims to characterise fungal and bacterial communities in sixty ponds distributed across three sub-basins of the Tietê River basin, located in the state of São Paulo, Brazil, and to assess the influence of land use and physicochemical parameters on the composition and diversity of these organisms. Sampling was carried out between November 2025 and January 2026, during the rainy season. From each pond, a combined water sample was obtained from four sampling points for environmental DNA analysis (eDNA). After filtration through 0.22 µm membranes, filters were placed in cryotubes, frozen in liquid nitrogen, and subsequently stored at -80 °C. In parallel, transparency, depth, and physicochemical water variables were measured, and additional samples were collected for the analysis of nutrients, metals, suspended solids, chlorophyll, and pesticides. For eDNA analysis, bacterial 16S rRNA and fungal ITS markers will be amplified, followed by next-generation sequencing and bioinformatic analysis. Preliminary results indicate variation among sub-basins, mainly in conductivity, turbidity, and suspended solids. Seventeen pesticide active ingredients, three degradation products, caffeine, and the antibiotic trimethoprim were quantified. Atrazine, tebuconazole, tebuthiuron, carbendazim (banned in Brazil since 2022), and caffeine were detected in all ponds. Lower microbial biodiversity and greater dominance of resistant taxa are expected in areas under extensive agriculture and with higher pesticide occurrence, highlighting the role of land use in shaping the aquatic microbiome.

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Keywords: Aquatic Microbiome, Metabarcoding, Pesticides, Tropical freshwater ecosystems.

Environmental compatibility of lunar regolith using *Aliivibrio fischeri* as a sensitive model organism and a natural soil microbial community as an adaptable biological entity

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Establishing sustainable life support systems for long-term lunar missions requires implementation of In-Situ Resource Utilization (ISRU) strategies, particularly the use of local materials such as lunar regolith for various applications. However, before such substrates will be employed, their environmental compatibility needs to be assessed. Previous toxicological studies on lunar regolith simulants (LRSs) have highlighted potential human health risks related to their inhalation; however, both their ecotoxicological effects and potential impacts on natural microorganisms are still unexplored. To address this gap, the present study performed an ecotoxicological screening of three high fidelity LRSs (LHS-1D, LHS-2, and LMS-1) using the Aliivibrio fischeri bacterium test (ISO 11348-3:2019). In an environmental compatibility context, the results were further deepened comparing them with those of other two test organisms belonging to different trophic levels (Lepidium sativum and Daphnia magna). Overall, LHS-1D showed detrimental effects on all tested organisms, and, for this reason, it was excluded from further experiments. Subsequently, aliquots of a soil were mixed with 70% of LHS-2 or LMS-1; the natural soil microbial community was analysed for 21 days in terms of total microbial abundance (DAPI counts) and viability (% live cells/live+dead). Although the natural microbial community abundance increased significantly in LRS presence, the percentage of cell viability was lower in regolith presence. This result suggests a selective pressure of LRSs on the microbial populations. Overall, the ecological plasticity of the natural soil microbial communities is confirmed, and this does not exclude bacterial adaptability to unexplored environments.

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Keywords: In-situ resource utilization (IRSU), Application for long term lunar mission, Ecotoxicological test, Microorganism plasticity

Impact of Plastic mulch film (polyethylene) on rhizosphere microbial community structure and functions

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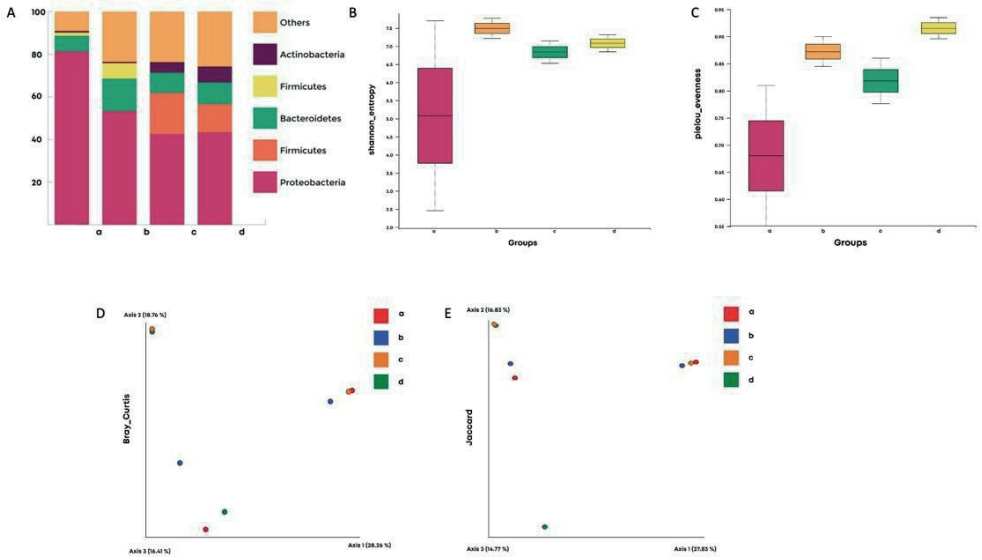
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Rapid population growth and food security is a serious concern in the developing countries calling for an urgent need to develop some ground-breaking and sustainable solutions to enhance agricultural productivity. In this regard exploiting the rhizosphere microbiome is not only the most appropriate foci but an innovative approach to develop agricultural solutions helpful to mitigate the issues of food security, climate change, crop productivity, land degradation and sustainability. The impact of plastic mulch films on the rhizosphere microbial communities is surprisingly less explored and needs further research. In the current study impact of plastic mulch film on the rhizosphere microbiome structure and function was studied. The study groups included control group (cucumber and strawberry intercropping without mulch film), cucumber and strawberry intercropping (with mulch film) and the mulch film itself. The microbial community composition was analysed using high throughput sequencing targeting the 16S rRNA region. For alpha and beta diversity analysis QIIME 2 and R software version 3.5.0 were used. The enzyme activity in soil was analysed using fluorescence microplate enzyme assays.

The results showed that application of polyethylene mulch film significantly altered the abundance and diversity of bacterial communities. The alpha and beta diversity indices showed significant differences ($p < 0.05$) in richness estimates for control and treatment groups. There was 38.57%, 42.86%, 22.86%, and 25.71% enhance in the enzyme activities for Alkaline phosphatase, Nitrate reductase, β-D-glucosidase and N-acetyl-β-glucosaminidase respectively. This study demonstrates that plastic mulch film has significant impact on the microbial population composition and functions in the rhizosphere selecting for the beneficial microbes and enhancing the functionality of the community. Current study advocates and provides empirical grounds for the application of the plastic mulch films in the sustainable agro-development and highlights how microbiome has the key role in the ecosystem

Keywords: Plastic, microbiome, soil, degradation


A
100
80
60
40
20
a
b
c
d
Others
Actinobacteria
Firmicutes
Bacteroidetes
Firmicutes
Proteobacteria
B
shannon_entropy
Groups
C
pielou_evenness
Groups
D
Bray_Curtis
a
b
c
d
E
Jaccard
a
b
c
d

Influence of cyanobacterial blooms and associated bioactive metabolites on fish gut microbiota

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Cyanobacteria are known to produce a wide diversity of structurally unique and biologically active natural products, including well-characterized cyanotoxins. However, growing evidence suggests that their effects extend beyond these compounds, highlighting the need to consider the broader diversity of bioactive metabolites. In freshwater ecosystems, organisms are exposed to multiple cyanobacterial genera, producing distinct cocktails of metabolites with potentially different ecological impacts.

Previous studies have demonstrated strain-specific differences in acute toxicity in *Microcystis*, but the consequences of exposure to diverse cyanobacterial assemblages on host–microbiota interactions remain poorly understood. My PhD research aims at investigating how different cyanobacterial morphotypes (filamentous, colonial…) and different metabolites cocktails (containing or not cyanotoxins) affect the gut microbiota and host physiology of fish models.

For this, fish (*Oryzias latipes* and *Danio rerio*) are exposed to selected strains characterized by contrasted and, in some cases, overlapping metabolite profiles. In particular, strains from different genera (*Microcystis aeruginosa* and *Planktothrix agardhii*) producing similar classes of metabolites (e.g., microcystins, aeruginosins, and cyanopeptolins, or alternatively aeruginosins, anabaenopeptins, and microginins) were included to disentangle the effects of metabolite composition from those related to cyanobacterial morphology. Additional strains of *Aphanizomenon gracile*, producing saxitoxin and/or puwainaphycins, further expanded the diversity of metabolite mixtures tested. We combined *in vivo* exposures of animals to simulated blooms with *in vitro* screening of the antibacterial activity from cyanobacterial extracts on isolated fish gut bacteria.

Preliminary results show that exposure to distinct cyanobacterial assemblages significantly reshapes fish gut microbiota composition and selectively affects key bacterial taxa. Despite similarities in metabolite families, microbial responses differ depending on the cyanobacterial species and strain. These findings highlight the role of metabolite diversity in modulating microbiota dynamics and emphasize the importance of integrating host–microbiota–environment interactions to better understand the ecological consequences of cyanobacterial blooms.

Keywords: Ecotoxicology; Aquatic animals; Cyanobacteria; Cyanotoxins; Intestinal microbiota

Integrated assessment of nano-formulated and conventional copper-based pesticides on microbiota across soil, aquatic organisms and mice within a One Health framework

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The extensive use of copper (Cu)-based pesticides in agriculture leads to soil accumulations, aquatic ecosystem contamination and human dietary exposure. To reduce these risks, Cu-based nanopesticides have been proposed as promising alternatives, offering higher biocidal efficacy at lower applications rates and potentially reduced environmental impact. This project funded by the *French Office for Biodiversity* (Ecophyto II+plan) aims to generate data, within a One Health framework, to assess risks associated with Cu nano-formulations on agroecosystems, aquatic ecosystems, and human health under relevant exposure scenarios. For this purpose, we compared the effects of a commercial $Cu(OH)_2$-based nanopesticide with those of an equivalent conventional Cu formulation using a multi-model approach. Representative biological models included soil for agroecosystems, *Xenopus laevis* and chironomids (*Chironomus riparius*) for aquatic ecosystems, and mice (*Mus musculus*) for human health. Given the antimicrobial properties of Cu-based (nano)pesticides, particular emphasis was placed on their effects on soil and intestinal microbiota across model species.

Cross-model analysis of microbiome responses to Cu-based pesticides was conducted to identify general response patterns, identify potential shared bioindicator taxa, and determine whether nano- pesticides induce specific changes in microbiome diversity, structure, and stability. In addition to host toxicity and Cu accumulation in the different biological models, the biocidal properties of both conventional and nano-sized Cu formulations altered microbiota composition and were associated with physiological changes in the host. Cu exposure significantly reduced microbiome alpha diversity and increased beta diversity, highlighting greater community dispersion across host-associated microbiota in all models, with stronger effects than those observed in soil microbiota. Moreover, the two commercial formulations induced distinct shifts in bacterial community composition.

Overall, our findings demonstrate that integrating microbiota analyses within a cross-species design reinforces the relevance of the One Health framework for evaluating the environmental and health impacts of Cu-based (nano) pesticides.

Beyond growth inhibition: lipidomic indicators of chemical stress in microalgae using high resolution mass spectrometry.

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Microalgae underpin aquatic food webs and are widely used as standard ecotoxicological test organisms, yet routine assays often rely on population-level endpoints (e.g., growth inhibition) that provide limited insight into sub-lethal physiology and mechanisms of action. To strengthen the role of microorganisms as ecotoxicological indicators for environmental risk assessment, we developed an integrated analytical workflow combining untargeted liquid chromatography– high-resolution mass spectrometry (LC–HRMS) lipidomics with rapid single-cell phenotyping by flow cytometry. Five laboratory reference algal strains spanning marine & freshwater environments, and prokaryotic & eukaryotic taxa (including widely used test organisms and toxin-producing groups) were analysed using biphasic extraction of non-polar lipids and polar metabolites. Full-scan LC– HRMS data were acquired in positive and negative ionisation at high mass resolution with data- dependent MS/MS for structural elucidation, followed by multivariate statistics (e.g., PCA) and library/database-supported annotation to identify discriminating molecular features. Lipidomic fingerprints clearly differentiated strains and highlighted compound classes driving inter-taxa separation. To evaluate ecotoxicological responsiveness, freshwater strains (*Microcystis* and *Chlorella*) were challenged with reactive oxygen species/superoxide generators, and coordinated shifts in the lipidome were quantified alongside flow-cytometric measures of cell status. Together, these orthogonal datasets provide sensitive, mechanistically informative indicators of chemical stress, complementing traditional growth endpoints and supporting more diagnostic interpretation of exposure and effect. This approach offers a scalable toolbox to refine microalgal ecotoxicity testing, improve assessment of mixture and emerging contaminant impacts, and contribute to reduction and refinement of animal testing by strengthening microbial and *in vitro* new approach methodologies (NAMs).

Keywords: Microalgae; Chemical stress; Sub-lethal endpoints; Lipidomics; Liquid Chromatography– High-Resolution Mass Spectrometry

A tiered approach combining nitrifier single-species tests and OECD 216 to assess pesticide effects on soil microorganisms

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Soil microorganisms play a crucial role in ecosystem functioning, yet their responses to pesticides are inadequately captured by current regulatory ecotoxicological tests. Recent studies by our group have proposed ammonia-oxidizing microorganisms (AOM) as soil microbial indicators of pesticide toxicity. Building on previous single-species tests (SSTs; Tier I) with nitrifying microorganisms and the OECD 216 soil N transformation assay (Tier II), we evaluated the use of a structured two-tiered approach to assess pesticide effects on soil microbiota. As a proof-of-concept, we assessed the toxicity of four pesticides (pyraclostrobin, metsulfuron-methyl, clopyralid and terbuthylazine) in SSTs with selected AOM strains, a nitrite oxidizing bacterium (NOB), and a synthetic microbial community (syncom) (Tier I), and a dose-response version of the OECD 216 test (Tier II). This setup allowed us to derive EC_{50} values to compare the level of conservatism of the two tiers (Tier I > Tier II) and perform risk assessment. Metsulfuron-methyl was the most toxic, with the lowest EC_{50} values observed in AOM and the syncom (0.03 - 0.04 mg L^{-1}), whereas substantially higher EC_{50} values were observed in soil (~56 mg kg^{-1} dws), highlighting the higher conservatism of the single-species approach. Risk assessment was conducted by comparing the PNEC with PEC_{soil} for the four pesticides. Unacceptable risk was identified based on SSTs (PNEC/ PECsoil < 1) for pyraclostrobin, metsulfuron-methyl and terbuthylazine; however, this was not confirmed in the soil-based OECD test, supporting our tiered approach. Overall, our study demonstrates that SSTs with selected AOM and NOB soil strains can serve as a Tier I screening tool complementing the Tier II soil-based OECD 216 assay (dose-response version), comprising a refined tiered risk assessment scheme for assessing pesticide effects on soil Microorganisms.

Acknowledgments: This work was financially supported by the project RATION that is funded by the European Commission (Grant Agreement No. 101084163).

Keywords: ammonia-oxidizing microorganisms (AOM), OECD 216, tiered risk assessment, pesticides, soil microorganisms

Pesticides Modify Bacterial-Fungal Interactions During Leaf- Litter Decomposition in Streams

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New agricultural practices increasingly use biopesticides as a more sustainable alternative. Biopesticides are defined as naturally occurring molecules that are designed to control pests while having a minimal impact on the environment and human health. They are generally more specific and readily biodegradable than conventional pesticides. However, their effects on non-target microorganisms remain poorly documented. This study evaluates the comparative effects of a biopesticide (kasugamycin), a conventional pesticide (copper sulfate) and their mixture on the interaction between the fungus *Paraconiothyrium variabile* and the bacterium *Shewanella putrefaciens*, both isolated from alder leaf litter submerged in a pond ecosystem. Microorganisms were exposed for 28 days in mono- and co-cultures to low and high concentrations of the fungicide kasugamycin (1–100 mg L^{-1}), copper sulfate (30–100 µg L^{-1}) and a mixture of both (30 µg Cu L^{-1} combined with 1 or 100 mg L^{-1} kasugamycin). Bacterial and fungal biomass, key functional endpoints (extracellular enzyme activities, respiration, litter mass loss), and tolerance acquisition to kasugamycin and copper sulfate during leaf litter decomposition were assessed. Our results show that 100 mg L^{-1} kasugamycin concentration mostly inhibits fungal biomass in monoculture after two weeks of exposure. In co-culture, bacterial- fungal competition amplifies this inhibitory effect, with a decrease in fungal biomass occurring one week earlier than in monoculture. A significant decline in respiration, but an acquisition of tolerance to kasugamycin, is also observed after 21 days of exposure to high-concentration in co-cultures. Analyses on copper sulfate and mixture treatments are ongoing and will be presented at the congress. Ecotoxicological responses to biopesticide differed between mono- and co-cultures decomposing alder leaf-litter, with microbial interactions exacerbating the fungicidal effect of kasugamycin. These findings highlight the importance of considering biotic interactions when assessing pesticide impacts on aquatic microbial Communities.

Keywords *:* Bacterial-fungal interactions ; leaf-litter decomposition ; conventional and bio-pesticide ; tolerance acquisition

Linking taxonomic and functional microbial signatures to trace arsenic sources during flood events in a mining-impacted watershed

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Floods are major drivers of particulate metal(loid) transport in mining-impacted hydrosystems. Identifying the origin of contaminated particles is therefore a challenge for post-mining management. When relying solely on geochemical tracers, such identification may lack sufficient resolution to discriminate multiple sources.

In the Salsigne mining district (Orbiel catchment, France), surface waters are contaminated by arsenic (As) originating from multiple sources (tailings and contaminated riverbed sediments). The relative contribution of these sources can vary significantly during hydrological events, making source apportionment complex. Improving the management of this site requires innovative approaches capable of capturing this spatial and temporal variability.

Previous work in another mining area demonstrated that the distribution of bacterial functional genes involved in arsenic cycling can be site-specific. This suggested that microbial signatures associated with soils and sediments may serve as tracers to determine the origin of particles at the scale of the Salsigne watershed.

In this context, the present study investigates whether combining functional bacterial markers with taxonomic diversity can enhance source discrimination and provide new insights into the biogeochemical processes governing arsenic dynamics in such polluted environments.

Reference samples from potential sources (soils and sediments) were collected under low-flow conditions, while water samples were collected at high temporal resolution during flood events at the catchment outlet. 16S rRNA gene metabarcoding confirmed temporal shifts in bacterial communities. In parallel, quantitative PCR assays targeting total bacteria, archaea, and genes involved in arsenic cycling - methylation (*arsM*), oxidation (*aioA*), efflux (*arsB*), and reduction (*arrA*) - are being developed.

This approach will enable the exploration of links between bacterial taxonomic diversity and functional potential and assess the extent to which functional genes complement taxonomic data for tracing contamination sources. Ultimately, this work will contribute to a better understanding of the coupling between hydrological processes, microbial dynamics, and arsenic transport in mining- impacted hydrosystems.

Keywords: microbial source tracking, mining pollution, arsenic, flood, functional genes

A Periphyton-Based Genomic Bioindicator of Surface Water Quality: Insights from a Field Data

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As part of the AquaBioSens project (Wallonia ERDF 2021–2027), freshwater biodiversity is being investigated to develop a bioindicator for monitoring water quality through genomic analysis of aquatic biofilm (periphyton) grown on standardized passive samplers, complementing existing indices such as the benthic diatom index.

An intensive one-year sampling campaign concluded in February 2026 at 12 sites (rivers and streams) in Wallonia, Belgium, including locations along the Vesdre river upstream and downstream of an area receiving untreated urban wastewater.

Physicochemical parameters and prokaryotic community compositions (16S rRNA gene, long- read Nanopore sequencing; Curry et al., 2022) were monitored across 118 samples (59 from water and 59 from periphyton). Based on the Nitrogen Trophic State Index (Carlson et al., 2007), samples were classified as oligotrophic (n=3), mesotrophic (n=9), eutrophic (n=34) or hypertrophic (n=13).

Beta diversity (NMDS, Bray-Curtis) revealed community clustering by sampling site and trophic level based on total nitrogen. Water quality upstream was notably better than downstream of the wastewater entry point, with communities gradually shifting as wastewater diluted along the river.

Redundancy Analysis (RDA, vegan v2.6.10, R) revealed that selected environmental variables explained 40.3% of the total variance in community composition (RDA1 = 19.7%, RDA2 = 11.6%; permutation test: $F = 4.21$, $p = 0.001$). The top 10 bacterial taxa contributing most to sample differentiation along the two first RDA axes are displayed.

IndVal analysis identified taxa significantly ($p < 0.01$) associated with trophic state: *Deinococcus budaensis* (oligotrophy), *Tychonema bourrellyi* (mesotrophy), and *Trichococcus pasteurii* (hypertrophy), the latter also highlighted by RDA as a key explanatory Taxon.

These analyses identified bacteria potentially serving as trophic state indicators in surface waters. Eukaryotic communities (18S rRNA gene; Guillou et al., 2012) will also be analyzed to complement this indicator.

Keywords: Bioindicator, Periphyton, Long-Read Sequencing

PICT approach applied to pharmaceuticals: influence of mixture effects on periphyton tolerance acquisition

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Assessing the ecological impacts of pollutants requires going beyond concentration measurements to account for ecotoxicological effects on exposed communities. The pollution-induced community tolerance (PICT) concept has emerged as a robust biomonitoring tool, enabling in situ evaluation of microbial community responses to toxic exposure, by providing effect-based, causal insights. The PICT approach has been mainly applied to assess the responses of periphytic microbial communities to various trace metals and herbicides but a few studies also suggest its potential applicability to pharmaceuticals.

Questions remain regarding the specificity of the measured PICT response, particularly whether tolerance acquired toward a model toxicant truly reflects prior exposure to that compound. Indeed, microbial communities in contaminated ecosystems are typically exposed to complex mixtures of toxicants, including multiple pharmaceutical classes.

To address mixture effects and potential co-tolerance processes, we conducted a 28-day microcosm study exposing periphytic communities to three commonly detected pharmaceuticals (the antibiotics sulfamethazine and ofloxacin, and the non-steroidal anti-inflammatory drug diclofenac) applied individually or in mixtures at environmentally relevant concentrations (18 nM for single compounds; 6 or 18 nM each in mixtures). We assessed tolerance acquisition using acute toxicity tests measuring phototrophic response to diclofenac (photosynthetic efficiency) and heterotrophic response to sulfamethazine (β-glucosidase activity).

For both types of tolerance measurements, the highest tolerance levels were observed after chronic exposure to mixture (18 nM), highlighting the importance of substance interactions during this phase. An increased phototrophic tolerance to diclofenac was observed in the following treatments: diclofenac (tolerance), ofloxacin (positive co-tolerance), and both mixtures (interactions between substances). The PICT hypothesis was not supported in heterotrophic communities chronically exposed to sulfamethazine, whereas communities from the ofloxacin treatment showed increased sensitivity to sulfamethazine (negative co-tolerance).

These results highlight potential co-tolerance mechanisms, but further investigation is needed to provide a more robust interpretation of community responses under multiple stress conditions.

Keywords: pollution induced community tolerance; co-tolerance; photosynthesis; β-glucosidase activity; biomonitoring

When pollutants collide: microbial life under multi-stressor pressure

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Marine sediments act as both sinks and secondary sources of environmental pollutants, posing risks to ecosystem integrity and human health. In natural environments, sediments and their microbial communities are rarely exposed to single contaminants but rather to complex mixtures of stressors with often non-additive effects. These interactions can be synergistic or antagonistic, altering microbial diversity, gene expression, and metabolic activity in ways not predictable from single- stressor studies. Understanding the effects of single and combined pollutant exposure on microbial communities, which play a crucial role in ecosystem functioning and resilience, is therefore essential. We conducted a 28-day lab-experiment using fresh marine sediments, exposing benthic microbial communities to three pollutants - tributyltin (TBT), octocrylene (a UV-B filter), and zinc (Zn) - individually and in combination, at environmentally relevant concentrations and at 100× higher levels. Changes in microbial community structure (bacterial and fungal; 16S and ITS amplicon sequencing) and function (enzyme activities and EcoPlates) were assessed. To compare responses of adapted versus less-impacted communities, sediments were collected from two contrasting sites in the Adriatic Sea (Croatia): the heavily polluted Šibenik Bay and the relatively pristine Zlarin Island. Microbial taxa exhibited site-specific responses to pollutant mixtures. *Halobacteriota* increased under UV-B treatments at both sites, more strongly in Zlarin, while *Bacteroidota* rose in Zlarin under TBT mixtures. *Flavobacteriaceae* increased under high-concentration mixed exposures at both sites, and *Methanosarcinaceae* was enriched under UV-B, particularly in Zlarin. Zlarin communities appeared more sensitive, whereas Šibenik communities showed greater resilience, likely reflecting long-term contamination. Respiration increased only in Šibenik at higher pollutant concentrations, while hydrolytic activity remained consistently lower than in Zlarin. In contrast, Zlarin samples exhibited enhanced biofilm formation, indicating a more dynamic response. Average well color development (AWCD) did not distinguish treatment effects, although it increased over time. These findings highlight how pollutant mixtures shape microbial community responses under realistic conditions and support the inclusion of microbial indicators in environmental monitoring frameworks.

Keywords: marine sediments, pollutants, microbial community, interactions, UV-B/TBT/Zn

poster presentation

Microplastic and herbicide degradation by the soil bacterium *Cupriavidus necator* JMP134

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Microplastics are increasingly recognized as emerging contaminants in agricultural soils, where they are found with pesticide residues and may alter microbial diversity and functions. In this study, we investigated the impact of bio-based and biodegradable microplastics on the biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used herbicide, by the bacterium Cupriavidus necator JMP134.

Soil microcosms were amended with increasing doses of microplastics and exposed to 2,4-D

herbicide. 14C-2,4-D mineralization was monitored by radiorespirometry and the abundance of tfd degrading genes involved in 2,4-D mineralization was quantified. Our results showed a clear decrease in 2,4-D mineralization with increasing microplastic concentrations. However, the abundance of tfd degrading genes remained stable across treatments, suggesting that, either these genes were not expressed in presence of microplastics or that 2,4-D was adsorbed onto microplastic surface.

Culture of C. necator JMP134 in a minimum medium with microplastics as the sole carbon source revealed a growth of the bacterium, indicating a potential ability to utilize microplastics as an alternative substrate. We therefore propose that, under certain environmental conditions, microorganisms may preferentially exploit either microplastics or herbicides as carbon sources, leading to reduced pesticide degradation despite the persistence of the degrading community.

These findings highlight the role of microplastics as modulators of microbial activity in soils and raise important questions regarding their impact on pesticide fate and side ecotoxicological effects on soil microbial functions.

Funding: from the Horizon2020 MINAGRIS project.

Keywords: biodegradation, soil bacteria, microplastics, herbicide

Updated mcrA reference databases for methanogen community profiling with amplicon sequencing

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The methyl-coenzyme M reductase subunit alpha gene (*mcrA*) is an important phylogenetic marker for high throughput ecological profiling of methanogenic archaea, central to industrial biological methane production and greenhouse gas emissions. Hence, it comprises a useful tool for ecotoxicology studies and microbial functional assays next to other nutrient cycling biomarkers like the ammonia oxidizer specific *amoA* gene. Yet, dedicated reference databases predate current relevant NCBI sequence accumulation and archaeal taxonomic revision. We present three updated *mcrA* reference databases: (i) one derived from NCBI-catalogued methanogen genomes (1,572 sequences); (ii) a database built by expansion of a previously published reference dataset, leveraging the NCBI nucleotide collection (27,942 sequences); (iii) a curated-taxonomy version of the latter. The updated amplicon databases provide a ~3.5-fold sequence richness expansion, extend genus-level richness from 31 to 83 taxa, more than 4-fold species-level richness, and incorporate novel lineages compared with the previous reference dataset (e.g. *Thermoplasmatota*-encompassed). The databases depending on the selected version, offer a deep coverage of to-date recorded *mcrA* variants, and/or thorough classification leveraging the phylogenomic annotation background of the host genome and the robustness of the near complete gene. All databases were formatted to support analysis with relevant contemporary software pipelines and packages. Overall, the generated databases facilitate a highly improved characterization of methanogen diversity and ecology, and comprise a highly useful tool for microbial ecotoxicology.

Keywords: *mcrA*; methanogenic archaea; amplicon sequencing; reference database; biomarker

Microbial eDNA signatures in the Adriatic: linking sediment signatures and sea urchin gut health to plastic pollution

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The Adriatic Sea faces significant ecological threats from (nano)microplastic (NMP) pollution. Following a "One Health" perspective, the ADRI-MICROBE project investigates interactions between NMP accumulation and microbial biodiversity. Building on research identifying sedimentary environmental DNA (eDNA) as a high-resolution archive for deciphering ecosystem shifts, this study characterizes the "plastisphere" within Adriatic sediments and its translocation into key bioindicators: sea urchins (*Paracentrotus lividus* and *Arbacia lixula*).

Tracing pollution history through sediment cores, we utilize 16S rRNA gene metabarcoding (Microgen) to profile microbial eDNA signatures in both sediments and sea urchin guts, assessing how NMP ingestion alters the host microbiome. Simultaneously, FTIR spectroscopy identifies the polymer composition of the ingested plastics. Fieldwork at three strategic locations - the Port of Šibenik, Krka estuary, and Zlarin Island - enables a comparison between highly impacted and pristine sites. By integrating sedimentary eDNA archives with contemporary microbiome analysis, this research provides a robust framework for monitoring long-term anthropogenic impacts, supporting the Marine Strategy Framework Directive (MSFD) and international conservation efforts.

Keywords: (nano)microplastics; microbial eDNA; sediment cores; sea urchin gut; Adriatic Sea

poster presentation

Assessing Fungicide Sensitivity in Aquatic Fungi:Development of Multi-Endpoint Bioassays

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Aquatic fungi play a central role in freshwater ecosystems by driving decomposition, nutrient cycling, energy flow, and community dynamics. In agricultural streams, these organisms are unintentionally exposed to fungicides, yet their sensitivity is not considered in current European Union regulatory frameworks due to the lack of standardized test systems. Consequently, potential chemical risks to aquatic fungi remain largelyunassessed.

This study aims to develop and evaluate bioassays for aquatic fungi to characterize their responses to fungicide exposure across multiple endpoints. Specifically, we seek to determine toxicity thresholds (e.g., EC_{50} values) for individual fungal species and to assess whether regulatory acceptable concentrations (RACs) or predicted no-effect concentrations (PNECs), derived from algae or aquatic invertebrates, are protective for aquatic fungi. To achieve this, we test oxygen consumption as a proxy for respiration using a 24-channel, non-invasive oxygen sensor system. In addition, growth-related endpoints are quantified via dry biomass and ergosterol measurements, complemented by further indicators of metabolic stress.

Preliminary results show distinct, species-specific respiration rates and a rapid decline in oxygen consumption for all species. Method development and optimization are ongoing. Overall, we expect that aquatic fungal species will exhibit differential sensitivity to fungicides, reflecting variation in their physiology and the mode of action of the compounds.

Pharmaceuticals in waste water treatment plants: characterizing the microbial diversities and the biodegradation under ketoprofen and oxazepam pressure

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Conventional wastewater treatment plants (WWTP) processes inadequately remove many pharmaceuticals because of the mixture's complexity and their recalcitrance. Microbial degradation of pharmaceuticals offers an eco-friendly, cost-effective alternative to conventional treatment methods. The goal of our study is to analyze the dynamics of microbial consortia under pharmaceutical stress, utilizing their selective pressure in isolating individual strains with biodegradation potential. Pharmaceutical levels were first characterized in two WWTPs receiving hospital and industrial influents. A targeted consortia enrichment approach was carried out using aerobic sludge from both WWTPs, ketoprofen (KETO) and oxazepam (OXA) were selected for the enrichments, representing both easily degradable and recalcitrant compounds found within these WWTPs. The enrichments were done at two distinct concentrations: 1 ppm and 1000 ppm of KETO and OXA separately, to select for high-tolerance and potentially degrading communities across these concentrations. Furthermore, enrichments were conducted with and without supplementary carbon sources (peptone and glucose) to select for both consortia capable of degrading low concentrations of OXA and KETO, as well as those utilizing these compounds as a sole carbon source.

We observed that microbial community composition was shaped primarily by the impact of individual pharmaceutical compound and its concentrations as well as the addition of supplementary carbon sources irrespective of the source WWTP. Successive enrichments yielded 18 ketoprofen microbial consortia: 11 of which achieved 15-74% degradation of 1000 ppm KETO. From these, 24 strains were isolated, eight degraded 1 ppm KETO at rates between 26-44%. Conversely, while OXA enriched consortia showed no degradation activity, four of the 28 strains isolated achieved novel 1ppm OXA degradation (30-37%). More isolated strains degraded KETO compared to the recalcitrant OXA. These results demonstrate that targeted enrichment is essential for unlocking the degradative potential of both consortia and isolates under pharmaceutical stress.

Funding: European union H2020 research under the Marie Sklodowska-Curie Grant Agreement No. 94516

Keywords: Pharmaceuticals, microbial communities, biodegradation, consortia, isolation

Linking Community-Level Metabolic Shifts to Pesticide Modes of Action in Aquatic Periphyton: A High-Throughput Metabolomics Approach

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Facing increasing chemical pollution of aquatic ecosystems, there is an emerging need to improve the risk assessment at microbiome level, as microbial communities are pivotal to ecosystem functions. Metametabolomics is a promising approach for detecting early, sensitive responses to chemical stress. We previously developed a high-throughput workflow for aquatic periphyton, utilizing robotic extraction, R package DRomics to evaluate aggregated meta-metabolome response and sensitivity threshold. Here, we validate this workflow by screening six pesticides with diverse modes of action: herbicide metolachlor; fungicides azoxystrobin and boscalid; insecticides fenoxycarb, imidacloprid and chlorpyrifos.

Metolachlor, azoxystrobin, and boscalid induced significant shifts in the periphyton metabolome, with 259-359 features following dose-response trends (*FDR* < 0.05). While the insecticides did not trigger significant global shifts, substantial numbers of features (19-600) showed dose-response relationships. Fenoxycarb exhibited the lowest median benchmark doses (BMD_{1SD}) of 6.0×10^{-3} mg/L, followed by azoxystrobin (1.8×10^{-1} mg/L), chlorpyrifos (2.8×10^{-1} mg/L) and imidacloprid (4.7 mg/L). Metolachlor and boscalid exhibited the highest median BMDs (16.4 mg/L). Class-specific BMD analysis identified fatty acids as primary targets for metolachlor. Specifically, very-long-chain fatty acids (VLCFAs) and polyunsaturated fatty acids decreased while short chain fatty acids and oxylipin accumulated, aligning with metolachlor's known inhibition of VLCFA synthesis. Other pesticides targeted diverse pathways: terpenoids (azoxystrobin, chlorpyrifos), shikimates and phenylpropanoids (boscalid, imidacloprid), and polyketides (fenoxycarb). The terpenoids induced by azoxystrobin or chlorpyrifos mainly showed bell trend, suggesting an antioxidant response activation followed by depletion. Boscalid and imidacloprid induced biphasic shikimate and phenylpropanoid responses, likely reflecting the defense response driven by the phenolic compounds and lignin production. Fenoxycarb trends were predominantly biphasic across all classes, indicating a defense or compensatory response to this insect growth regulator. This workflow successfully evaluates aggregated meta- metabolome response of periphyton, and links metabolic shifts to specific pesticide modes of action.

Keywords: high-throughput metabolomics; periphyton; chemical risk assessment

Direct vs digestate-mediated contamination of soil with LDPE: Evaluation of microplastic and additives toxicity in a multi species-context and functional effects on key microbial processes.

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We now know that there is more plastic in the soil than in the oceans. This pollution is particularly prevalent in agricultural soils, primarily as result of the application of organic amendments such as digestates containing microplastics (MPs) and associated additives. Low-density polyethylene (LDPE) MPs are the most frequently detected and their accumulation may threaten the environmental quality and functions of soil. In this context, the objectives of this study were:(1)to evaluate the impact of 0.1% LDPE-MPs produced with known additives at two concentrations (1X and 10X) on two keystone annelid species exposed alone or simultaneously, with or without plant,(2)to evaluate the effect of 0.1 and 1% LDPE-MPs with 1X and 10X additives applied via digestate on soil respiration and a key process of N-cycling.
When comparing the effects of LDPE on *A. caliginosa* and *E. albidus* either alone or together, with or without a plant (*Lolium perenne*) – we observed significant biotic interactions under uncontamined conditions. Plants reduced *E. albidus* growth and *E. albidus* reduced *A. caliginosa* biomass. Interestingly, exposure to LDPE-MPs 10X mitigated these antagonistic effects but led to a disruption of N cycling as evidenced by elevated soil ammonium concentration and higher denitrification activity.
When the soil is amended with contaminated digestate, respiration increases by up to 90% for LDPE- MPs 10X and more than 60% regardless of MPs concentration. This can be explained both by the addition of a carbon source via digestate and by the possible use of additives as a carbon source. A 10 to 20% decrease in denitrification is observed whether LDPE is added with or without digestate.
Our results highlight both the importance of using multi-species models to detect the indirect effects of MPs on soil function and the contrasting effects of organic amendment on the toxicity of microplastics.

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Keywords: Microplastics; Additives; Soil functions; Digestate; multi-species

poster presentation

Using PICT approach to diagnose chemical pressure in aquatic ecosystems:towards an operational framework

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Microbial communities have strong potential for the ecological monitoring of contaminated aquatic ecosystems, yet remain underexploited in current biomonitoring frameworks. Their short generation times and rapid responses to environmental changes make them highly sensitive indicators of chemical pressures. Chronic exposure to toxic substances can lead to the development of community tolerance, typically associated with shifts in structure and functioning, with tolerant taxa replacing sensitive ones.

This property underpins the Pollution-Induced Community Tolerance (PICT) approach, which links integrated microbial responses to the nature and intensity of chemical pressures. PICT is increasingly recognized as a relevant tool to assess the ecological effects of contaminants (*e.g.*, herbicides, metals, pharmaceuticals). However, its operational implementation is still limited by the lack of a standardized interpretation framework enabling direct application by water managers.

The DiagnoPICT project, funded by the French Biodiversity Agency, addresses this challenge by evaluating PICT as a diagnostic tool for chemical pressure in rivers. The approach combines, at a large spatial scale (27 river sites across the Auvergne Rhône-Alpes region), tolerance measurements of microbial communities to model substances (*e.g.*, copper, sulfamethazine, diclofenac) with the characterization of chemical contamination profiles across water, sediment and periphyton.

Our results reveal marked spatial variability in contamination profiles along with contrasted microbial tolerance patterns. Variance partitioning analyses showed that microbial tolerance was primarily explained by site-specific contamination profiles. However, tolerance responses were not consistently associated with the *in situ* concentrations of the corresponding compounds, suggesting the occurrence of co-tolerance and complex adaptive mechanisms within microbial communities.

Overall, these findings highlight the capacity of PICT to integrate chronic exposure to complex contaminant mixtures and support its potential as a diagnostic tool. They provide a strong basis for the development of an operational framework, although further work is needed to incorporate key environmental drivers, particularly physicochemical characteristics of aquatic systems.

Keywords: microbial community, periphyton, sediment, tolerance, xenobiotic

Ecotoxicological impact of PFAS on microbial communities and plants during phytoextraction assays

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants of global concern, frequently accumulating in soils at high concentrations, particularly at sites impacted by firefighting activities such as airports. While phytoremediation represents a promising mitigation strategy, the selection of suitable plant species and the ecotoxicological effects of PFAS on soil biota remain poorly understood.

This study investigated (i) the impact of PFAS on soil microbial communities (bacteria and fungi) and plant oxidative stress, and (ii) PFAS dynamics in planted soils through the assessment of phytoextraction capacities. A controlled pot experiment was conducted using one uncontaminated soil and three contaminated soils, including one historically polluted soil and two soils spiked at increasing concentrations (1X and 10X). Three plant species with phytoremediation potential—hemp, sugar beet, and sunflower—were evaluated.

Marked differences in plant biomass, mycorrhizal colonization, microbial activity, enzymatic functions, and community diversity were observed between contaminated and control soils. However, these variations were primarily explained by differences in soil physicochemical properties (e.g. pH, organic matter content, and cation exchange capacity) rather than PFAS contamination. Along a defined contamination gradient (0, 55.5 and 555 µg PFAS/kg soil dw), only limited PFAS-specific effects were detected, including a decrease in leucine aminopeptidase activity and shifts in bacterial community composition, notably an increase in Proteobacteria. Plant identity further modulated soil responses. Sunflower, characterized by higher biomass production, reduced alkaline phosphatase and leucine aminopeptidase activities. In addition, each plant species induced distinct changes in fungal community structure. PFAS accumulation also differed among species, with hemp and sugar beet exhibiting higher bioconcentration factors than sunflower. Phytoextraction was effective for all four PFAS (PFOS, PFOA, PFHxS, PFPeA), with similar capacities in spiked and historically contaminated soils.

Keywords: PFAS, microbial communities, plants, stress, bioaccumulation

Biodegradation of hydrophobic volatile organic compounds: bioavailability,kinetics and microbial community

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Hydrophobic volatile organic compounds (HVOC) having low aqueous solubility that limits their bioavailability for microbial degradation. This complicates the study of biodegradation and associated kinetics leaving a significance data gaps under regulatory frameworks. This is particularly relevant for fragrance compounds and natural complex substances such as essential oils, which motivated the BioSINC project, “Biodegradability of Intrinsically Natural and Complex Substances”, coordinated by the CIHEF (Interprofessional Committee for French Essential Oils) and funded by FranceAgriMer.

This study investigates biodegradability of terpenes compounds that are HVOC; menthol, menthyl acetate, β-caryophyllene and δ-cadinene. Biodegradation by activated sludge collected from Bellevigny, France was assessed by OECD 301F guideline, a standardized method for evaluating biodegradation capability based on biochemical oxygen demand over 28days. To address the bioavailability limitation inherent to HVOC, silicone oil was incorporated as non-aqueous phase liquid reservoir, sustaining compound availability to microorganisms throughout the assay.

In the presence of silicone oil, biodegradation exceeded 60% within 28 days for the compounds, meeting the biodegradability threshold. To our knowledge, this represents the first demonstration of silicone oil enhanced biodegradation of these HVOCs. 16S rRNA sequencing of the microbial communities identified biodegraders affiliated with *Proteobacteria* and *Bacteroidota*, among others, providing insight into the microbial diversity.

Based on these findings, future work will employ metatranscriptomics to characterize gene expression during non-biodegradable compounds, identifying metabolically active pathways and functional genes involved in biodegrdation of these industrially relevant compounds.

Keywords: hydrophobic volatile compounds, biodegradation; bioavailability; 16S rRNA, Activated sludge

Port eco-exposome influence on bioaccumulation, metabolome and microbiome diversity and function in coastal fish across development

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Port infrastructures, by reducing habitat complexity, represent a major threat to juvenile fish in these nursery areas. Ecological restoration is widely implemented to mitigate these impacts. However, organisms inhabiting restored structures are often exposed to chronic,low-level anthropogenic pollution. This study aims to evaluate how the port eco-exposome influences metal accumulation, microbiota diversity and function, and the juvenile fish Metabolome.
Research was conducted at two contrasting nurseries, an artificially restored port and a natural area. The eco-exposome was characterized by metal contamination and bacterial communities. In parallel, analyses were performed on Diplodus vulgaris juveniles from the 2023 and 2024 cohorts throughout their development.Port waters contained higher levels of heavy metals and urban-associated microbial communities, along with a greater abundance of pollution-related genes compared to the natural coastal site. These patterns were influenced by seasonal variation and climatic events. Juvenile fish from the port accumulated more metals and displayed distinct metabolic and microbiota profiles compared to those from the natural nursery across development, whereas no significant differences were observed in the abundance of resistance-associated genes. Instead, a decrease in these genes at both sites was observed in the later fish developemental stage. Metabarcoding analyses revealed taxa enriched in the port-exposed juvenile microbiota, known for their antibacterial activity and ability to degrade complex compounds. In parallel, metabolomic analyses revealed expression changes in stress-associated metabolites, including amino acids and lysophosphatidylcholines, in port-exposed juveniles at intermediate and late developmental stages.
Overall, these findings highlight the need for a holistic framework including both environmental biogeochemical characterisation and multi-omics approaches to better assess the exposome

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Keywords: Port eco-exposome, Juvenile fish, metal accumulation, microbiota, metabolome

From microbiome shifts to functional evidence: quantitative indicators for environmental assessment of chemical stressors

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Animal microbiomes respond to chemical stressors, including pollutants and natural toxins. Yet microbiome data are rarely used in environmental assessments because changes in diversity or community composition are difficult to interpret. A central challenge is to translate microbial composition into functional evidence of disruption, adaptation, detoxification, or risk amplification.

I propose a quantitative framework that links taxonomic profiles to functional traits, including resistance, degradation, toxin transformation, nutrient cycling, and host-supporting metabolism. These traits are then connected to host physiology or ecosystem processes. The framework will be illustrated with examples from controlled laboratory testing and Baltic Sea monitoring. Daphnia will serve as a laboratory model for linking microbiome responses to toxicity endpoints, while copepods and amphipods will provide pelagic and benthic examples from the Baltic Sea. Because copepods and amphipods are regularly monitored in aquatic systems, they offer a practical means of linking microbiome-based indicators to existing environmental assessment frameworks.

The aim is to support microbiome-based indicators that can distinguish harmful impairment from functional resilience or altered chemical risk, while remaining compatible with environmental monitoring and assessment frameworks.

Keywords: microbiome-based indicators, environmental assessment, aquatic monitoring, invertebrates, host-microbiome interactions

Periphyton based Effect Directed Analysis to link the chemical exposome to functional impairment of microbial ecosystems

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Complex mixtures of organic contaminant in aquatic systems may pose ecological and health risks, even at trace levels. Traditional monitoring often fails to capture mixture effects or identify key toxicity drivers. Effect-Directed Analysis (EDA) reliably identifies (un)known drivers of (eco)toxicity at contaminated sites but is currently limited to single-species bioassays. Periphytic biofilms, comprising autotrophic and heterotrophic microorganisms, are highly sensitive to environmental stress and excellent indicators of ecosystem health. This project introduces a novel ecologically relevant EDA approach using freshwater periphyton, integrating early molecular (meta-metabolome) responses and physiological endpoints (respiration, enzymatic and photosynthetic activity) to assess complex mixture effects at the community level and identify responsible Eco-toxicants.

The biofilm-based EDA approach integrates: (i) sample preparation, including solid phase extraction of chemical mixtures from surface waters and Ultra High-Performance Liquid Chromatography (UHPLC) fractionation to reduce mixture complexity; (ii) community bioassays to evaluate the effects of whole extracts and their corresponding fractions; and (iii) cutting edge chemical analysis using UHPLC-HRMS to identify key drivers of toxicity in bioactive extracts and fractions.

To develop biofilm-based EDA diagnostic tool, fluvial biofilms were collected from a pristine stream and cultured on glass tiles in 6-L microcosms simulating oligotrophic conditions. Two dose-response experiments were performed by exposing biofilm to synthetic model mixtures (one mimicking whole environmental extract, another mimicking subsequent fraction) of prevalent surface-water contaminants known to affect biofilms. Endpoints were assessed during short-term exposures (4–72 h) at eight concentrations range (0-100 µg/L). The most appropriate functional endpoints and exposure conditions will be selected for the validation of the biofilm-based EDA approach in real environmental mixtures. By linking bioassay responses with environmental chemicals, this study explains mixture effects, establishes cause–effect relationships between the chemical exposome and ecological impairment, and further prioritizes hazardous chemicals for regulation.

Funding: the Pharm-ERA Marie Skłodowska-Curie Actions Network.

Keywords: Complex Mixtures of Chemical Contaminants, Ecosystem Health, Effect-Directed Analysis, Microbial Communities, Metametabolomics.

Alteration of prey-predator interactions due to pesticide pollution: its impacts on the nitrogen cycle

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Pesticide pollution represents a major environmental concern due to its impact on ecosystem diversity and functioning. In soils, protists are key predators of bacteria, affecting community structure and nutrient cycling. However, the effects of contamination on trophic interactions remain poorly understood. This study aimed to evaluate the impact of pesticide pollution on prey-predator interactions and the nitrogen cycle. We performed a microcosm experiment using autoclaved soil inoculated with an artificial community of nitrifiers, composed of ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB). After community establishment, we introduced axenic cultures of the ciliate *Tetrahymena pyriformis* and the flagellate *Poterioochromonas* sp. Two pesticides, metsulfuron-methyl and etridiazole, were tested independently at two dose levels (low and high). Microcosms without protists and/or without pesticides served as controls. Sampling was performed at 0, 7, 14, 21 and 28 days of incubation. NH_4^+ and NO_3- concentrations were measured, along with the abundance of AOB, AOA, NOB, and each protist species via qPCR. Our results indicated that both protist presence and pesticide application affected NH_4^+ oxidation and NO_3- production ($p < 0.05$). A negative correlation was observed between AOB and *Tetrahymena* densities ($r = -0.7$, $p < 0.05$), while no correlation was observed for other groups, indicating predator preference for AOB, which dominated ammonia oxidation in this system. Pesticide application. both as single factor and in interaction with predators, significantly affected community structure. Both pesticides affected the correlation between predators and AOB depending on the pesticide, highlighting the importance of considering microbial interactions in the context of anthropogenic pollution.

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Keywords: trophic interactions, ammonia-oxidizing microorganisms, predator selectivity, pollution

A new framework for ecosystem assessment through the integration of eDNA inventories with graph theory and niche modeling

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Rethinking the ecological significance of network structure (or topology) in the area of massive environmental DNA (eDNA) data represents a major challenge in ecology. This challenge arises from the observation that network structure fundamentally influences ecosystem functioning. However,translating ecological network analysis into quantitative tools for ecological assessment is still inits infancy. We hypothesized that a tool integrating relevant network topological metrics could reflect anthropogenic pressure, such as chemical pollution. To address this challenge, we present an innovative approach using eDNA inventories and combining graph theory(specifically graphlet analysis), niche modelling, and machine learning to produce a predictive model for ecological assessment. We validated our approach using 599 phytoplankton inventories collected from 186 lakes spanning a gradient of chemical pollution. Here, graphlet-based network topological metrics explained 67% of the chemical gradient variability, demonstrating their effectiveness as predictive tools for ecological assessment. We also showed a link of these network topological metrics with ecological processes. This framework offers a scalable approach that can be transposed to a wide range of ecological situations to obtain assessment tools derived from graphlet-based network topology, paving the way for a more comprehensive understanding of ecosystems functioning.

Acknowledgments: We thank the French water agencies and the private firms responsible for monitoring under the WFD for agreeing to collect the samples necessary for this study.

Funding:INRAE and the Pôle ECLA R&D.

Keywords: environmental DNA, ecological networks, graphlet analysis, machine learning, ecological assessment

Contaminants impact on diatom resting spores

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In natural environments, microbial cells are known to switch from growing phases to resting phases when conditions become challenging for growth due to biotic and abiotic factors. The entrance in resting phase is a plastic response that allows cells to remain viable while waiting for environmental conditions to improve and the opportunity to restart their growth. Resting phases are important for species persistence, biodiversity maintenance and population dynamics through space and time. However, a considerable knowledge gap exists regarding how chemical contaminants may induce or interfere with microbial resting stage formation.
Species in the marine planktonic diatom genus *Chaetoceros* can form resting spores that are morphologically distinct from vegetative cells. In our work we investigated the ability of different contaminants (diuron, diclofenac and ethinylestradiol) to induce spore formation, alter spore viability and germination in strains of *C. socialis, C. affinis, C. didymus, C. pseudocurvisetus.*
C. socialis and *C. pseudocurvisetus* known to form spores under environmental constraints, also formed spores under contaminants pressure. Spore viability and germination varied depending on the contaminant. Diclofenac, a contaminant reported to be detoxified by diatom cells, did not have significant effect on spore viability and germination with respect to control treatment (spores obtained under nitrogen starvation). In contrast, ethinylestradiol, with its intracellular accumulation and alteration of lipids storages, reduced spore viability and germination capacity. Whereas diuron (a photosystem II inhibitor) enhanced spore formation but did not alter their viability or ability to germinate even after prolonged exposure (up to 1 month).
The study indicated that contaminants may interfere with the natural succession of growing and resting phases in diatoms and that by affecting resting phases, may alter long term population dynamics.
Acknowledgments: This work is part of the PhyCoRest project, supported by the European Union-Next generation EU. PNRR Young researchers (YOUR II) -MSCA-GEA.
Keywords: Contaminants, Diatoms, Life stages, Spores, Propagule

New approaches in genomics of microbial ecotoxicology

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Microorganisms have evolved multiple adaptive mechanisms to cope with exposure to adverse conditions, including at the genome scale. Such genome-wide mechanisms involve variations in replicon copy number or genome conformation. We aim to characterize the dynamics of these two features to pollutant exposure using Methylobacterium as a model and focusing on the genotoxic effect of dichloromethane (CH3Cl) degradation.An elevated chromosome copy number per cell (polyploidy) can be advantageous to overcome stresses, by favoring DNA repair, storing nutrients and providing a reservoir of potentially beneficial mutations. We are upgrading a classical approach to quantify ploidy by taking advantage of absolute quantification by droplet digital PCR (ddPCR) to assess pollutant effects on copy number of replicons.

Chromosome conformation (3D shape) can also be modulated as an adaptation mechanism. Several types of chromosome tertiary structures have been identified at various scales, from long-range interactions between specific loci to large interaction domains or global nucleoid compaction. Their occurrence often depends on environmental conditions. We are implementing Chromosome Conformation Capture (3C) methods to gain insight on pollutant effect on genome tertiarystructures.

We envisage that the response of these two genomic features could be leveraged as novel approaches for the assessment of pollutant toxicity and for the detection of environmental contaminant.

Keywords:Ploidy, chromosome conformation, exposome, genotoxicity

poster presentation

Low concentrations of 'inert' surfactants inhibit nitrifying microorganisms

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Under emerging frameworks such as the European Union Soil Monitoring Law, soil microbial biodiversity is recognised as a key component of soil health. Ammonia-oxidising bacteria (AOB) and archaea (AOA) serve as bioindicators due to their role in the first step of nitrification. This highlights the need to understand how anthropogenic chemicals, e.g., agrochemicals, affect nitrification. Surfactants, widely used co-formulants in agrochemicals, are typically labelled 'inert', but have increasingly shown to alter microbial community composition and function, raising concerns for soil health. Using a high-throughput in vitro assay combined with a synthetic microbial community of nitrifiers (SynComNi), we assessed the inhibition and toxicity of three 'inert' surfactants in agrochemicals: pelargonic acid (PA), dodecyltrimethylammonium chloride (DTAC), and linear alkylbenzene sulfonates (LAS). EC50 values were determined for soil AOB (Nitrosospira multiformis) and AOA (Candidatus Nitrososocmicus franklandianus and Nitrososphaera viennensis) strains. The SynComNi, composed of the AOB N. multiformis and the nitrite-oxidising bacterium (NOB) Nitrobacter winogradskyi, enabled the assessment of the toxicity of surfactants throughout the complete nitrification process. Growth and activity were quantified via nitrite and nitrate production, alongside gene abundance (amoA, nxrB) using qPCR. All surfactants showed concentration-dependent inhibition. DTAC and LAS showed the highest toxicity (EC_{50}: 1.099-68.78 µM), whereas PA was less toxic (EC_{50}: 1800-2000 µM). AOA were the most sensitive, with N. viennensis showing the lowest EC50 values. In the SynComNi, DTAC showed higher toxicity compared to individual strains, with complete inhibition occurring at 16.79 µM. Higher concentrations of LAS resulted in a delayed lag phase for AOB and inhibited the growth of NOB at 34.39 µM, with complete inhibition at 68.79 µM. PA showed lower toxicity toward AOB compared to the individual assays, although it inhibited NOB at concentrations ≥ 1980 µM. These results demonstrate that 'inert' co-formulants inhibit nitrifying microorganisms, with implications for nutrient cycling and soil health.

Keywords: Ammonia-oxidising microorganisms, nitrification, surfactants, herbicides, synthetic microbial community

OECD 216: A Case Study Exploring Reliability, Sensitivity and Test Design for Soil Microbial Pesticide Risk Assessment

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The OECD 216 nitrogen transformation test underpins regulatory assessment of plant protection products (PPPs), yet questions remain regarding its reliability, sensitivity, and representativeness. Here, we present an integrated, forward-looking suite of studies on OECD 216 reliability, endpoint sensitivity, and methodological development. Our work showcases OECD 216 as a test system with a continued and important role in the future of soil microbial PPP risk assessment.

We show that OECD 216 reliability may be constrained by soil-dependent dynamics, characterised by distinct “dip” and “rise” nitrate response patterns. These behaviours introduce variability that can obscure true treatment effects and complicate interpretation. Addressing and minimising these dynamics in OECD 216 studies improves test performance and increases confidence in study outcomes.

We then compare the sensitivity of the OECD 216 functional endpoint with metrics exploring microbial community composition derived from amplicon sequencing. While compositional endpoints may offer greater resolution of microbiome responses, their sensitivity and consistency vary substantially across compounds and methods of endpoint derivation, and they do not systematically outperform functional measurements. This indicates that functional endpoints remain a necessary and robust component of PPP regulatory assessment.

Finally, we show that transitioning from the current two-dose OECD 216 test design to a dose–response study approach represents a sensible and pragmatic improvement for PPP risk assessment. By capturing the full ecotoxicological response, dose–response studies better characterise non-monotonic responses and provide a more robust basis for regulatory decision-making. We also identify the further work required to support a well-considered and successful transition to dose–response OECD 216 studies for PPP risk assessment.

Through addressing the key criticisms of the OECD 216 study system, our work shows how improved reliability, demonstrable sensitivity, and dose–response methodologies can combine to deliver a more robust and ecologically meaningful soil microbial PPP risk assessment.

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Keywords: OECD 216, risk assessment, soil microbiology, ecotoxicology, pesticides

Native bacteria mitigate diuron and copper toxicity to microalgae isolated from a chronically contaminated coastal lagoon.

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In natural environments, microalgae are invariably associated with bacteria, yet the role of these microbial consortia in modulating microalgal physiological responses to chemical stress remains largely unexplored. This study investigates whether native bacterial communities enhance the tolerance of field-isolated microalgae to toxic compounds. Microalgae-bacteria consortia were isolated from the Mediterranean coastal Or Lagoon (South of France), chronically impacted by herbicides and trace metals. The chlorophyte *Chlamydomonas* sp. Or2023a and the diatom *Entomoneis* sp. Or2023b were isolated and axenised. Both axenic and xenic cultures were exposed to the herbicide diuron and the trace metal copper in 72-h dose- response assessments, following the evaluation of the bacterial diversity of their native phycosphere. Both isolates displayed elevated intrinsic tolerance, particularly *Entomoneis* sp., whose effective concentration inhibiting 50% of growth (EC50) values (77 µg L^{-1} for diuron; 109 µg $L^{-1}$for free ion copper) exceeded most published benchmarks for marine diatoms, likely reflecting adaptation to chronic chemical exposure. Beyond this baseline, native bacteria further modulated sensitivity in a species- and compound-specific manner. In *Chlamydomonas* sp., bacteria mitigated diuron toxicity (EC50 from 6 to 11 µg L^{-1}), though at the cost of reduced growth under non-stressed conditions, suggesting a growth-defence trade-off. In *Entomoneis* sp., bacteria alleviated copper toxicity (~30% EC50 increase) and induced hormetic growth responses, consistent with copper bioavailability regulation by metal-tolerant *Rhodobacteraceae* dominating its phycosphere. These findings demonstrate that microalgal sensitivity to contaminants is jointly shaped by environmental history and native microbial consortia, underscoring the need to incorporate field-isolated algal-bacterial assemblages into ecotoxicological frameworks

Keywords: microbial ecotoxicology, microalgal-bacterial consortia, field-isolates, axenization, marine environment

From the Atlantic to the Mediterranean: how environmental factors, colonisation kinetics and polymer type shape diatom diversity in the plastisphere

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Diatoms, despite being the main autotrophs and key early colonizers in biofilms on plastics, currently remain poorly studied compared to other microbes. The identification of the main and specific drivers of their communities at different spatial and temporal scales especially lacks to better understand the dynamics of plastic pollution in oceans.

249 plastics were immersed at 6 locations across the Atlantic Ocean and Mediterranean Sea in coastal systems of 5 countries. Spatial and seasonal dynamics of plastic-attached diatom communities were investigated by incubating high-density polyethylene (HDPE) at all sites for 84 days. Temporal dynamics (7 days to 12 months) at each season were also investigated with HDPE, polypropylene and polystyrene in Toulon (NW Mediterranean, France). Amplicon sequencing of the rbcL gene allows to determine diatom diversity in parallel to the measure of physico-chemical parameters of the seawater.

Geographic location was the primary driver of diatom diversity, followed by season and incubation time, with intra-site variations always lower than inter-sites ones. Intra-site variations were mostly due to relative abundance changes while inter-sites variations corresponded to shifts between species. Moreover, biofilms from the Atlantic sites shared neither species nor ASVs, unlike biofilms from the Mediterranean. Pioneer abundant taxa changed from one season to another while mature taxa tended to converge irrespective of the season. Community shaping was observed at different taxonomic levels, down to the genetic variant, highlighting the extent to which genetic divergence occurs. Finally, the nature of plastics plays a minor role, as shown previously for bacteria and fungi in the plastisphere. These results will allow us to better understand the influence of plastic pollution on ocean dynamics (productivity, transfert of pollutants, etc).

Keywords: Plastisphere, marine diatoms, biofilm, plastic polymer, marine pollution

Karst microbiome dynamics as tracers of water circulation and contamination pathways in complex hydrosystems

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While the appropriate use of antibiotics in human and veterinary medicines is one of the most important measures to tackle antimicrobial resistance (AMR), an ecological and integrative approach considering interconnections between human or animal health and environment is to be developed to act on overall epidemiological cycle of AMR. AMR have been widely studied in wastewater and polluted hydrosystems but more scarcely in natural hydrosystems, particularly those supplying drinking water. Drinking water supply in Mediterranean regions relies heavily on karst aquifers. In these areas, climate change is increasing the frequency and intensity of extreme rainfall. During floods, intensified exchanges between surface and groundwater and rapid water circulation can enhance contaminant transfer toward karst springs used for drinking water supply.

A multidisciplinary study conducted within the PEPR K3 project (ANR-22-PEXO-0012) investigated the impact of flood events on bacterial dynamics within the Lez karst aquifer (southern France). The objective is to contribute to a better understanding of the transfer of bacteria from diffuse and concentrated infiltration zones to karst springs. Water samples were collected during flood events at representative sites across the aquifer, including temporary streams, infiltration points, boreholes, temporary and perennial springs. Hydrochemical characterization included measurements of physicochemical parameters and conventional geochemical tracers (major and trace elements). Bacterial diversity was analyzed using 16S rRNA gene metabarcoding, while fecal contamination indicators and emerging antibiotic resistance genes were quantified by qPCR (*bla*OXA-48, *bla*NDM, *bla*KPC).

The results reveal strong spatial variations in bacterial communities linked to hydrological conditions and recharge processes. Several emerging resistance markers were detected along groundwater flow paths, highlighting rapid transfer during high-flow conditions. These findings demonstrate the relevance of combining microbial and hydrochemical approaches to characterize contamination dynamics in karst systems and may contribute to the development of early warning systems for drinking water resource management under increasing hydroclimatic pressure.

Keywords: groundwater microbiome; karst; microbial tracers; anthropic contamination

poster presentation

Impact of glyphosate and its transformation product, AMPA,on soil microbial communities

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Agricultural intensification has relied on massive pesticide use, leading to significant environmental consequences, including soil and water contamination and biodiversity loss. Among these pesticides, glyphosate is one of the most widely used herbicides worldwide and is increasingly associated with adverse effects on non-target organisms. Due to the persistence of glyphosate and of one of its main transformation products, aminomethylphosphonic acid (AMPA), both are frequently detected in agricultural soils and aquatic environments, raising environmental concerns.

In this context, we evaluated the ecotoxicological impacts of glyphosate, a glyphosate-based formulation, and AMPA on soil microbial communities using a microcosm approach. Three agricultural soils with contrasting physicochemical properties were exposed to agronomic (x1) and 10-fold (x10) doses. Endpoints were selected to provide an integrated assessment of microbial community structure and soil functioning after short-term (7 days) and longer-term (49 days) incubation periods. Microbial diversity and community composition were assessed using high- throughput sequencing targeting prokaryotes (16S rRNA gene) and eukaryotes (18S rRNA gene and ITS region). In addition, their effects on soil functioning were evaluated through a range of activities involved in carbon and nitrogen cycling. The capacity of soil microbial communities to degrade glyphosate and AMPA was further assessed by measuring the mineralization potential of ^{14}C-labelled glyphosate or AMPA using radiorespirometry assays. Finally, multivariate statistical analyses were performed to integrate these endpoints and identify potential biomarkers of soil exposure to glyphosate and AMPA. This work will contribute to a better understanding of the effects of glyphosate and AMPA on the diversity and functioning of soil microbial communities. Results are currently being analysed and will be presented at the conference.

Funding: the French National Research Agency (ANR) through the AAPG 2025 call for research proposals (grant ANR-25-CE34-7565).

Keywords: microbial ecotoxicology, glyphosate, AMPA, microbial communities, soil functions

Bacterial Community Composition and Structure in Urban Ponds and their Potential for Organic Contaminant Degradation

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Urban ponds are highly dynamic biodiversity hotspots that provide various ecosystem services. However, they are often exposed to a large number of chemicals and other anthropogenic stressors, potentially affecting the overall aquatic biodiversity. In particular, microbial communities, key players of biogeochemical processes in aquatic ecosystems, are indicators of chemical pollution with pollution-sensitive and less sensitive species. Here, I will present the impact of chemical pollution on the diversity and taxonomic composition of bacterial communities along an urbanization gradient in the Berlin-Brandenburg area, and their potential to degrade organic contaminants. Water samples from 38 ponds were analyzed using 16S metabarcoding approach to evaluate the impact of various environmental factors on bacterial community patterns. In addition, we also set up a microcosm experiment using microbial inocula of selected ponds to analyze the potential of pond bacterial communities to degrade organic contaminants and to test whether exposure to these contaminants results in specific adaptation of bacterial communities to chemical pollution. The results enable us to better understand how microbial community composition responds to chemical pollution and shed light on the resilience and adaptability of pond ecosystems to environmental stressors.

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Keywords: Urban ponds, Microbial community, Biodegradation, Organic contamination

Evolution of energetic metabolism and arsenic resistance genes in microbiome of mine residues during phytostabilization

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Arsenic, a toxic metalloid released into the environment mainly through industrial activities such as mining, poses significant environmental challenges. Phytostabilization, which employs resistant plants to immobilize or stabilize contaminants in the soil, is a strategy to prevent their spread through erosion or aerial dissemination, at a moderate cost. However, the impact of phytostabilization on arsenic speciation remains complex and difficult to predict. The oMIMo project (ANR-22-CE04-0012-0), supported by the French National Research Agency, aims to develop a predictive reactive transport model, explicitly integrating microbially- mediated geochemical reactions related to arsenic, iron and sulfur metabolisms. This model is informed by comprehensive analyses of a two-year pilot experiment at a metric scale. The metric mesocosm was filled with 1200 kg of arsenic-contaminated tailings from an old tin mine. Baseline monitoring of the bare tailing was performed during 6 months then assistedphytostabilization was started by amending the half part of the tailing with limestone and compost, and Festuca rubra sowing. Solid samples for metagenomic analysis were collected every 6 months. Whole Genome Sequencing (WGS) metagenomics (17 samples with 250 Mbp Illumina sequencing) and metaproteomics (27 samples on an Astral MS instrument) were performed, focusing on pre-compiled redox genes and subjected to global meta-analysis. The characterization of microbiota energetic metabolism and its effect on arsenic speciation and solubility will be analyzed by our group to inform the predictive model.Preliminary metagenomics results on T0 versus 18 months timepoints at surface depth show that the genes of interest belong mostly to Pseudomonadota and Actinomycetota, with a decrease in the abundance of Actinomycetota over time, and that the most abundant mechanism of arsenic resistance is the oxidation of arsenite.We expect our meta- and multi- omics results to deeply inform the microbial-profiles in terms of taxonomic and functional evolution, along the depth profile and over time.

Keywords: Arsenic contamination ; Phytostabilization ; Soil microbiome ; Metagenomics

Evaluation of Biological Quality Elements Using Periphyton Genomics: From Community Profiling to Novel Biological Indices for in-Field Assessment

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There is a growing need for effective methods to assess the impacts of environmental stressors on freshwater biodiversity. The AquaBioSens project (Wallonia-ERDF/JTF 2021–2027) addresses this need by developing a periphyton-based (aquatic biofilm) genomic indicator. Periphyton communities play a central role in aquatic ecosystem functioning and respond rapidly to environmental stressors, making them powerful indicators to detect pollution and to support ecological evaluations.

Periphyton samples grown on passive samplers were collected from various waters different in terms of physicochemical conditions and pollution pressures they are exposed to. Periphyton communities were characterized using long-read amplicon sequencing (*16S* and *18S* rRNA). Physio-chemical analyses of the water were conducted, and water quality indices (WQI) calculated (e.g., chemical status, WQI-CS). Key shifts in microbial community composition in response to changes in water quality class were identified through regression analysis, integrating α-diversity metrics (e.g. Shannon) as well as taxon-specific associations (IndVal).

Fifty-one indicators of good status (19 bacteria, 32 microeukaryotes) and 17 of poor status (15 bacteria, 2 microeukaryotes) were identified using the WQI-CS to classify water quality. Diatoms (e.g., *Cymatopleura*, *Navicula, Amphora, Cocconeis*), and green algae (e.g. *Desmodesmus*) were indicators for good status, with the species *D. Subspicatus* and *N. cryptotenella* correlating to α-diversity (R^2 0.89, $p < 0.001$, $\beta > 0$; and R^2 0.42, p 0.05, $\beta < 0$). Bacteria were indicators of poor status (e.g., *Phaeodactylibacter, Novosphingobium,* and *Blastocatella),* with *B. fastidiosa* correlating with α-diversity (R^2 0.22, $p < 0.01$, $\beta < 0$).

Ongoing work will include additional WQIs (e.g., trophic indices), additional taxonomic markers (e.g., *rbcL*, *COI1*), and unsupervised statistics w/o a priori WQI classification (e.g., NMDS, RDA), and complemented with controlled mesocosm experiments. The final set of bioindicator will be converted into a novel biological indice for rapid in-field biodiversity diagnostics, supporting biological water quality monitoring and ecological assessments.

Keywords: Periphyton; Biological Water Quality; Bioindicator; Water Quality Indice; Long-read sequencing;

From PAH pollution to microbial hazards: cross-domain network reorganization in urban anthrosols

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Urban anthrosols, including street deposits accumulating on roadsides, sidewalks, and vegetated urban infrastructures, represent overlooked environmental matrices exposed to chronic chemical contamination and recurrent biological inputs. However, little is known about how organic pollutants influence microbial assembly processes and cross-domain ecological interactions within these anthropogenic habitats. This field study, conducted across contrasting urban sub-catchments of Lyon (France) within the OTHU urban water long-term field observatory, investigated the effects of polycyclic aromatic hydrocarbon (PAH) contamination on bacterial and fungal communities inhabiting street deposit-derived anthrosols. Chemical analyses were combined with quantitative PCR, microbial source tracking, high-resolution amplicon sequencing (16S rRNA gene and fungal ITS marker), null-model community assembly analyses, and cross-domain amplicon sequence variant (ASV) network inferences. PAH contamination emerged as a major driver of microbiome organization. Increasing PAH levels were associated with a marked decline in microbial biomass, particularly in fungi, while alpha-diversity remained relatively stable. Community assembly was globally dominated by stochastic processes, but bacterial assemblages exhibited increasing heterogeneous selection under higher PAH loads, indicating stronger local environmental filtering. Cross-domain network analyses revealed a non-linear reorganization of microbial interactions along the pollution gradient, with intermediate contamination maximizing modularity and severe contamination promoting more cohesive, less vulnerable, and highly cooperative networks. These stress-adapted networks were dominated by fungal hub taxa and enriched in hydrocarbon-degrading and pathogen-associated bacterial lineages. In parallel, microbial source tracking revealed significant human and canine fecal inputs correlated with opportunistic pathogen loads. These findings demonstrate that PAH pollution not only reshapes microbial community assembly but can also promote the ecological integration and persistence of microbial hazards in urban anthrosols, highlighting the relevance of cross-domain microbiome analyses for urban ecotoxicology and One Health risk assessment.

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Keywords: Urban microbiome; Anthrosols; Polycyclic aromatic hydrocarbons (PAHs); Fungal-bacterial interactions; Urban health hazards

Combined effects of microplastics and organic pollutants on soil microbial functions and plant growth in agricultural soil

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Agricultural soils are increasingly exposed to microplastics and organic pollutants, yet their combined effects on microbial functions, plant-associated microorganisms and crop performance remain poorly understood. As contaminant mixtures may disrupt nutrient cycling and plant–microbe interactions, ammonia-oxidizing microorganisms (AOMs) and arbuscular mycorrhizal fungi (AMF) are relevant microbial indicators, linking nutrient cycling, plant nutrition and soil health. This study investigated the effects of different microplastic types and two organic pollutants, albendazole, an anthelminthic, and pyraclostrobin, a fungicide, on microbial and plant responses in a pot experiment.

Spring onions were grown in pots containing agricultural soil from Crete, Greece, with no prior microplastic contamination. Soil was amended with LDPE-, PBAT- or starch-based microplastics at 0.1% and 1% w/w, with or without albendazole and pyraclostrobin. Untreated pots served as double controls. Treatments were sampled at 45 and 90 days to assess potential nitrification, nitrate levels, AOM abundance, plant biomass, AMF colonization and AOM/AMF community diversity.

Preliminary results showed a significant negative interactive effect of microplastics and the organic pollutant mixture on potential nitrification, while nitrate levels were affected in specific combined-exposure treatments, particularly under LDPE and pollutant co-exposure, with partial recovery at 90 days. In contrast, no significant effects were detected on AOM abundance based on qPCR. Plant effects were transient, with reduced shoot biomass at 45 days followed by recovery at 90 days. AMF hyphal colonization was not significantly affected, although arbuscular colonization showed treatment-dependent responses at the early sampling point.

Overall, these preliminary findings suggest that combined exposure to microplastics and organic pollutants can affect soil microbial functions and plant performance. Ongoing sequencing analysis will clarify whether functional changes are associated with shifts in AOM and AMF communities.

Acknowledgments: This work is performed within project MINAGRIS (Grant Agreement 101000407) funded by the European Union's Horizon 2020 research & innovation Programme.

Keywords: microplastics; soil microbial functions; ammonia oxidizers; arbuscular mycorrhizal fungi; plant growth

Evolution of microbial communities, geochemistry and plant stress in two arsenic- rich mine tailings phytostabilized with *Festuca rubra*

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Securing mine tailings represents a major environmental challenge, and phytostabilization is a moderately cost-effective option for mitigating the dispersal of contaminated particles. In the interdisciplinary oMIMo project (ANR-22-CE04-0012-0), a controlled scenario of assisted phytostabilization was studied in 20 L pots in a greenhouse, using two arsenic-rich tailings from a former silver–lead mine and a tin mine. The tailings differed in mineralogy and texture, with the silver–lead mine wastes being coarser and containing higher amounts of iron oxide minerals. These residues, planted or not with *Festuca rubra*, were amended with compost and limestone to provide nutrients to the plants and increase the pH. The monitoring, carried out every six months over two years, combined the evolution of the prokaryotic community (abundance by Most Probable Number- MPN -of bacteria involved in As, Fe and S cycles, and diversity of the active community through 16S rRNA sequencing), the geochemistry of interstitial water and the toxicity of As and metals for plants. Porewater composition strongly differed between the two mine wastes but were not significantly influenced by plants. Silver-lead mine tailings showed high Pb and low As concentrations in porewaters, whereas the opposite was observed in the tin mine residues. The active prokaryotic community diversity, initially dominated by bacteria from the compost, evolved differently depending on the residue, and growth of Cyanobacteria on the surface of all pots was observed. Results of MPN tests suggest that plants promoted the growth of bacteria involved in As biogeochemical cycle. Anaerobic FeIII-reducing and SO_4-reducing prokaryotes were more abundant in the tin mine tailings than in the silver-lead residues. The omega3 index, bioindicator of plant stress, seems to indicate a greater toxicity of the silver-lead mine tailings for *F. rubra*. Globally, results show that both microbial communities and plant stress are linked to specific tailings characteristics.

Keywords: mine wastes, phytostabilization, metals, arsenic, microbial communities, Plant stress

poster presentation

Co-stressor dynamics reprogram arsenic detoxification under salt stress In *Chlamydomonas reinhardtii*

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While arsenic toxicity and the activation of canonical detoxification pathways are well understood in phototrophs under stable laboratory conditions, such as in the model limnic microalga *Chlamydomonas reinhardtii*, their regulation under multiple, concurrent environmental stresses remains poorly resolved. Such co-stressor interactions can reshape cellular physiology, with consequences that are difficult to predict from single-stressor studies alone. To resolve how combined salt (NaCl) and arsenate (As(V) exposure reshapes cellular physiology, we integrated growth characterizations, photosynthetic performance studies, and multi-omics in *C. reinhardtii*. We found that chronic co-exposure to As(V) and salt did not act in additive ways on growth across a wide range of concentrations (0-50 mM NaCl, and 0.064-10 µM As(V)), in that salt caused the most detrimental response, while the addition of As(V) did not enhance the growth defect more than salt alone. Only salt deteriorated photosynthetic performance in the tested concentration range, while arsenate did not. Despite the absence of a clear growth phenotype, our data suggest metabolic rewiring of arsenic handling during salt co-exposure. Proteomics and lipidomics indicate a collapse of major detoxification pathways, with possible intracellular accumulation of arsenate. Specifically, we suspect disruption of the canonical detoxification cascade, in which arsenate is normally reduced to arsenite for further processing via methylation and partial conversion into complex organic arsenicals such as arsenolipids. These downstream transformations were blocked in the presence of salt co-stress, with strongly reduced rates of arsenosugar phytol formation. Together, these results point to a systemic breakdown of the typical arsenic response pathway under combined stress while preserving cellular viability. The apparent reconstitution of arsenical processing may change the way it is mobilized and biotransformed in the environment, challenging unidirectional concepts of toxin coping. This highlights the relevance of co-stressor dynamics for freshwater systems increasingly affected by saltwater intrusion and arsenic input.

Keywords: Arsenic, *Chlamydomonas reinhardtii*, Co-Stress

poster presentation

Pollution Footprints in Paradise: Impacts of Hydrocarbons, PCBs, and Metals on a Polynesian Atoll Soil Microbiomes

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Industrial activities can lead to environmental degradation, with soil contamination by organic and metallic pollutants. The French Ministry of the Armed Forces funds a research program aiming to characterize the diversity of plants and the soil microbiome at sites contaminated by organic and metallic pollutants, with the objective of proposing phytoremediation solutions.
The first mission, which took place in 2025, involved characterizing the study sites and taking bare and rhizospheric soil samples from a contaminated area and some control sites. Plant diversity was assessed, contamination was measured, and microbial abundance and diversity were estimated using qPCR and Illumina sequencing targeting bacterial 16S rDNA and fungal ITS.
Analysis of soil samples revealed the presence of organic contaminants, such as alkanes, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs), as well as metal contaminants, such as zinc, cadmium, lead, and copper. These contaminants exhibited highly heterogeneous spatial concentrations. No significant differences in microbial abundance were observed between the different conditions. Alpha diversity analyses showed high bacterial and fungal richness and diversity indices. The specific environment of the study site, such as the climate and calcareous soil substrate, appears to primarily influence both bacterial and fungal communities. Indeed, bacteria such as Solirubrobacteraceae or Xanthobacteraceae and fungi such as Chaetomiaceae or Calcarisporiellaceae families, dominated the communities and are typical taxa adapted to arid or nutrient-poor mineral soils, and with some being thermotolerant. The impact of different plant types was not significant, whereas contamination mainly affected bacterial diversity: the relative abundance of the Tistrellales order decreased and that of the Bacillales order increased in contaminated soil samples. This work improves our understanding of contamination impacts on microbial communities in the soils under investigation, a largely understudied topic.

Keywords: Contaminants; Sequencing; Microorganisms; Pacific Island

Iron-dependent modulation of Cr(III) toxicity in *Chlamydomonas reinhardtii*

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Chromium is a widespread environmental contaminant mainly present as hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)). Although Cr(VI) toxicity has been extensively characterized, the biological effects of Cr(III) remain less understood. Because Cr(III) shares structural and biochemical similarities with Fe(III), we investigated whether iron availability modulates Cr(III) toxicity in the green microalga *Chlamydomonas reinhardtii*. In iron-replete medium, Cr(III) induced limited toxicity but significantly altered iron homeostasis. Exposure to Cr(III) increased the expression of iron uptake markers including *FEA1*, *FOX1*, and *FTR1*, suggesting the induction of intracellular iron deficiency. Under iron-deficient conditions, Cr(III) toxicity was markedly enhanced, leading to impaired growth, increased cell size, elevated ROS production, and chlorophyll reduction. ICP-MS analyses revealed that Cr(III) accumulation increased in iron- deprived cells compared with iron-supplemented conditions. Considering the naturally low bioavailability of iron in many aquatic environments, our findings highlight the potential ecological relevance of Cr(III) toxicity under environmentally realistic iron-limited conditions.

Keywords: *Chlamydomonas reinhardtii*, chromium, iron

Chemical contamination gradients drive spatio-temporal dynamics of diatom communities in marine biofilin in a highly anthropized Mediterranean bay

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Marine biofilms are complex microbial communities colonizing submerged surfaces. Diatoms, the main autotrophs in these communities, are recognized for their sensitivity to environmental variations and are widely used as bioindicators in freshwater ecosystems. However, their biomonitoring potential in marine ecosystems remains relatively unexplored. The Bay of Toulon (NW Mediterranean Sea) is divided in two parts: a highly anthropized coastal ecosystem exposed to chronic chemical contamination from multiple sources (the small bay) and a less impacted one (the large bay). This study aimed to investigate the spatial and temporal structuring of marine diatom biofilm communities, evaluate their sensitivity to environmental and anthropogenic gradients, and explore their potential as biomonitoring tools. Fourteen sites, distributed across the large and the small bay, were selected for spatial analysis, and five were additionally monitored throughout four seasons for temporal analysis. At each site, HDPE plates were immersed for one month allowing *in situ* biofilm colonization, from which DNA, trace metal and organic contaminants were analysed. Diatom communities were characterized using *rbcL* metabarcoding and taxonomically assigned with the Diat.barcode database. Spatial analyses revealed significant differences among sampling sites (PERMANOVA, p = 0.001), with PCoA ordination showing site-specific clustering patterns. Especially, sites located within the small bay exhibited relatively similar community patterns, potentially reflecting the influence of local anthropogenic pressures on biofilm structuring. These biofilms also showed significant higher metal bioaccumulation, especially for copper, lead and chrome. Moreover, Temporal analyses highlighted a significant seasonal effect on diatom community composition, notably in winter (PERMANOVA, p = 0.001), indicating pronounced temporal turnover throughout the year. These results demonstrate that marine diatom biofilm communities can respond to environmental variability even at relatively small spatial scales, supporting their potential as sensitive bioindicators of marine water quality. Ongoing chemical analyses will further explore relationships between organic contaminant bioaccumulation and diatom community structuring.

Keywords: Diatom, Marine biofilms, Anthropogenic pressures, Bioindication, marine water quality

poster presentation

Integrating DNA Metabarcoding into Diatom-Based Regulatory Frameworks: Bridging Biodiversity Analysis and Water Quality Assessment

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Diatoms are widely used for ecological assessment of freshwater systems, traditionally relying on microscopy identification. However, this approach depends largely on taxonomic expertise and is constrained by counting limits, overlooking rare taxa. While having limited impact on water quality assessment, it may affect biodiversity analyses.To address these limitations, we compared periphytic diatom assemblages upstream and downstream of local discharges in four Flemish rivers (Belgium), assessed by both microscopy and rbcL metabarcoding methods. RbcL biofilm sequences were taxonomically assigned using the Diat.Barcode database. Ethanol-preserved biofilm aliquots (70%, 1:4 v/v) were analyzed following Van de Vijver & Wetzel (2024).Species overlap between methods was low (15–19%), underscoring methodological discrepancies. Metabarcoding detected higher species richness at most sites (7out of 9; $p<0.05$) but lower Shannon diversity (8 out of 9; $p<0.05$), likely driven by dominance of high-biomass taxa (e.g. Melosira varians). It also identified two differentially abundant taxa (ALDEX2, $p<0.05$) between upstream and downstream sites, whereas microscopy detected none.

The EKC index, used for diatom-based water quality assessment in the Flemish region and based on the proportion of impact-sensitive and impact-tolerant taxa, differed significantly ($p<0.05$) between methods, with rbcL metabarcoding indicating moderate to good status and microscopy indicating insufficient to good status. As EKC was developed for microscopy-based data, its application to metabarcoding outputs may require recalibration. Nevertheless because read abundance is often linked to DNA quantity and organism biomass, metabarcoding reads may still capture ecologically relevant functional patterns driven by dominant taxa, supporting their use beyond numerical abundance.Our results demonstrate that microscopy and metabarcoding provide complementary but partially overlapping insights into diatom communities. While microscopy seems to be more aimed towards abundance-based assessments, metabarcoding captures more biomass-related signals. Integrating both improves detection of rare and cryptic taxa and strengthens freshwater biomonitoring frameworks

Keywords: Water Framework Directive; biomass scaling; functional trait dominance; molecular bioassessment; cross-method comparability.

Impact of metolachlor and metolachlor ESA on microbial and denitrifying activity in soils and river sediments in an agricultural watershed in Brittany (France)

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The occurrence of chloroacetanilide herbicides and their transformation products raises concerns regarding their effects on microbial processes. We investigated the impact of metolachlor and its metabolite, metolachlor ethane sulfonic acid (ESA), on microbial activity and denitrification in soils and river sediments from an agricultural watershed in Brittany (France), where elevated concentrations of these compounds were detected in ground and river water. It is expected that ESA exerts stronger inhibitory effects on microbial and denitrifying activity, as well as on microbial diversity. In addition to assessing ecotoxicological impacts, the degradation dynamics of both compounds were evaluated in soils and sediments.

Experiments were conducted using topsoil and deeper soil layers, incubated for 65 days, to determine whether higher organic carbon availability in topsoil enhances metolachlor degradation to ESA and influences microbial activity. River sediment incubations were performed in flow-through reactors over 45 days to assess nitrate reduction under exposure to both compounds at different concentrations. Microbial activity was quantified via CO_2 production, while denitrification was assessed through N_2O production using the acetylene inhibition technique. Preliminary results indicate that low concentrations of metolachlor slightly reduce nitrate reduction in sediments. Ongoing metabarcoding analyses will further elucidate potential shifts in microbial community composition.

Keywords: Metolachlor, Metolachlor ESA, denitrification, soil microbial activity, agricultural watershed.

Impact of Quaternary Ammonium Compounds (QACs) on the environmental denitrifier *P. veronii*

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Quaternary ammonium compounds (QACs) are widely used as disinfectants in domestic, medical, industrial, and agricultural settings through sprays, wipes, and surface-cleaning products. Their continuous release via wastewater and agricultural run-off into aquatic environments raises concerns regarding impacts on environmental microbial communities, including bacteria involved in nitrogen cycling. In this study, we evaluated the toxicity of several structurally distinct QACs toward *Escherichia coli* and the environmental denitrifier *Pseudomonas veronii* by determining EC50 values under aqueous exposure conditions. In addition, environmentally relevant concentrations and sub-EC50 exposures were used to assess their effects on denitrifying activity in *P. veronii*, together with possible changes in membrane lipid status.

The tested compounds included benzalkonium chlorides (BAC C12, C16, C18), dialkyldimethylammonium compounds (DADMAC C8, C16), and alkyltrimethylammonium compounds (ATMAC C16) with different alkyl chain lengths. Contrary to expectations based on conventional disinfectant studies, shorter-chain and more water-soluble QACs showed stronger inhibitory effects than longer-chain compounds. These preliminary results suggest that solubility and bioavailability may play a role in determining the environmental effects of QACs in aquatic systems, compared to the use and application as a disinfectant.

Keywords: Quaternary ammonium compounds (QACs), antimicrobial toxicity, denitrifying bacteria

Metagenomic and Genome-Resolved Insights Of Marine Biofilms Resistome Across Contrasted Coastal Environments

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Marine biofilms rapidly colonize immersed artificial surfaces and act as sentinels of environmental conditions. In coastal areas exposed to anthropogenic activities, marine biofilms may provide valuable insights into local metal contamination patterns through variations in their microbial composition and their resistome. We hypothesized that biofilms exposed to various metal contaminations exhibit distinct patterns of metal resistance genes (MGRs) that reflect local toxicity. To investigate this hypothesis, PVC plates were deployed for one month in five harbours across three different oceanographic basins. Biofilm DNA was extracted and the metagenomes were sequenced, yielding over 100 million short-reads per sample. Overall, 893 bacterial families were detected by taxonomic assignments of the raw reads using Kaiju against the NCBI non-redundant database. Some of this families have also been recovered with the reconstruction of metagenome- assembled genomes (MAGs) with CONCOCT, rendering 135 medium-to-high-quality prokaryotic MAGs (<15% contamination, >50% completeness). Both contigs >1kb and MAGs were aligned against the BacMet and PanRes databases with DIAMOND for the detection of MRGs. While ubiquitous marine taxa (e.g., *Roseobacteraceae* (~19%), *Flavobacteriaceae* (~11%), and *Alteromonadaceae* (~4%)) dominated globally, overall community composition and MRG profiles diverged significantly based on site-specific pollution and physico-chemical gradients. The analyses revealed a common set of core functions involved in metal response (23 out of 83) across all sites, with a portion of these MRGs being site-specific. Furthermore, 44 of the 83 MRGs identified in the contigs were also detected in the MAGs, where multidrug resistance (*ruvB*, *actP*, *actR*, *hmrR*), hydrogen peroxide (*sodB*), and arsenic resistance (*arsH*) were the most frequently detected genes. Among these genes, *ruvB* was associated with 12 bacterial families in MAGs, including the *Alteromonadaceae* and *Hyphomonadaceae*. We propose that MRGs associated with the plastisphere are promising as an additional analysis for characterizing the effect of chronic metal pollution in marine microbial communities.

Keywords: Marine Biofilms; Metagenomes; MAGs; Metallic Pollution; metal resistance genes

Interpreting toxicant-induced microbiome shifts in *Daphnia magna* requires baseline characterization

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Environmental contaminants can alter the gut microbiome of aquatic organisms; however, interpretation of microbiome responses remains challenging because microbial composition is shaped not only by toxicological exposure, but also by laboratory-associated factors and genetic background.

Daphnia magna is a keystone species in aquatic ecosystems and a widely used model organism in ecotoxicology. Its microbiome is increasingly recognized as both a modifier of toxicant responses and a potential biomarker of exposure. However, before microbiome shifts can be interpreted as exposure-related effects, the baseline range of microbiome variability in laboratory D. magna populations must first be characterized.

Here, we evaluated whether publicly available 16S rRNA sequencing datasets contain a reproducible microbiome signal that could serve as a baseline for interpreting toxicant-induced shifts in D. magna. We curated metadata relevant to microbiome-aware ecotoxicology, including clone identity, culture conditions, exposure regimes, sample type (gut versus whole body), stressor class, and amplified 16S rRNA variable region. In total, 53 studies were identified, of which 43 contained metadata suitable for ecotoxicological interpretation.

Most studies targeted either the V4 (52%) or V3–V4 (47%) regions of the 16S rRNA gene. Although these datasets collectively provide a substantial foundation for comparative microbiome analysis, differences in amplified variable regions reduce cross-study comparability. Studies using the V4 region captured a broader diversity of Daphnia clones and exposure types, whereas V3–V4 amplicons generally provide improved genus- and species-level taxonomic resolution. As a result, methodological variation in sequencing approaches may confound interpretation of microbiome differences across studies. Nevertheless, the inclusion of both antibiotic and non-antimicrobial contaminants creates opportunities to compare direct microbial disruption with broader toxicant-associated dysbiosis.

Despite increasing interest in microbiome-mediated ecotoxicology, no synthesis has yet evaluated whether existing Daphnia microbiome datasets are sufficiently standardized to establish reproducible baseline microbial communities across laboratory conditions.

Keywords: gut microbiome; daphnia magna; stress; 16S rRNA1

poster presentation

Sublethal pesticide exposure and the honey bee gut microbiota: interactions and impacts on bee health

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Bees are essential pollinators whose health is increasingly threatened by agricultural intensification and widespread agrochemical exposure. While pesticide risk assessment largely focuses on acute toxicity, chronic sublethal exposure can also impair immune competence, pathogen resistance, and foraging behavior. Because these same functions are partly supported by the specialized honey bee gut microbiota, pesticide-induced perturbation of this community may contribute to sublethal effects on the host. Conversely, the gut microbiota may modulate pesticide toxicity through biotransformation or by altering their persistence and bioavailability.

Here, we use the honey bee gut microbiota to investigate reciprocal pesticide–microbiome interactions. Building on a high-throughput *in vitro* screen of agrochemical effects on bee-associated bacterial isolates, we exposed gnotobiotic honey bees colonized with a defined synthetic community to selected agriculturally relevant pesticides at sublethal concentrations corresponding to 1% of the reported bee LD50. Microbial community dynamics were followed across exposure and recovery using qPCR and full-length 16S rRNA amplicon sequencing.

Overall, the synthetic gut community was largely resilient at these concentrations, although selected pesticides caused subtle strain-specific shifts in community assembly. Follow-up experiments comparing mono-colonization with *Gilliamella apicola*, a conserved honey bee gut bacterium, to full-community colonization at higher pesticide doses further suggest that community context can buffer pesticide sensitivity of vulnerable strains. In parallel, targeted and untargeted LC-MS analyses revealed pesticide-dependent differences in gut metabolite profiles and pesticide persistence, providing a basis to explore microbiota-mediated pesticide biotransformation and broader metabolic changes *in vivo*. Finally, pathogen challenge experiments with *Serratia marcescens* showed that some pesticide treatments increased infection frequency, suggesting that sublethal exposure may weaken microbiota-mediated colonization resistance. This provides an initial readout for exploring how pesticide-induced microbiota perturbations may affect broader bee health outcomes. Together, our results support the gut microbiota as a key factor in modulating the sublethal impacts of agrochemical exposure on bees.

Keywords: Gut microbiota, Pesticides, Biotransformation, Bee health, Pathogen resistance

Influence of Hydrological Connectivity and Surface Inputs on Groundwater Microbial Communities Across Contrasted French Aquifers

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Groundwater is the main water resource used for human activities, notably producing drinking water.Today, within the European Water Framework Directive (WFD; 2000/60/EC), assessment of groundwater quality status relies only on hydrochemical monitoring while a broad range of geochemical processes controlling nutrient cycling, contaminant attenuation, redox properties, hence groundwater quality, are controlled by microbial activity. The spatial and temporal dynamics of microbial diversity and functions remain poorly characterized in aquifers. Few studies have investigated groundwater microbial communities across multiple aquifers to evaluate whether they could be good indicators for the quality status of groundwater, revealing their functioning. The study investigated several French aquifer systems with a gradient of hydrological connectivity between surface and groundwater, ranging from porous-fissured systems (Beauce aquifer, Crépieux-Charmy alluvial aquifer, East Lyon aquifer, Crieu alluvial aquifer) to strongly karstified systems (Bauges massif and southern Ardèche). Microbial biofilms were studied in situ using a methodology based on the incubation of clay beads as artificial substrates directly in boreholes during several months. Biofilm development was investigated through biomass measurements, microbial activity assays, and metabarcoding. Water samples were simultaneously collected to measure several physico-chemical parameters, nutrient concentrations, and dissolved organic carbon. Preliminary results revealed that microbial enzymatic activities of biofilms were higher in aquifers highly connected to the surface than in more isolated systems. This suggests that surface-groundwater connectivity, and notably the associated dissolved organic and nutrients fluxes, had an effect on groundwater microbial activities. Hydrological connectivity appeared to be a major driver controlling groundwater microbial communities, suggesting that microbial-based monitoring could be used to assess vulnerability of groundwater systems to surface pressures. Metabarcoding analyses are currently in progress to further characterize these communities and their potential responses to surface-groundwater exchanges.

Keywords: groundwater – microbiology – connectivity – bioindicators - metabarcoding

Understanding the effects of perfluoroalkyl substances (PFASs) on bacteria

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Per- and polyfluoroalkyl substances (PFASs) are a class of very diverse chemicals found in products of daily use, that are highly persistent and encountered everywhere in the environment. They accumulate/biomagnify within the natural food chain and show a relatively high toxicity including the alternative products developed after the ban of the legacy compounds. Most toxicological studies have been performed at a macroscopic/organism level (growth, reproduction, size of the leaves…) mainly on aquatic ecosystems and to a lesser extent on terrestrial ones. Studies on human health are nevertheless gaining in importance. Few studies have used OMICS to analyses at the cellular level and globally the pathways perturbed by these chemicals. Most importantly, very few studies were performed on bacteria and have mainly deal with them as biomass/biofilm in PFAS dissemination studies, and analysed changes in bacterial communities diversity or in global activities such as methane production, catalase, phosphatase, urease activities. To fill in the gap, we propose in our project to analyse in details by proteomics the response to various PFASs of the model gram-positive bacterium *Bacillus subtilis* that is mainly encountered in soil upper layer and contributes to plant rhizosphere. Some species of *B. subtilis* can also colonize fresh and salty water as well as gastrointestinal tract of different animal species including human. In parallel, we will also study the gram-negative bacteria *Pseudomonas* describe to be able to biodegrade PFOA. In particular we will focus on sporulation, quorum sensing that can have impacts on antibiotic gene resistance transfer, biofilm and toxins production.

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Keywords: PFAS, B. subtilis, Pseudomonas, Proteomic


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Session 3

Natural Toxins - Microbiota

The Good, the Bad and the Biodegradable: Rethinking Plastics in Agriculture

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Microplastics have emerged as a major environmental concern in terrestrial ecosystems, with agricultural soils representing one of the largest sinks due to the extensive use of plastic mulch films. While plastic mulching improves crop productivity and resource-use efficiency, the long-term accumulation and fragmentation of mulch residues into microplastics can alter soil quality, plant performance, and ecosystem functioning. Our previous research demonstrated that both conventional polyethylene and biodegradable plastic mulch residues influence plant growth, rhizosphere microbial communities, soil physicochemical properties, and key ecosystem functions, highlighting the complex impacts of plastic residues on the soil–plant system.

In this presentation, I will provide an overview of these findings and present recent results investigating the effects of biodegradable plastics and their additive interactions with emerging chemical contaminants. The new research explores how these combined stressors influence plant growth, rhizosphere microbial community composition and rhizosphere chemistry, with a particular focus on belowground plant–microbe interactions. Understanding the combined effects of biodegradable plastics and co-occurring contaminants is essential, as agricultural soils are increasingly exposed to multiple environmental stressors rather than individual pollutants. These findings contribute to a more comprehensive understanding of the ecological risks associated with plastic use in agriculture and provide new insights for developing sustainable plastic management strategies and protecting soil health in agroecosystems.

Keywords: biodegradable plastic; emerging chemical contaminants; plant growth, rhizosphere microbial community composition and rhizosphere chemistry

Biopesticide-induced responses in periphytic microbial communities: new insights from an untargeted meta-metabolomic approach

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As demand grows for more environmentally sustainable agricultural practices, naturally derived substances and biopesticides have gained increasing attention. However, like conventional pesticides, these compounds can be dispersed across different environmental compartments and affect non-target organisms, including aquatic microbial communities that play key roles in ecosystem functioning (e.g. trophic relationships and biogeochemical cycles). This study aims to contribute to the risk-benefit assessment of biopesticide use by investigating the responses and tolerance mechanisms of periphytic microbial communities exposed to kasugamycin and copper. Standard ecotoxicological analyses — covering physiological (photosynthetic and heterotrophic enzymatic activities), structural (biomass, algal composition), and tolerance (Pollution-Induced Community Tolerance, PICT) descriptors — were combined with an untargeted LC-HRMS/MS metabolomic approach, offering new insights into the molecular phenotype of entire communities and the biochemical processes modulated in response to contaminants. Community-level responses were monitored throughout a 28-day chronic exposure. Kasugamycin exposure significantly increased phototrophic biomass and, at the highest concentration, inhibited community photosynthetic activity. Copper drove a shift in algal community composition — with declining green algae and rising diatom abundance — coupled with a reduction in heterotrophic enzymatic activities. Both biocides induced a transient acquisition of tolerance. Untargeted metabolomic analyses revealed significant effects of exposure duration and contaminant identity on community metabolism. Altogether, these results contribute to elucidating the cascade of responses triggered by kasugamycin and copper, deepening our understanding of the adverse and tolerance outcome pathways (AOPs/TOPs) in periphytic microbial communities. Future work could further explore molecular descriptors as potential biomarkers for monitoring and safeguarding the ecosystem functions supported by these communities.

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Keywords: microbial communities; biocides; risk assessment; aquatic environment

Effects of pelargonic acid on soil microbial community abundance, diversity and function

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Pelargonic acid (PA), a non-selective herbicide of natural origin, is the most widely marketed plant-derived herbicide; however, its effects on non-target soil microbial communities remains poorly documented. This study assessed the effect of PA on soil microbial communities using agricultural soil microcosms (n = 175, 5 replicates per treatment). Treatments consisted of a pure PA formulation (with Tween-20), a commercial PA formulation (Roundup® Rapid EC), glyphosate (RoundUp® Top) as a conventional herbicide reference, and two controls (sterile water and Tween-20). All treatments were applied at agronomic dose, 23 L ha-1 for PA formulations and 3.525 L ha-1 for glyphosate, according to supplier recommendations. Soil samples were collected at 1, 2, 3, 8, 9, 10, and 30 days post-application to monitor microbial abundance, taxonomic diversity, and general enzymatic activity. PA formulations induced a rapid and significant increase in CO_2 production, associated with a rise in bacterial abundance (16S rRNA gene copies), representing an initial response phase. A subsequent phase was marked by a decline in overall enzymatic activity and a decrease in fungal abundance (18S rRNA gene copies) that persisted until day 30, consistent with the known antifungal properties of PA. At the bacterial taxonomic level, notably Pseudomonas, exhibited an early increase following application, without resilience over the incubation period. Fungal communities experienced a delayed compositional shift, primarily driven by unclassified fungi. In contrast, glyphosate treatment showed stable taxonomical composition over time compared to Control, with only minor fluctuations in microbial activity and no significant effect on microbial respiration. Overall, the results demonstrate that both pure and commercial PA formulations induce comparable, significant, and persistent shifts in soil microbial community structure and function, raising questions about the ecological consequences of this natural herbicide on soil communities.

Keywords: microbial ecotoxicity; soil microorganisms; natural herbicides; pelargonic acid; glyphosate

Fit-for-purpose risk assessment for low-risk pesticides

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In recent years, in response to the European Green Deal, and particularly the Farm to Fork Strategy, the Biodiversity Strategy, and the Sustainable Use of Pesticides Directive, the European Commission has introduced measures to reduce the use of chemical plant protection products and to promote the market uptake and use of low-risk pesticides (LRPs). Several EU-funded projects, together with a range of relevant regulatory initiatives, are currently ongoing in this area. New Approach Methods (NAMs) are expected to play a key role in future Integrated Approaches to Testing and Assessment by integrating data from multiple methods. Traditional animal-intensive testing approaches are often time-consuming, costly, and limited in predictive capacity. In contrast, NAMs, such as in vitro, in chemico, and in silico methods, combined with high-throughput screening and omics technologies, provide more direct insight into molecular and cellular pathways related to efficacy, toxicity and environmental fate. In addition, specific NAMs could be more appropriate for specific type and sub-type of low-risk pesticides compared to the conventional methods currently used. This presentation reviews the current trade and regulatory landscape for LRPs. Within the EFSA-funded project “Develop a stepwise approach for a fit-for-purpose risk assessment, in particular for low-concern active substances and uses”, our consortium developed a risk assessment scheme based on Problem Formulation (PF) and the Pathways to Harm (PH) model. For this scheme, generic PH were developed for each hazard–protection goal combination. Owing to its inherent flexibility, the scheme can be applied across all major categories of LPRPs and is intended to harmonise their assessment. It was tested through case studies representing the principal types of LRPs, including botanicals, semiochemicals, microbial metabolites, inorganics, peptides, and dsRNA. The presentation will also discuss recent advances in microbial metabolites and NAMs for their efficacy and risk assessment.

Keywords: Microbial metabolites; problem formulation; risk assessment; NAMs; Waiving criteria

Biological Nitrification Inhibitors: Curse or Blessing for the Soil Microbiota?

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Biological nitrification inhibitors (BNIs) are plant-derived compounds that suppress soil nitrification by targeting ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and comammox bacteria. As interest in sustainable agricultural practices increases, BNIs are considered environmentally friendly alternatives to synthetic nitrification inhibitors (SNIs). However, their behaviour in soil systems, and particularly their broader ecological impact, remains insufficiently understood. This study evaluates the ecotoxicological effects of two BNIs, MHPP and MBOA, in comparison with the widely used SNI DMPP. Soil microcosm experiments were conducted using an acidic soil over a 30-day incubation period, with compounds applied at six concentrations. Nitrification rates and the abundance of ammonia-oxidizing microorganisms were assessed at 0, 5, 10, 20, and 30 days. Results showed inhibition of nitrification across treatments, with DMPP exerting the strongest effect. BNIs exhibited more variable responses, with greater inhibition at higher concentrations but no clear linear dose-response relationship. Abundance and diversity data of microbial communities are currently being analysed and will be presented at the conference. Amplicon sequencing is being used to identify potential adverse effects on the communities of bacteria, fungi and protists and eventually derive ecotoxicological threshold values (Hazard Concentration 5% - HC5 ; Non Observed Effect Concentration, NOEC) for nitrification inhibitors using Species Sensitivity Distributions (SSDs) and Principal Response Curves (PRCs). By integrating sequencing data with metabolic activity results, we provide a balanced assessment of whether may induce off- target effects on soil microbial communities beyond nitrification Inhibition

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Keywords: biological nitrification inhibitors, soil ecotoxicity

High-throughput Microtox screening of non-target effects of biological nitrification inhibitors

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Biological nitrification inhibitors (BNIs) are increasingly proposed as nature-based tools to reduce nitrogen losses in agricultural systems. However, their specificity towards nitrifying microorganisms is seldom evaluated. Here, we screened a diverse set of BNIs for potential non- target effects using a Microtox dose–response assay with Aliivibrio fischeri, a widely applied high-throughput model in microbial ecotoxicology.

Most BNIs tested reduced A. fischeri growth in a concentration-dependent manner. In particular, methoxybenzoxazolinone (MBOA) and several isothiocyanates - previously identified as key nitrification-inhibiting metabolites in soil - showed strong toxic effects. Although the assay uses a marine bacterium, Microtox responses are generally comparable to soil-based microbial toxicity assays and enable detection of non-specific toxic effects in organisms that are not targets of nitrification inhibition. Compounds with weaker effects in the assay, including caffeic acid, linolenic acid, MHPP and umbelliferone, are nonetheless reported to inhibit a broad range of non-nitrifying microorganisms.

These findings indicate that non-target microbial effects are common among currently described BNIs. We suggest that widely used discovery approaches relying on ammonia-oxidizing cultures may unintentionally favor generally toxic metabolites, while overlooking molecules that specifically interfere with nitrification processes. Incorporating parallel screening against both target and non-target microorganisms during BNI discovery could help mitigate this bias. Such dual testing strategies are becoming increasingly feasible and may be essential to ensure the ecological reliability of BNIs intended for agricultural use.

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Keywords: Biological nitrification inhibitors, Microtox assay; (Aquatic) ecotoxicity; Non-target effects; High-throughput screening

Probing for bioavailable nitrification inhibitors in rhizosphere soil using a novel, high-throughput *Nitrosomonas Europaea* solid-phase contact bioreporter assay

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Nitrate leaching and nitrous oxide emission driven by nitrification in agricultural soils are negatively affecting the environment and climate. Biological nitrification inhibition (BNI) holds a potential to reduce these harmful nitrogen losses by inhibiting ammonia oxidation to nitrite, limiting downstream conversion by nitrite oxidizing and denitrifying bacteria. Recently the BNI trait was successfully transferred into modern wheat (*Triticum aestivum* L.) cultivars from *Leymus racemosus*, facilitating the breeding of novel BNI-wheat lines. Previously, high throughput phenotyping of BNI in plants has relied on hydroponic-based systems, which are well suited for high-throughput, however, offering little resemblance to the applied soil environment. Therefore, we developed a high- throughput solid-phase contact assay for quantifying BNI activity directly in soil samples using a transgenic bioluminescent ammonia oxidizing bacterium, *Nitrosomonas europaea* (pHLUX20) as test organism. Implementing this assay, a pipeline for phenotyping BNI in the rhizosphere of soil grown wheat seedlings was designed and validated in pot experiments with spring BNI-wheat lines and their non- BNI parental lines. Rhizosphere soil from three confirmed BNI-wheat lines (Roelfs-BNI, Vorobey-BNI, and Borlaug-BNI) inhibited bioluminescence from the *N. europaea* bioreporter compared to their parental lines and unplanted soil. The Roelfs-BNI rhizosphere showed a 22.9% to 30.3% inhibition compared to no inhibition in the non-BNI Roelfs parental line across three independent experiments. Additionally, ammonium fertilization increased the BNI activity in the rhizosphere of BNI-wheat. This method presents a novel pipeline for efficient high-throughput BNI phenotyping in soil, facilitating testing under environmental and ecological relevant conditions.

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Keywords: Bioavailability, biological nitrification inhibition, ammonia-oxidizing bacteria, nitrification inhibitors, soil toxicity

Response of a soil microbiome-plant system to drought stress under different inputs of organic amendments

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Drought is a key abiotic stressor for terrestrial ecosystems, which negatively affects microbial activity and plant productivity. Applying compost can improve soil structure, porosity, water retention and carbon content. However, depending on the origin of its feedstock (e.g. agro-zootechnical or municipal waste), a compost may contain various contaminants, including pharmaceuticals and antibiotic resistance genes (ARGs) from manure. This study aimed at investigating how a plant–microbiome system responded to drought stress after compost application. Four composts from different decentralised composting plants were applied to soil microcosms. Each microcosm contained soil amended with one of the compost. Overall, organic amendments increased microbial abundance and activity, with significant higher values in rhizosphere than in bulk soil (unplanted conditions).

Under un-stressed conditions, the compost with pig manure, yard trimmings and olive pomace, due to its high phosphorus and labile carbon content, promoted the highest plant biomass values. Differently, under drought, it resulted the worst for plant growth, possibly due to the lowest levels of humic acids, which protect plants against abiotic stress. Poultry manure compost also introduced high levels of ARGs, which increased over time (*sul1* and *sul2*) under drought stress.

Under drought stress, the compost containing donkey manure, municipal solid waste and yard trimmer was the best. In fact, it helped rosemary plants to face drought stress and it introduced only a limited number of ARGs, which decreased over time.

These findings show how composting is an effective process for waste valorisation and for producing a potential organic fertiliser or soil improver. However, to evaluate how combine organic waste of different origin in this process is a key point for producing a good quality compost suitable for specific agricultural purposes.

Keywords: Compost, Drought tolerance, Microbial activity, ARGs, Circular economy

Contrasting levels of BNI activity in *Trigonella foenum-graecum* root exudates reveal distinct metabolomic profiles

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Biological nitrification inhibitors (BNIs) are natural compounds released via plant roots that suppress the activity of ammonia-oxidizing microorganisms (AOM). They offer a sustainable alternative to synthetic nitrification inhibitors (SNIs) for reducing nitrogen (N) losses and enhancing nutrient use efficiency in agroecosystems. Although BNIs are considered an evolutionary adaptation of plants to retain nitrogen as NH4+ in N-limited soils, the specific environmental factors promoting their production and exudation remain poorly understood. We investigated the BNI potential of Trigonella foenum-graecum, a medicinal feedstock crop characterized by an active secondary metabolism. Specifically, we selected the “Grevena” cultivar identified through preliminary screening as a high BNI-producing cultivar and aimed to identify the cultivation factors that maximize BNI potential. Plants were grown in a sterilized sand–vermiculite mixture under urea fertilization (25 mg N kg-1), at three pH levels (neutral, alkaline and acidic) and two moisture regimes (80% and 30% water-holding capacity), to assess the combined effects of ammonium fertilization, pH, and drought stress on BNI release. Hydrophilic and hydrophobic root exudates were extracted from plant roots and screened for their inhibitory activity against three soil ammonia-oxidizing bacteria (AOB) (Nitrosomonas communis, Nitrosomonas ureae, and Nitrosospira multiformis) and two ammonia-oxidizing archaea (AOA) strains (Candidatus Nitrosocosmicus franklandianus and Nitrososphaera viennensis), using a fast-track bioassay. Root exudates from urea-fertilized plants grown under neutral pH and well-watered conditions showed significantly higher AOM inhibition, while drought stress under the same conditions significantly reduced inhibition levels. AOA consistently exhibited higher sensitivity than AOB, with inhibition mainly driven by the interaction between urea fertilization and pH. These findings highlight the strong environmental regulation of BNI activity. Ongoing metabolomic analysis of the root exudates coupled with their inhibition profiles will identify molecules that are involved in the BNI activity observed.

Acknowledgment: I acknowledge MINAGRIS EU Horizon 2020 Programme under Grant Agreement number: 101000407se chemical composition underlying BNI activity.

Keywords: fenugreek, root exudates, biological nitrification inhibitors, ammonia-oxidizing microorganisms, metabolome

Chemical profiling and antimicrobial activity of birch bark and propolis extracts obtained using sustainable solvents

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Birch bark and propolis are rich sources of bioactive compounds with significant biological potential. In this study, a representative ethanolic extract was analyzed by RP-HPLC-DAD to characterize the chemical profile of the raw materials, enabling the identification of phenolic compounds (e.g. chrysin, galangin, pinocembrin, pinobanksin) and pentacyclic triterpenes (e.g. betulin and betulinic acid), which are characteristic constituents of propolis and birch bark, respectively. Furthermore, a total of nine extracts were prepared from birch bark (n=6), propolis (n=1), and their combination (n=2) using sustainable extraction solvents, including three hydrophilic and three hydrophobic solvents. Hydrophilic compounds were extracted using water, aqueous solutions of β-cyclodextrin and hydroxypropyl-β-cyclodextrin, while lipophilic compounds were obtained using sunflower oil and hydrophobic natural deep eutectic solvents (HNaDESs), specifically capric acid:lauric acid (1:2) and thymol:lauric acid (1:2). The formation of HNaDESs was confirmed by FT-IR spectroscopy. The obtained extracts were evaluated for their antimicrobial activity against a diverse panel of microorganisms, including Gram-negative bacteria (Escherichia coli, Salmonella enterica, Pseudomonas marginalis, Pseudomonas sp., Ochrobactrum anthropi, Citrobacter freundii, Acinetobacter sp., and Roseomonas sp.) and Gram-positive bacteria (Staphylococcus sp. and Staphylococcus aureus). Antiyeast activity was assessed against Candida boidinii and Wickerhamomyces anomalus. The results demonstrated notable antimicrobial activity across several tested microorganisms, which can be attributed, at least in part, to the use of natural deep eutectic solvents (HNaDESs). These solvents likely enhanced the extraction efficiency of bioactive compounds and contributed to the overall antimicrobial potential of the extracts. The findings highlight the promising role of HNaDES-based extraction systems in the development of sustainable and effective natural antimicrobial agents. Further studies are required to evaluate their incorporation into delivery systems, and their potential applications in food matrices, agriculture, and cosmetics.

Keywords : birch bark, propolis, cyclodextrin, HNaDES, antimicrobial activity

Impact of biological agents and biopesticides derived from tomato by products on human health and environmental risk assessment

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In recent years, research has increasingly focused on reducing chemical inputs in agriculture and valorizing agro-industrial by-products. Tomato processing residues represent a promising source of beneficial microorganisms (biological control agents; BCAs) and bioactive compounds. These biological agents can promote plant growth, exert antimicrobial activity, and enhance the shelf life of agricultural products, while plant-derived extracts may function as growth promoters and biopesticides.This study aims to isolate and evaluate biological and chemical agents derived from tomato by-products, with the goal of developing formulations to control plant pathogens and pests, thereby reducing reliance on synthemtic pesticides. Their potential impacts of these agents on human health and the environment will also be assessed, including ecotoxicological effects on non-target organisms.This work is part of the SALSA project ("Sustainable applications of leftover tomato as agroecological solution to improve crop protection in agriculture"), which seeks to develop bio-based solutions for plant protection, foster collaboration among farmers, researchers, and industry, and support the transition toward more sustainable and resilient agricultural systems.

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Keywords:Tomato by-products, biological agents, biopesticides, human health,environmental risk assessment

Exogenous application of dsRNA induces soil-dependent changes on fungal but not on bacterial and protists communities

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Double-stranded RNA (dsRNA)-based RNA interference (RNAi) is a promising, sequence-specific approach for targeted control of pests and pathogens with expected minimal off-target effects. However, the persistence of exogenously applied dsRNA in soil has not been studied sufficiently as yet, while its impacts on the soil microbiota remains unknown. We evaluated the effect of a 297 bp dsRNA targeting the *Leptinotarsa decemlineata* actin gene on the soil microbiota. Specifically, dsRNA was applied either naked or formulated with chitosan at three concentrations (0, 240, 2400 ng g^{-1} soil) across three soils with varying pH (acidic 4.0, neutral 6.7, alkaline 8.3). Impacts on soil microbial communities (fungi, bacteria, protists) were assessed over time (0, 7, 28 days post application) using amplicon sequencing. Soil type was the main driver of microbiome structure, explaining 56.8, 25.7 and 49% of the variation of the fungal bacteria and protists communities ($p < 0.001$), followed by time, while dsRNA dose and chitosan showed lower or no impact. Based on this data were analyzed per soil. Responses to dsRNA differed strongly among microbial groups. Fungal communities were the most responsive particularly in the acidic soil, where both time ($p < 0.001$, 17.7%) and dsRNA dose ($p < 0.001$, 13.5%) significantly influenced community composition. Bacterial communities remained largely stable, with no consistent response to dsRNA or formulation. Protist communities exhibited the highest variability, with community structure influenced by a combination of time, dose, and formulation. Overall, our findings suggest that soil is the primary determinant of soil microbiome responses to exogenous dsRNA, while dsRNA itself exert limited effects on soil microbiota. On going research will shed light into the actual mechanism of the negative effects of dsRNA on the abundance of specific soil fungi.

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Keywords: ds RNA, soil microbiota, protist, fungi, bacteria

Microbial metabolites used as biopesticides

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The need to reduce dependence on chemically synthesized pesticides, together with the increasing demands of modern crop protection, has led to the development of various biopesticide-based approaches, including microorganisms, synthetic microbial communities (SynComs), and microbial metabolites. The formulation of these plant protection products remains a major challenge, as the active ingredient(s) may be either the microorganism itself or the microbial metabolites responsible for the biological activity. These products can benefit agricultural crops by promoting plant growth and/or by protecting plants against pests, pathogens, and weeds. Primary and secondary microbial metabolites, including amino acids, carbohydrates, lipids, organic acids, phenolics, peptides, lipopeptides, alkaloids, polyketides, and volatile organic compounds represent important classes of compounds with potential applications in agriculture as biopesticides and/or biostimulants. Most of them exhibit nematicidal, fungicidal, and insecticidal activities and their risk assessment procedure needs to be adapted. Metabolites can be classified based on both their origin and their function with a common distinction made between primary and secondary metabolites. High-performance liquid chromatography (HPLC), combined with different detectors such as UV–Vis, diode array (DAD), or mass spectrometry (MS), has been widely used in metabolomic fingerprinting studies. Untargeted analysis provides a comprehensive approach for exploring microbial metabolomes, while targeted and suspect screening strategies can be applied to identify characteristic patterns, markers, and bioactive compounds that may support efficacy and risk assessment. To this end, the present work aims to present a general analytical workflow based on LC-QTOF-MS for metabolomic fingerprinting and the characterization of bioactive compounds. The proposed approach outlines the main steps of analysis, from data acquisition to processing and interpretation, combining untargeted analysis with target and suspect screening strategies. This conceptual framework highlights the potential of LC-QTOF-MS as a powerful tool for exploring metabolite profiles and supporting biopesticide research and sustainable agricultural practices.

Keywords: Microbial metabolites; metabolomics; efficacy; risk assessment; crop protection

Determination of the background levels of microorganisms used as plant protection products in EU soils using shotgun metagenomics and q-PCR approaches

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Soil microorganisms can support multiple ecological functions and importantly could relief plants from abiotic and biotic stress. Hence soil constitutes a rich pool of microorganisms that have the potential to be used as microbial pesticides in the frame of a continuously evolving pesticide market that is supported by emerging EU policies aiming to support the rapid introduction in the market of innovative microbial products. In this frame it is of out most importance to determine if microbes used as plant protection agents in Europe are present in agricultural soils and what is their background levels. This knowledge will facilitate risk assessment and determine if the release of these microbes in a plant protection context constitute a major ecological threat. In this frame we aimed to determine the background level of two microbial strains, *Paecilomyces lilacinus*, a nematode-trapping fungal strain, and the plant growth-promoting and biopesticide *Bacillus velezensis,* used as model organisms in the frame of the EU project RATION. To achieve this goal we collected 340 soil samples from the LUCAS (Land Use and Coverage Area survey) 2018 initiative, all derived from cropland and processed them for DNA extraction. Two different analytical approaches are employed to determine the background levels of these two microorganisms (i) at strain level, using shotgun metagenomics combined with recruitment analysis using the reference genomes of the specific strains and (ii) at species level using qPCR with primers already validated for their specificity against *P. lilacinus* and *B. velezensis*. We anticipate that this study will provide valuable data that will facilitate the access to the market of safe microbial pesticides. This work is in progress and the results will be presented at the Conference.

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Keywords: Shotgun metagenomics, microbial pesticides, q-PCR, *Bacillus velezensis*, *Paecilomyces lilacinus*

Screening novel winter wheat breeding lines for biological nitrification inhibition using a soil-based phenotyping pipeline

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Nitrate leaching and nitrous oxide emissions from agricultural soils represent major environmental and climate challenges. Biological nitrification inhibition (BNI) offers a promising nature-based solution to reduce these nitrogen losses. The BNI trait has been successfully transferred from *Leymus racemosus* to modern wheat (*Triticum aestivum* L.), creating BNI translocation lines that can facilitate further breeding of the BNI trait in wheat. To advance this breeding effort, Sejet Plant Breeding developed a panel of novel winter wheat lines generated by crossing elite cultivars with BNI translocation lines.

Here, we report the application of a recently developed high-throughput, soil-based BNI phenotyping pipeline that relies on a bioluminescent *Nitrosomonas europaea* bioreporter strain (pHLUX20) to screen a panel of novel winter wheat lines at the seedling stage for their ability to exude biological nitrification inhibitors into rhizosphere soil. The novel wheat lines were generated by crossing elite winter wheat cultivars with BNI translocation lines to bring the BNI trait into winter wheat. Known BNI-wheat (Roelfs) and corresponding non-BNI parental wheat lines to newly generated lines were used as BNI controls.

Results revealed substantial variation in BNI activity among the screened lines, indicating that the trait was not uniformly retained after crossing. Notably, several crossed lines exhibited BNI activity exceeding that of their non-BNI parental lines, suggesting that the BNI trait can successfully be crossed into new wheat cultivars. These findings highlight both the promise of integrating BNI into elite germplasm and the development of a novel, robust soil-based phenotyping tool to guide selection in plant breeding programs.

Session 4

Biodegradation of Pollutants

Emergent interactions shape the effects of metabolites on the assembly of microbial communities

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Microbial communities drive essential processes in natural and industrial settings, from nutrient cycling and host health to wastewater treatment.Environmental compounds are a key lever on these systems: prebiotics can steer communities toward desirable states, while antibiotics and other xenobiotics reshape them in ways we fail to anticipate. Both cases pose the same unsolved problem: we cannot predict how adding a given ompound will restructure a community. Efforts to relate the number of supplied resources to community diversity have yielded conflicting results, with studies reporting increases, decreases, or no change in richness as nutrient complexity grows. We argue this inconsistency arises because taxonomic richness poorly captures functional structure, and because a compound's effect depends on the metabolic context in which it acts. Here we test whether the effect of a chemical on community assembly is predictable across metabolic environments, mapping the chemical composition of an environment to the composition of the community assembling within it. We assembled communities in 84 environments spanning combinations of sugars, organic acids, amino acids, alcohols, and aromatic compounds, quantifying the compositional shift elicited by six focal metabolites across twelve metabolic backgrounds. We find that a metabolite's effect is not idiosyncratic, but varies systematically with the community it is added to: for all six compounds, the magnitude of the compositional shift is a simple, largely linear function of the community state assembled in the background environment alone. A compound's effect in a novel environment is therefore predictable, despite the complexity of the underlying species–species and species– environment interactions. Consumer-resource models show these patterns emerge robustly from the architecture of metabolic networks, particularly species' resource utilization preferences. Preliminary evidence suggests the same emergent patterns govern the effects of pollutants and antibiotics, pointing to general principles. These findings open a path toward both predictive engineering ofcommunities and anticipating the impact of chemical perturbations.

Acknowledgments: This work was supported by the Zero Emission Biotechnology Program at TU Delft.

Keywords: Microbiome; microbial communities; pollutants; antibiotics; metabolites

Unraveling the fate of phenanthrene in a microbe-poplar system: linking the active microbiome to metabolic pathways

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Phytoremediation relies on the collective actions of plants and their associated microbiome, which function as a holobiont. However, identifying the specific microbial groups that actively degrade polycyclic aromatic hydrocarbons (PAHs) remains a significant methodological challenge. In this study, we employed a combination of DNA Stable Isotope Probing (DNA-SIP) and targeted metabolomics to elucidate the fate of 13C-labelled phenanthrene during the growth of Populus x canadensis.

Combining isotopic tracing with high-resolution mass spectrometry revealed that phenanthrene undergoes significant biotransformation after root absorption. A distinct spatial distribution of 13C-metabolites was observed across plant compartments: primary oxidation metabolites accumulated in the roots, while downstream metabolites from subsequent biodegradation steps were translocated to the aerial parts (stems and leaves). This supports the concept of a 'green liver' effect.

Simultaneously, the incorporation of 13C into microbial DNA enabled us to identify active bacterial degraders over a 24-day period. We characterised a clear succession dynamic: primary phenanthrene degraders (e.g. Massilia and Micrococcaceae) dominated the rhizospheric soil and root compartments after eight days, followed by the emergence of late-stage degraders (e.g. Mycobacterium and Sphingomonadaceae) on days 16 and 24. Notably, despite the presence of specific fungal endophytes (e.g. Fusarium and Diaporthe), fungal communities exhibited negligible 13C incorporation. This suggests that if fungi are involved in phenanthrene degradation, it relies on co-metabolism or stress alleviation rather than direct assimilation.

By linking the identity of active degraders to the kinetics of metabolite formation, our approach successfully deciphers functional partitioning within the holobiont. These findings emphasise that the poplar-microbe system is an active and selective driver of bioremediation, rather than merely a passive accumulation sink.

Keywords: DNA-SIP, Phytoremediation, 13C-phenanthrene, Active microbiome, Metabolomics

Surfactants in glyphosate-based herbicides may stimulate glyphosate-degradation in agricultural soil

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Glyphosate-based herbicides (GBHs) are the most widely used herbicides globally.Toincrease efficacy, formulations contain a variety of adjuvants. For example, surfactants are commonly used to improve spread and facilitate uptake, while being generally referred to as 'inert' ingredients. The toxic effect of glyphosate (GLP) itself on bacteria has been demonstrated in multiple studies, yet, little is known about the impact of these additives. Prior research on oil-degrading bacteria and wastewater microbial communities, however, has suggested that dispersants can have a direct impact on key microorganisms. Here, we examined the impact of surfactants often found in GBHs on both isolates and soil microbial communities. Growth experiments were conducted with avariety of surfactants and GBHs on GLP-degrading bacteria, isolated from agricultural soil. Ecotoxic effects of single surfactants only emerged at concentrations far exceeding the realistic pplication rates (≥ 15-30 mg/L), whereas lower concentrations (≤ 15 mg/L) appeared to stimulate GLP degradation. Currently, further conditions are being tested, including the combined application of surfactants. To assess complex soil microbiomes, microcosms were set up under oxic and anoxic conditions, comparing pure GLP and a commercial formulation. Under oxic conditions, GLP was rapidly degraded (> 80% within 3 days), with minimal differences between treatments. Transcriptome data, however, revealed higher expression of GLP/glycine oxidases in the GBH treatment. Contrary to previous assumptions, GLP degradation also occurred anaerobically. In accordance with the previous findings, adjuvants in the GBH stimulated degradation (> 70% in 21 days) compared to pure GLP (< 50% in 21 days). 16S rRNA gene sequencing data suggested that these differences are due to a change in activity,rather than community composition.
Collectively, thesedata indicate that surfactants support GLP degradation, although further research is required to verify these findings both in- and outside of the laboratory.

Keywords: Glyphosate; Surfactants; Pollutants; Microcosm; Metagenomics

Revealing marine plastic degraders using stable isotope tracers

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Plastic biodegradation in natural environment is performed by the microbial biofilm living on its surface (so-called “plastisphere”), whose molecular mechanisms remain a black box. Here, we present the DNA-stable isotope probing (DNA-SIP) coupled with -OMICs techniques as a powerful tool to describe the microorganisms actively involved in plastic biodegradation. We produced two types of ^{13}C-labeled polyhydroxyalcanoates with short chain length (^{13}C-scl-PHA) (Odobel et al., 2025)and medium chain length (^{13}C-mcl-PHA), which were incubated as sole carbon source with natural marine plastispheres. Rapid biodegradation into carbon dioxide (CO_2) was found for scl-PHA, where much longer biodegradation rates were observed in the case of mcl-PHA. DNA-SIP coupled with 16S rRNA metabarcoding identified different active biodegraders according to thepolymer types (mainly *Marinobacter sp.*, Cellvibrionaceae and *Alteromonas sp.* for scl-PHA and *Leptonema sp.* for mcl-PHA). Further analysis of ^{13}C-labeled metagenome- assembled genomes (MAGs) enables us to reconstruct several genomes, some of which revealed numerous copies of depolymerases. This revealed different combinations of depolymerase gene organization, forming homodimers and sometimes fused heterodimers, increasing the biodegradation efficiency of these species and give them a selective advantage in the community. Overall, these results illustrate the potential of the stable isotope tracers to explore the functional reservoir of the plastisphere for discovering new processes involved in the plastic end-of-life in the marine environment.

Funding: ANR-PLASTIMAR (ANR-23-CE34-0011-01) Biotic and abiotic factors driving plastic litter biodegradation in the marine environment

Keywords: Plastisphere, DNA-SIP, metabarcoding, metagenomics

Mapping reciprocal metabolic interactions between the Honeybee Gut Microbiota and an Agrochemical Library

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Commensal bacteria in the animal gut play an indispensable role digesting food and synthesizing beneficial metabolites. Their extensive metabolic repertoire also enables the biotransformation of synthetic compounds including drugs, pollutants, and agrochemicals. Pollinators, such as bees, are particularly exposed to agrochemicals through foraging activity. Therefore, investigating the mechanistic interplay between agrochemicals, bees and their microbiota is crucial to understand the full impact of chronic, sub-lethal exposure that is now common across diverse ecosystems. Limited findings already indicate that the honeybee gut microbiota is both perturbed by certain pesticides, while also biochemically altering others. Whether these microbial biotransformation's are beneficial or harmful to their bee host remains unknown. Unfortunately, the vast array of agrochemicals makes comprehensive experiments documenting interactions with microbes in the bee gut challenging.

We screened reciprocal interactions of chemical toxicity and microbial biotransformation of an agrochemical library against 20 isolate strains and synthetic communities representing the species level diversity of the honeybee gut microbiota. We measured in vitro toxicity of 1054 pesticides, antibiotics, and other agrochemical compounds, finding that 13% of the chemicals inhibit bacterial growth at environmentally relevant concentrations. Amongst the largest chemical classes in our screen, fungicides were substantially more likely to be toxic to the microbiota (6% of interactions) than herbicides or fungicides (1% of interactions). Sequencing microbial communities further revealed evidence of both cross-sensitization and cross- protection to toxic agrochemicals.

In parallel, we found significant biotransformation by at least one strain in 15% of the 656 measurable, non-toxic agrochemicals. Biotransformation was enriched in sulfonylurea and cyclohexanedione oxime herbicides as well as in organothiophosphate insecticides. Well known biotransformation reactions, such as nitroreductase activity, were prevalent. However, we also found numerous instances of unreported degradation and conjugation reactions.

Together, our results highlight the gut importance of the gut microbiota in modulating agrochemical impacts on bees.

Keywords: gut microbiota, pollinators, agrochemical, biotransformation

Microbial Degradative Capacities of *Rhodococcus* sp. as a Strategy to Reduce Pesticide Metamitron Pollution in Agricultural Systems

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Despite ongoing efforts to reduce pesticide inputs, synthetic herbicides and their transformation products remain a major source of environmental contamination. Metamitron, extensively used in sugar beet cultivation, was among the most marketed active substances in Belgium in 2020 (114,700 kg sold). Although its reported field half-life is relatively short, metamitron is rapidly transformed into desamino-metamitron, a metabolite that is substantially more persistent and frequently detected in soils and surface waters, raising concerns about long-term ecotoxicological effects.

In this study, we first sought to identify microorganisms capable of efficiently degrading metamitron under environmental conditions. Enrichment cultures were established from pesticide-exposed matrices, including biobed substrates, using mineral media supplemented with metamitron as the sole carbon and/or energy source. Degradation kinetics were monitored by high-performance liquid chromatography (HPLC), leading to the isolation of fifteen metamitron-degrading strains. Among them, strain 8M, identified as a *Rhodococcus* sp., completely degraded 50 $mg \cdot L^{-1}$ metamitron within 48 hours, representing one of the fastest degradation rates reported to date.

In a second step, transformation products generated by strain 8M were characterized. Metamitron metabolites were structurally identified by ^1H and ^13C nuclear magnetic resonance (NMR), and analytical standards of the main metabolites were produced to confirm their identity and fate. Using these standards, we demonstrated that strain 8M not only transforms metamitron but is also capable of degrading desamino-metamitron, the major persistent metabolite.

Finally, soil microcosm experiments conducted with agricultural soil showed that bioaugmentation with strain 8M significantly enhanced metamitron dissipation compared to non-inoculated controls. Together, these results link microbial degradation kinetics, metabolite identification, and successful soil bioaugmentation, highlighting the potential of strain 8M for applied bioremediation strategies targeting herbicide persistence and associated ecological risks.

Keywords: sustainable agrosystems, pesticides, bioremediation, degrading microorganisms, bioaugmentation

Mobilization of genes encoding potential PFAS-degrading enzymes:positive genetic selection in cyanobacteria and potential for bioremediation

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that disrupt multiple aspects of microbial physiology. In this study, we screened all complete bacterial genomes in the RefSeq database for homologs of 43 candidate PFAS-degrading enzymes. The genetic context of enzymes were screened for mobile genetic elements (MGEs), indicating positive genetic selection. Among the most compelling findings was a conserved fluoroacetate dehalogenase gene in 14 *Microcystis* cyanobacteria genomes, consistently flanked by MGEs and genes involved in stress response, photosynthesis, and toxin-antitoxin systems. All these cyanobacteria were originally isolated from PFAS-polluted surface waters. This genomic architecture suggests an evolutionary adaptation to mitigate PFAS-induced stress—especially photosynthetic inhibition and membrane disruption—rather than for metabolic utilization. The presence of MGEs and insertion sequences further supports positive selection and potential horizontal gene transfer.

Interactions between potential catabolic enzymes in *Microcystis* and PFAS ligands were modelled with state-of-the-art AI- and physics-based simulations. Subsequently, we cultured selected *Microcystis* strains with PFAS and quantified the effect on growth. These findings position *Microcystis* and related cyanobacteria as potential candidates for PFAS bioremediation. Unlike traditional degraders that rely on PFAS as a carbon source, *Microcystis* may degrade PFAS to alleviate physiological stress, offering a novel detoxification-based mechanism. Harnessing such stress-adapted phototrophs could enable the development of environmentally integrated, light-driven bioremediation strategies for PFAS-contaminated aquatic ecosystems.

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Keywords:PFAS, bioremediation, evolution, cyanobacteria

Response of marine bacteria to contaminants of emerging concern: resistance, biodegradation and surfactant-mediated interactions

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Contaminants of Emerging Concern (CECs) are anthropogenic chemicals increasingly detected in aquatic environments due to incomplete removal by conventional treatment processes, raising concerns about ecosystem and human health. While the impacts of CECs on marine eukaryotes are well documented, their interactions with marine bacteria, particularly regarding resistance and biodegradation, remain insufficiently understood. This study investigated the resistance and biodegradation potential of marine bacterial strains isolated from marine sediments, including *Priestia sp.* 35 OD PABA G14, *Rhodococcus* sp. 23 AHTN G14, and *Marinobacter hydrocarbonoclasticus* SP17, toward diverse hydrophobic and hydrophilic CECs such as synthetic musks, UV filters, pesticides, and pharmaceuticals. *Priestia* sp. and *Rhodococcus* sp. showed high resistance to all of the hydrophobic tested CECs even up to 500 $mg.L^{-1}$. Only Ketoprofen was toxic to bacterial cells, particularly to *Rhodococcus* sp. starting at concentration as low as 4 $mg.L^{-1}$. Furthermore, *Priestia* sp. and *Rhodococcus* sp. strains exhibited high biodegradation potential, especially for hydrophobic compounds. A positive relationship between bacterial resistance and biodegradation potential was observed, especially for hydrophobic pollutants. Additionally, the influence of environmentally relevant concentrations of surfactants—SDS, CTAB, and Triton X-100—on biodegradation was evaluated. Surfactant effects were selective and depended on both bacterial strain and contaminant properties. CTAB notably enhanced degradation of galaxolide by SP17 while inhibiting octocrylene degradation by *Priestia* sp, highlighting contrasting strain-specific responses. Distribution analyses revealed hydrophobic compounds were predominantly associated with cell-bound fractions, whereas hydrophilic compounds remained mainly extracellular, influencing their bioavailability and degradation. Overall, these findings emphasize the crucial role of microbial specificity, contaminant properties, and surfactant interactions in shaping marine bacterial biodegradation processes, offering insights for developing improved bioremediation strategies for CEC removal in marine environments.

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Keywords: biodegradation, pollutants toxicity, marine bacteria and surfactants

Unveiling the potential of fungi as bioremediation agents against CECs

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Life on earth depends on freshwater, but this resource is more and more under pressure. Continuous discharge of polluted wastewater can cause significant environmental impacts on surface and groundwater, mainly due to the so-called Contaminants of Emerging Concern (CECs). Europe is taking action, with proposal aiming at the “polluter pays principle”, forcing pharma and cosmetic companies to cover the extra costs to clean urban wastewater. Wastewaters treatment plant, and especially their sludge, are an accumulation point of micropollutants, causing their complex microbial community to adapt. SusWater project aims to study this biodiversity and its powerful enzymatic cascade. The fungal community of the contaminated sludge was studied using liquid enrichment technique, using CECs commonly found in wastewaters treatment plants. Overall, 104 fungi were isolated, mostly belonging to the Ascomycota phylum. Surprisingly Basidiomycetes (9%) and Mucoromycetes (7%) were quite abundant. The isolated fungi were tested for their capabilities to degrade CECs. The removal efficiency was evaluated with a proper HPLC-UV detection method. Fungi isolated from this extreme environment must have developed peculiar metabolic skills and were indeed capable of degrading the target CECs. Notably, the degradation yields were strain dependent, but also CECs dependent. For instance, fungi easily attacked estriol and diclofenac, while fungi were almost ineffective against carbamazepine. Overall, 30% of fungi halved the concentration of one or more CECs with few strains capable of removing more than 70% of the CECs. The two best performing strains were studied in more complex systems, where the transformation of CECs is followed in real wastewaters. Immobilization and free fungi were used, and the optimal solution was scale up to reactors. The fungal treatment was able not only to transform most of the target pollutants but also to improve the water quality from an ecotoxicological point of view.

Keywords: Fungi, CECs, urban wastewaters, ecotoxicology, reactors

PFAS-driven restructuring of sediment fungal microbiota: selecting tolerant fungi for future bioremediation

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Per- and polyfluoroalkyl substances (PFAS) are persistent emerging contaminants that can alter microbial communities and ecosystem functioning, yet their effects on sediment-associated fungal microbiota remain poorly explored. As microorganisms play a central role both as indicators of pollutant impact and as potential mediators of pollutant transformation, fungal communities from PFAS-contaminated environments may provide valuable models to investigate pollutant-microbiota interactions and identify candidates for future bioremediation.

This study aimed to investigate the structure and response of sediment fungal microbiota exposed to PFAS and to select tolerant strains with potential biotechnological relevance. Sediment samples were collected from three sites along a watercourse in the province of Turin, Italy, characterized by moderate PFAS contamination. Fungal diversity was assessed through an integrated culturomics and metabarcoding approach. In parallel, enrichment experiments were performed under three PFAS conditions: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and an equal-weight mixture of perfluorobutanoic acid (PFBA), perfluorobutanesulfonic acid (PFBS), perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA). The enrichment consisted of three consecutive steps with increasing PFAS concentrations, 10, 100 and 200 mg/L, in which PFAS represented the only carbon source supplied to the system. This design imposed a progressive and selective chemical pressure aimed at reshaping the fungal microbiota and favouring PFAS-tolerant taxa.

Direct isolation from sediments yielded approximately 200 fungal isolates, mainly belonging to Ascomycota and evenly distributed across the three sites. After enrichment, only about 20 strains were recovered. Metabarcoding revealed a decline in alpha diversity and a marked restructuring of fungal communities throughout the enrichment procedure, indicating a strong PFAS-driven selection. The selected tolerant fungi will be tested in future degradation assays and characterized through omics-based approaches, contributing to the assessment of PFAS impacts on fungal microbiota and to the development of microbial resources for pollutant biotransformation.

Keywords: PFAS; fungi; biodegradation; fluvial sediments; mycology

From Pollution to Remediation: Tolerance to Multiple Pollutants and Functional Potential of Marine Sediment Fungi

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Results from the MicroLink project revealed that sediments within ports and bays along the eastern Adriatic coast represent highly contaminated marine environments, characterized by elevated concentrations of organotin compounds (TBT), heavy metals (including mercury), and high sediment toxicity as indicated by the Microtox test. These conditions impose strong selective pressure on benthic microorganisms and may promote the development of adapted communities with enhanced tolerance and pollutant transformation potential.

The aim of this study was to isolate and functionally characterize marine sediment fungi in order to assess their bioremediation potential. Sediments were collected from four heavily impacted sites: Marina Mandalina (Šibenik Bay), the Port of Šibenik, Vranjic Bay, and the Port of Split. A total of 51 culturable fungal isolates were obtained and identified by amplification and sequencing of the ITS rDNA region using primers ITS4 and ITS5. In parallel, fungal community structure, including non-culturable taxa, was analyzed using amplicon sequencing of the V7–V8 region of the 18S rRNA gene.

Functional characterization of the isolates included screening for tolerance to multiple pollutants, including eight heavy metals, TBT, the UVB filter octinoxate, and polycyclic aromatic hydrocarbons (fluoranthene and anthracene). Metal-tolerant isolates were further screened for organic acid production. In addition, fungal isolates tolerant to PAHs were evaluated for enzymatic activities relevant to pollutant degradation, specifically laccase and manganese peroxidase.

The results indicate the presence of highly adapted fungal communities exhibiting pronounced tolerance to diverse environmental stressors and expressing functional traits associated with pollutant transformation. These findings provide a foundation for selecting promising fungal candidates and support their potential application in marine sediment bioremediation strategies.

Keywords: marine fungi, sediment pollution, bioremediation potential

From Pollution to Solution: The Hunt for Nature's Dehalogenases

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Many organohalogenated compounds (OHCs) have been synthesized during the last century to answer human health, industrial or agronomical needs. Their widespread use and uncareful disposal near inhabited area and water supply leads to severe environmental contamination and high human exposure. These acute/chronic exposures, even at low concentrations, have been linked to several health issues in both wildlife and humans. OHCs' stability made a great argument in favour of their use in different applications such as pharmaceuticals, pesticides or waterproofing agents but nowadays represents the main problem in their ecological impacts.
In order to degrade these pollutants, one approach is to find new enzymes, called dehalogenases, able to catalyse the hydrolyzation of the carbon-halogen bond and in this way, produce more bioavailable and *in fine* less toxic degradation products. However, new enzyme screening tools are needed, as known dehalogenases remain ineffective against many OHCs. Moreover, classical approaches such as genome mining mainly identify similar enzymes with co nserved activities, limiting the discovery of novel functions. To overcome these limits, an original fluorescent based screening method has been developed, allowing a quick and easy detection of dehalogenation activities. This method is based on a change of fluorescence induced by the dehalogenation in a coumarin probe. In this study, with the goal of identifying new microorganisms and enzymes for the degradation of OHCs, microbial samples from chlordecone and PFAS contaminated areas have been isolated using soluble halogenated carbon source to mimic the presence of pollutant. Combining innovative fluorescent screening for dehalogenase detection with genome sequencing and analytical monitoring of pollutant degradation, we are now characterizing more than 100 microbial strains and consortia of interest. This talk will describe our cutting-edge approach and how our first results, using our newly synthetized coumarin probes, can bring new tools in microbial remediation and help tackle OHCs pollution.

Keywords: dehalogenase, bioremediation, chlordecone, PFAS, fluorescent screening

Isolation and characterization of a *Piscinibacter* sp. bacterium able to degrade the nitrification inhibitor DMPP

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Nitrification inhibitors (NIs) such as 3,4-dimethylpyrazole phosphate (DMPP) are widely used in agriculture to retard the microbial conversion of ammonium to nitrate, improving nitrogen use efficiency. Although DMPP's half-life is highly variable and affected by factors such as temperature, humidity, clay content, and organic matter, it is generally considered non-persistent in soil at recommended application rates. However, to date, no microorganism capable of biodegrading it has been reported.

In the present work, different soils were used as potential sources of microbial degraders. To stimulate the growth of microorganisms able to degrade DMPP, an enrichment approach was followed, involving long-term, cyclic application of the NI in soil, followed by transfer to liquid cultures using different growth media inoculated with the treated soils. Degradation of DMPP (2.5 μg mL^{-1}) occurred only in liquid cultures of the minimal medium MSM+N inoculated with soil collected froma rice field in Italy with no prior exposure to NIs. DMPP was almost completely depleted from the medium within 35 days. After plate-spreading, colony selection and degradation testing, a pure bacterial culture showing accelerated degradation (almost complete depletion within 7 days) of DMPP was derived. Amplification and sequencing of the full length 16S rRNA gene revealed the highest match (98%) to *Piscinibacter caeni* strain MQ-18. Whole-genome sequencing is underway to elucidate the degradation mechanisms, while additional experiments are on-going to further characterize the degrading ability (i.e. effects of DMPP concentration, medium pH and growth temperature).This is the first report of isolation of soil microbes degrading Nis like DMPP.

Acknowledgements: This project was supported under the "3rd Call for H.F.R.I. Research Projects to support Post-Doctoral Researchers"(#7840: "FRIDA: From Inhibition to aDAptation: Exploring the interplay between nitrification inhibitors and the soil microbiome towards a sustainable agriculture").
Keywords: Nitrification inhibitors, DMPP, biodegradation, Piscinibacter

Identification of bacteria and metabolic genes involved in lindane degradation using DNA-SIP and metagenomics

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Soils contaminated with hexachlorocyclohexane and its γ-isomer lindane represent a major environmental issue due to the persistence, bioaccumulation, and toxicity of these organochlorine pesticides. Microbial bioremediation is considered a promising remediation strategy and constitutes the main objective of the MIBIREM project. Although several lindane-degrading bacteria have been identified, the active degraders within environmental communities and the catabolic pathways involved remain poorly characterized.

This study aimed to combine DNA stable isotope probing (DNA-SIP), metabarcoding, and metagenomics to identify active lindane-degrading bacteria in contaminated soils and link their genomic potential to in situ activity. Two contaminated soils, from Sabiñánigo (Spain) and Bitterfeld (Germany), were incubated in triplicate microcosms containing 10 ppm of ^{13}C-labeled lindane for 10, 20, or 30 days. Parallel controls with unlabeled lindane were included. Independent microcosms were harvested at each time point.

Biodegradation kinetics were monitored by GC-MS TOF analysis of ^{13}C-lindane and its labeled degradation metabolites. Genomic DNA was extracted and separated by ultracentrifugation in isopycnic CsCl gradients. Although isotope labeling of DNA remained relatively weak, qPCR quantification of 16S rRNA genes enabled the identification of ^{13}C-enriched DNA fractions, particularly after 30 days of incubation. Metabarcoding of 16S rRNA genes was performed on four fractions from each replicate and soil. Comparative analyses between labeled and unlabeled treatments identified bacterial taxa significantly enriched in heavy fractions. In the Sabiñánigo soil, one ASVs affiliated with *Sphingobium* was particularly abundant. Shotgun metagenomic sequencing was subsequently performed on ^{13}C-enriched DNA fractions following whole-genome amplification. Metabolic genes involved in lindane degradation were investigated in the Sphingobium MAGs.

Keywords: lindane, soil, DNA-SIP, metagenomic, biodegradation pathway

Genome-based analysis of *Bacillus velezensis* Bm3 isolated from a vulnerable dioxin-contaminated site in central Syria reveals mechanisms of dioxin degradation

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Dioxin contamination represents a serious global environmental threat, particularly in developing and climate-vulnerable regions. In a previous study, we isolated and characterized several bacterial strains from a climate-vulnerable, dioxin-contaminated site in central Syria, a region characterized by extreme drought and increasing temperatures. Among these isolates, *Bacillus* sp. Bm3 exhibited remarkable aerobic biodegradation activity toward 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), underscoring its potential as an effective bioremediation agent in dry and warm contaminated environments.

To further elucidate its TCDD-degrading capacity, we report here the whole-genome sequence of strain Bm3, phylogenetically identified as *Bacillus velezensis*. Genomic analyses reveal substantial genetic and functional potential for TCDD degradation, supporting the conclusion that *B. velezensis* Bm3 represents a promising candidate for the bioremediation of TCDD-polluted sites in arid and climate-vulnerable regions.

This study evaluates the potential of *Bacillus velezensis* Bm3, isolated from a dioxin-contaminated site in central Syria, for the aerobic biodegradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Quantitative analyses demonstrated a strong biodegradative capacity, with 71% and 76% of the initial TCDD concentration removed after 5 weeks. Whole-genome sequencing revealed a 5.29 Mbp genome encoding 5,411 predicted proteins, providing a solid genetic basis for this activity. A two-step degradation pathway is proposed, initiated by cytochrome P450–mediated hydroxylation of TCDD to dihydroxy-hydrodibenzo-p-dioxin, followed by conversion to the key intermediate catechol. This compound is subsequently cleaved by catechol 2,3-dioxygenase, yielding acetaldehyde and pyruvate. Gene expression and protein accumulation analyses supported this mechanism, showing strong induction of a specific cytochrome P450 isoform (CYPBM3_v1_ua3131) and cat-2,3-diox transcripts. These findings identify *B. velezensis* Bm3 as a promising candidate for TCDD bioremediation in warm, dry environments.

Keywords:Dioxin, TCDD, biodegradation, *Bacillus velezensis* Bm3, Whole genome

Bacterial defluorination from fluoroacetate to short-chain PFAS

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Microbial degradation of per- and polyfluoroalkyl substances (PFAS) represents a potential strategy for remediation of these persistent and toxic environmental contaminants [1]. Short-chain PFAS (C<7) are particularly challenging to remediate due to their high mobility [2].

Incubation of PFAS-contaminated environmental samples with fluorinated compounds yielded cultivable microorganisms with defluorinase activity toward fluoroacetate. Ongoing analyses of bacterial diversity and community composition aim to evaluate the effects of PFAS amendments and to identify taxa associated with defluorination, as well as potential taxonomic bioindicators of PFAS exposure.

The fluoroacetate-degrading strain *Caballeronia* sp. S22 [3] was investigated in parallel. Its genome encodes a gene cluster putatively involved in fluorinated compound metabolism [4], harbours several previously uncharacterised defluorinases, and shows high resistance to fluoroacetate and fluoride.

Adaptive laboratory evolution experiments involving prolonged exposure to fluoroacetate, 2-fluoropropionate, and difluoroacetate yielded stable variants of strain S22 with higher defluorination activity and an extended range of short-chain fluorinated substrates, including some that can be used as sole carbon sources for growth. Ongoing transcriptomic analyses aim to investigate how these phenotypes relate to substrate-dependent gene expression and to identify associated regulatory and metabolic pathways.

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Keywords: PFAS; Biodegradation; Defluorination; Defluorinases

Mechanism-First AI Framework for Discovering FAcDs for PFAS Bioremediation

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants due to the exceptional stability of the carbon–fluorine (C–F) bond. Microbial fluoroacetate dehalogenases (FAcDs) provide a potential biocatalytic route for PFAS transformation; however, naturally occurring variants are largely limited to simple monofluorinated substrates. Understanding the mechanistic limitations underlying this substrate specificity remains a key barrier to advancing PFAS bioremediation. Here, we present a multi-tiered "Mechanism-First" computational framework to identify and evaluate FAcD variants with potential activity toward highly fluorinated compounds. We integrate the Boltz-2 biomolecular foundation model with docking, molecular dynamics simulations, and hydration thermodynamics analysis to systematically assess enzyme–ligand compatibility across 27 major perfluorinated pollutants. Candidate complexes were evaluated based on mechanistic and structural criteria, including catalytic geometry, active-site interactions, and stability. Top-ranked variants were further validated through long-timescale simulations and binding free-energy estimation, while WaterMap analysis revealed that the displacement of high-energy active-site water molecules is a key thermodynamic driver of substrate binding. This workflow yields a data-driven shortlist of FAcD candidates with predicted activity toward diverse PFAS structures. Ongoing experimental validation aims to confirm degradation potential and refine mechanistic insights. More broadly, this approach provides a generalisable framework for mechanism-guided discovery of next-generation enzymes for environmental bioremediation.

Keywords: Perfluoroalkyl substances; Fluoroacetate dehalogenase (FAcD); Microbial biodegradation; Mechanism-guided discovery; Molecular Dynamics Simulation.

Rhizosphere microbiome engineering and root exudate metabolites as strategies to enhance the bioremediation of petroleum hydrocarbons in polluted soils

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Nature-based solutions for historically polluted sites remediation include the enhancement of the degradative potential selected in the soil microbiome over time. Biostimulation of degradative microorganisms can be achieved by adding specific compounds directly to the soil or it can be mediated by plants, that act synergistically with the soil microbiome in rhizoremediation approaches, often prompted by the addition of selected microorganisms.

Here, sunflower was employed alone or supplemented by degradative strains to optimize petroleum hydrocarbon (PHC) remediation of an industrial polluted soil. The dynamics of the rhizosphere microbiome and the abundance of PHC catabolic genes were monitored on the soil metagenome, while the efficacy of microbial inoculation in boosting PHC removal was chemically assessed. Moreover, leveraging untargeted metabolomic data, the role of specific root exudate metabolites as possible biostimulants of PHC degradation was evaluated *in vitro* providing them to the microbial cells detached from the target soil.

In soil, we observed that sunflower recruited a rhizosphere community diverse from that evolving over time in unplanted microcosms. Moreover, bioaugmentation with degrading strains further steered the structure of the rhizosphere microbiome, increasing the abundance of catabolic genes and the removal of PCHs compared to non-inoculated plants. *In vitro*, soil-detached microbiome showed improved PHC degradation when supplemented by the tested metabolites, suggesting that these plant-derived molecules trigger degradation pathways of the native bacterial populations naturally selected in the contaminated soil.

Our findings demonstrate that the understanding of ecological interactions in the rhizosphere, mediated by invading degrading strains and by plant via root exudation is essential for optimizing rhizoremediation interventions.

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Keywords: rhizoremediation; biostimulation; plant-microbe interactions

Adaptive Laboratory Evolution (ALE) towards microbial degradation of polyethylene terephthalate (PET)

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Polyethylene terephthalate (PET) is one of the most widely used plastics and is highly resistant to biodegradation due to its aromatic structure and physicochemical properties. Developing efficient biological strategies for PET degradation remains a key challenge in environmental biotechnology. This study explores adaptive laboratory evolution (ALE) as a non-genetic approach to enhance microbial PET degradation.

Environmental bacterial and yeast isolates from Arctic soil were subjected to long-term cultivation with PET as the primary carbon source. Repeated passaging under selective pressure promoted microbial adaptation to this recalcitrant substrate.

Biodegradation performance was assessed by comparing parental and evolved strains using growth assays on PET-derived monomers. Structural changes in PET surfaces were analyzed using scanning electron microscopy, and degradation products were quantified by HPLC.

The findings demonstrate that ALE can improve microbial interactions with resistant plastics and represents a promising strategy for sustainable plastic waste management.

This work was supported by the Wrocław University of Environmental and Life Sciences (Poland) as part of the research project no N060/0004/25.

Keywords: Adaptive Laboratory Evolution (ALE), polyethylene terephthalate (PET),biodegradation, microplastics, environmental microbiology

Improvement of polycaprolactone (PCL) microbial degradation by Adaptive Laboratory Evolution (ALE)

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Plastic pollution remains a major environmental challenge, even in the case of biodegradable polymers such as polycaprolactone (PCL), whose degradation efficiency is often limited by microbial capabilities. This study investigates adaptive laboratory evolution (ALE) as a strategy to enhance microbial degradation of PCL.

Bacterial and yeast isolates originating from Arctic soil were subjected to ALE using PCL as the sole carbon source. Serial passaging under increasing selective pressure enabled the selection of adapted strains with improved metabolic performance, capable of growth in PCL supplemented minimal medium.

The biodegradation potential of evolved strains was compared with parental strains using qualitative and quantitative assays, including spot tests on PCL-containing media, biofilm formation, and release of monomers measured by HPLC. Enzymatic activity was evaluated through esterase assays and hydrolysis of PCL emulsions.

The results indicate that ALE is an effective, non-genetic approach for improving microbial degradation of biodegradable plastics and provides a useful framework for studying polyester biodegradation mechanisms.

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Bacterial Species of a Waste Dumpsite exhibit Heavy Metal Bioremediation Proficiency

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Heavy metal pollution has significantly impacted on the environment with poor waste management systems. Toxic metals such as Lead (Pb), Cadmium (Cd) and Arsenic (As) pose long-term ecological, human and public health risks. This study investigated responses of bacterial species isolated from a solid waste dumpsite in Ibadan, Nigeria to heavy metal exposures. Using conventional methods, four bacterial isolates were identified - Bacillus cereus (BC), Bacillus subtilis (BS), Staphylococcus aureus (SA) and Staphylococcus epidermidis (SE) and exposed to Lead (Pb) (0, 100, 200 and 600 mg-1L), Cadmium (Cd) (0, 25, 50 and 100 mg-1L) and Arsenic (As) (0, 125, 250 and 500 mg-1L) concentrations. Growth rates and residual metal concentrations were monitored at 35° C of incubation and determined at two days interval for 10 days using UV spectrophotometer at 560 nm and Atomic Absorption Spectrophotometer respectively. On exposure to all As, Cd and Pb concentrations, Bacillus cereus and Staphylococcus aureus exhibited distinct growth P>0.05 comparable to their controls while Bacillus subtilis and Staphylococcus epidermidis revealed similar trend to Pb, Cd and As except As at 500 mg-1L and Cd at 100 mg-1L. With heavy metal remediation potentials, on exposure to the highest concentrations of Pb (600 mg-1L), Cd (100 mg-1L) and As (125 mg-1L), all 4 bacterial species demonstrated remarkably high remediation efficiency. Findings revealed remediation percentage ranges between 98.29 to 99.35 % for Pb, 93.24 to 99.76 % for Cd and 95.27 to 98.87% for As, with remediation proficiency order of Pb>Cd>As. Furthermore, higher remediation percentages were recorded on the 10th day compared to the 6th day of incubation. Overall, the heavy metal remediation performance of the species was: BS>SE>SA>BC. This study highlights the robust and inherent traits of these indigenous bacterial species from waste dumpsites for eco-friendly bioremediation and restoration of heavy metal-contaminated environments.

Keywords: Bacteria; Eco-friendly; Heavy metals; Remediation proficiency; Solid waste dumpsite

Biodegradation of Atrazine : Insights from Consortia and Isolated Strains in Full-Scale wastewater treatment plants

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Atrazine (ATZ) is a persistent herbicide frequently detected in ground and surface waters. Despite its ban in the European Union, its environmental persistence and classification as an endocrine disruptor necessitate effective remediation strategies. Microbial degradation offers a sustainable approach for ATZ removal. In our study, we hypothesized that utilizing microbial sources with inherent metabolic versatility such as activated sludge from waste water treatment plants (WWTP) would provide a broader genetic reservoir for potential ATZ degradation. To test this, a consortia enrichment approach was carried out using activated sludge from WWTP. Enrichments were conducted under divergent selective pressures (1 ppm and 1000ppm ATZ) to select for high-tolerance and potentially degrading communities across these different concentrations. Additionally, media were prepared with and without supplementary carbon (peptone/glucose) to select for consortia capable of ATZ degradation at low concentrations and as a sole carbon source, respectively. Regardless of the WWTP source, distinct variations in microbial community composition were observed, driven primarily by ATZ concentration and supplementary carbon source availability. Through successive enrichment cycles, eighteen distinct consortia were obtained. However, the degradative activity was concentration dependent. Only consortia enriched at 1000 ppm demonstrated degradative activity, with six consortia degrading 1000 ppm ATZ at 17-70% rate. Conversely, consortia enriched at 1 ppm showed no detectable degradative activity on 1ppm ATZ. Subsequently, five strains were isolated exclusively from the ATZ-degrading enrichments that lacked asupplementary carbon source. These isolates degraded ATZ (1 ppm) at rates of 16-30%, with the transformation product 2-hydroxyatrazine identified during the process. These findings demonstrate that a consortia enrichment strategy utilizing varied selective pressures effectively unlocks functional genetic reservoirs essential for obtaining both consortia and isolates capable of ATZ degradation.

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Keywords: Atrazine, biodegradation, microbial communities

Bioaugmentation with a synthetic community mitigates petroleum hydrocarbon- induced stress in sunflower by shaping the rhizosphere microbiome

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Rhizoremediation exploits plant–microbe interactions to clean-up petroleum hydrocarbons (PHCs) pollution in soil. Here, we investigated a bioaugmentation strategy with a synthetic community (SynCom) in sunflower for the rhizoremediation of a historically PHC-polluted soil. Through a culturomics approach, a bacterial collection was established by exploiting the capacity of sunflower root system to select potential hydrocarbon-degrading and plant-growth- promoting rhizobacteria to withstand PHC phytotoxicity in contaminated soil. Based on a screening for beneficial traits, a four-member SynCom was assembled consisting of three *Pseudomonas* strains and a *Stenothrophomonas* one. In a preliminary trial, SynCom seed coating significantly improved sunflower germination and increased seedling biomass in polluted soil. This beneficial effect was further confirmed in a 21- days pot experiment under outdoor conditions, where SynCom-inoculated plants showed a 43% higher biomass compared to untreated control plants. However, chemical analysis on soil did not display a significant reduction in HC concentration. Accordingly, qPCR evaluating the abundance of genes involved in PHC degradation did not show variations upon the SynCom treatment. Considering these findings, 16S rRNA amplicon sequencing was performed and beta-diversity analysis highlighted significant differences in the structure of rhizosphere bacterial populations in SynCom-inoculated compared to control plants. In SynCom-treated plants, differential abundance analysis showed an increase of bacterial families known to be enriched in PHC polluted soil like *Immundisolibacteraceae*, of well described plant growth promoting *Pseudomonadaceae* and in *Opitutaceae*, potentially acting as stress-mitigating bacteria. These patterns suggest that SynCom inoculation may modulate rhizosphere microbial assembly to favour plant fitness, supporting its potential role in mitigating PHCs stress in sunflower plants.

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Keywords: Petroleum hydrocarbons; rhizoremediation; SynCom; bioaugmentation

Assessing the potential of dichloromethane degradation in environmental samples through the absolute DNA quantification of two biomarker loci

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Dichloromethane (DCM, CH_2Cl_2) is massively used as a solvent and classified as probably carcinogenic to humans. DCM can be used as a carbon and energy source by phylogenetically diverse Gram- positive and Gram-negative bacterial taxa via the highly sequence conserved DCM-dehalogenase. This enzyme, encoded by dcmA, catalyzes the conversion of DCM into hydrochloric acid and formaldehyde, a metabolite that fuels the methylotrophic metabolism. Alternatively, DCM degradation can be catalyzed under anoxic conditions via a distinct pathway mediated by methyltransferases encoded by the mec genecluster. This makes dcmA and the mecgene cluster relevant functional markers for assessing DCM degradation potential in environmental samples. The primary objective of this work is to develop a strategy to assess the abundance of dcmA and mec in environmental DNA (eDNA). To enable absolute quantification of dcmA and mec copy numbers, a method based on droplet digital PCR (ddPCR) was developed. This analytical method, offering higher sensitivity and precision compared to quantitative PCR (qPCR), is particularly well-suited for complex environmental matrices due to its robustness to inhibitors typically found in eDNA samples. After optimizing ddPCR conditions for these two loci, the limit of blank, limit of quantification, and dynamic range were experimentally determined. Absolute quantification was then performed on eDNA from various environmental microcosms inoculated with marine sediments, activated sludge or a multi-contaminated aquifer.This work provides a methodological framework for precise assessment of DCM degradation potential in environmental samples. It paves the way for future field studies, aimed at quantifying the abundance and dynamics of DCM degradation potential, ultimately supporting more effective bioremediation efforts.

Keywords:Bioremediation; Dichloromethane; Methylotrophy; Microbial ecology; Pollution

Novel HRMS Approaches for Identifying Transformation Products of the Antiparasitic Toltrazuril, Combined with Predictive Tools to Assess Their Environmental Fate.

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Toltrazuril is a widely used veterinary anticoccidial drug whose environmental transformation and fate remain insufficiently characterized. This study applies advanced high-resolution mass spectrometry (HRMS) workflows combined with in silico prediction tools to comprehensively investigate the formation and identification of toltrazuril transformation products (TPs) in environmental matrices.

A suspect and non-target screening strategy was implemented to detect TPs, integrating HRMS/MS data with computational fragmentation tools, including CFM-ID, SIRIUS, and MZmine, to support structural elucidation. In parallel, transformation pathways were explored using prediction tools to generate candidate TPs, enabling a more targeted and confident annotation process. The combination of experimental and predictive approaches enhanced the coverage of detectable transformation products and improved identification confidence.

The identified TPs were further evaluated using the TyPol classification framework to assess their environmental fate and potential risks based on physicochemical and structural descriptors. This integrative workflow allowed prioritization of transformation products according to persistence, mobility, and potential ecotoxicological relevance.

The study highlights the added value of combining HRMS-based non-target screening with predictive modeling and classification tools for a more comprehensive assessment of Contaminant transformation in environmental settings Overall, this work contributes to the development of advanced methodologies for the identification and prioritization of transformation products, supporting improved environmental risk assessment of veterinary pharmaceuticals. Acknowledgments & Funding: This research was supported by the European Union, in the context of the Pharm-ERA doctoral network. The authors acknowledge Universita Catolica del Sacro Cuore and AEIFORIA SRL for providing analytical facilities and technical support.

Keywords: Toltrazuril, Transformation products, HRMS non-target screening, Environmental fate

Bioremediation of PFOS using bacteria isolated from fiberbank sediments

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Perfluorooctane sulfonate (PFOS), a highly persistent compound within the per- and polyfluoroalkyl substances (PFAS) group, remains a major concern in wastewater treatment due to its resistance to conventional removal and degradation methods. This study investigates the potential for PFOS remediation using bacteria species isolated from fiberbank material on samples derived from a wastewater treatment plant in Östersund, Sweden.

A total of 44 bacterial isolates obtained from two fiberbanks outside the cost of Sundsvall Sweden were screened for their tolerance and potential sorbtion and utilization of PFOS. Each isolate was cultivated in minimal salt media supplemented with 10 mg L^{-1} PFOS, alongside control conditions. Growth experiments were conducted on both agar plates and in liquid broth, where PFOS served as the sole carbon source. In addition to individual cultures, mixed-culture systems were evaluated to assess possible synergistic interactions among isolates. Parallel experiments were also performed under anaerobic conditions to evaluate the influence of oxygen availability on PFOSremoval.

Cultures were incubated over multiple weeks, with samples collected at 7-day intervals. Residual PFOS concentrations in the media were quantified using liquid chromatography–mass spectrometry (LC-TQMS) to monitor potential sorbtion, degradation or transformation of PFOS. Several isolates demonstrated the ability to grow in PFOS-amended media, indicating tolerance, while mixed cultures exhibited enhanced growth compared to individual strains, suggesting cooperative mechanisms. Any reductions in PFOS concentrations may be likely largely due to sorption onto bacterial cells or potential degradation, which requires further investigation.

Ongoing analysis focuses on confirming PFOS concentration reduction in the media and identifying if the change in concentration has its origin from sorbtion, degradation or transformation of PFOS. Metabolic pathways involved in eventual degradation or transformation will also be investigated. These findings support the potential application of native microbial communities in developing sustainable, biological remediation strategies for PFOS-contaminated wastewater systems.

Funding: European Union

Keywords: PFOS, bioremediation, wastewater treatment, bacterial isolates, PFAS

Isolation of PFAS-degrading bacteria and assessment of microbial diversity in contaminated soil in North Italy

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The widespread contamination of soil and groundwater by per- and polyfluoroalkyl substances (PFAS) presents a major environmental challenge due to their persistence and toxicological risks. The high C-F bond strength makes PFAS non-biodegradable in the environment, and despite some in vitro evidence, their biodegradation remains an ongoing challenge, especially on soil. This research aims to address the challenge of PFAS contamination through two main objectives. First, we focus on isolating and identifying microorganisms capable of degrading PFAS from legacy industrial contaminated soil. The second objective investigates the microbial diversity present in soils, sampled from heavily contaminated sites in northern Italy. The first objective involves the application of sequential enrichment approach targeting two PFAS compounds (PFOA and HFBA), followed by molecular and non-molecular microbiological techniques, to identify and characterize microbial strains with biodegradation potential. The second objective of this study was to analyze the soil microbiome from heavily contaminated sites in the Veneto Region (Italy). High throughput 16S amplicon sequencing was employed to characterize microbial diversity, providing insight into the potential for PFAS bioremediation. Following whole genome sequencing, 22 bacterial strains were identified. All genomes were subsequently screened against this database, revealing putative dehalogenase- related genes in every isolate. Despite promising in vitro results, the mechanisms underlying PFAS biodegradation remain an ongoing challenge.

Keywords: PFAS, Soil, Biodegradation, Bioremediation, contamination

From agricultural waste to functional nanomaterials: ecotoxicity and bactericidal activity of green synthesized copper oxide and magnetite nanoparticles using grapevine cane extracts

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The valorization of agricultural waste for the synthesis of nanoparticles represents a sustainable strategy with strong potential in biomedicine, among others. This study evaluates the ecotoxicity and bactericidal activity of copper oxide and magnetite nanoparticles obtained from extracts from four different grapevine cane varieties, an agricultural residue that is usually burned, for the green synthesis of copper oxide and magnetite nanoparticles. The findings provide an integrated perspective on their effectiveness and environmental safety. Different synthesis conditions were compared in order to optimize nanoparticle formation and properties. The resulting nanomaterials were characterized in terms of morphology using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), as well as structural and compositional features by X-ray diffraction (XRD), and zeta potential analysis. In addition, the antimicrobial activity of the synthesized nanoparticles was evaluated, together with a detailed assessment of their environmental toxicity, including their effects on biological model systems relevant to aquatic ecosystems, in order to evaluate their potential ecological risk. The ecotoxicological response showed clear dependence on both synthesis conditions and grapevine variety, indicating that small variations in precursor composition and synthesis parameters can significantly influence biological impact. The results revealed distinct bactericidal activities, along with differences in environmental toxicity, depending on both the synthesis conditions and the grapevine cane variety used for the extracts.

Overall, this comparative approach allowed the selection of the most suitable nanoparticles depending on the intended application. These include microbial elimination and adsorption of pollutants such as antibiotics, heavy metals, and other emerging contaminants. This work demonstrates that grapevine cane residues can be effectively transformed into functional nanomaterials, contributing to waste valorization and pollution mitigation strategies.

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Keywords: Green synthesis; nanoparticles; grapevine residues; copper oxide; magnetite.

Ecological Engineering of herbicide- and antibiotic- degrading microbial consortia using fragmentation and coalescence approaches

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The repeated use of xenobiotic compounds in agroecosystems—such as chemically synthesized pesticides in conventional agriculture and veterinary antibiotics used in livestock farming or introduced through organic soil fertilization—results in their transfer, to surface and groundwater, contaminating both water and sediment compartments. These xenobiotics and their transformation products, resulting from a range of abiotic and biotic transformation and degradation processes, can have adverse effects on the organisms living in these environmental compartments and on the ecosystem functions and services they support. Consequently, the development of bioremediation approaches, based on the use of pesticide and antibiotic biodegradation capacities of bacterial guilds could be an innovative solution to improve the resilience and sustainability of agroecosystems by reducing the persistence of these xenobiotics—pesticides and antibiotics—in agricultural soils and receiving aquatic systems.

Here, we have focused on two xenobiotics used in agricultural practices: a recently banned herbicide that is still found in soil, isoproturon (IPU), and a widely used veterinary antibiotic that is frequently detected in rivers: sulfamethazine (SMZ). We present an innovative ecological engineering approach that does not require the isolation and laboratory cultivation of degrading strains. By combining fragmentation of a soil exposed to IPU and a sediment exposed to SMZ source degrading communities and coalescence of fragmented communities of interest, we built simplified microbial consortia able to degrade both IPU and SMZ. We then evaluated the efficiency of these multidegrading microbial consortia into contaminated soils and sediments, with some consortia demonstrating effectiveness in both environments, as well as their unintended effects on the diversity and structure of native environmental communities.

Our approach provided us a better understanding of both the microbial architecture of xenobiotic degradation and of the process of coalescence between microbial communities. Also, the engineered multidegrading microbial consortia demonstrated better performance in terms of pollutant degradation than isolated degrading strains when tested in environmental conditions

Keywords: Bioremediation ; Microbial Consortia ; Herbicide ; Antibiotics

Identification and large-scale functional characterization of phytochelatin synthases across the fungal kingdom

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Phytochelatins are cysteine-rich peptides synthesized from glutathione by phytochelatin synthases (PCS) and are known to play a central role in heavy metal detoxification, particularly for cadmium. While their function has been extensively characterized in plants, PCS remain poorly described in fungi, where they are often considered rare and poorly conserved.

In this study, we performed a large-scale identification of putative PCS genes across a large set of 2,000 fungal genomes using conserved domain searches and sequence homology approaches. A total of 352 candidate PCS genes were identified and subjected to phylogenetic analysis, revealing a broad but heterogeneous distribution across fungal lineages.

To assess their functional relevance, a selection of 50 representative PCS genes from major phylogenetic clusters were cloned and expressed in *Saccharomyces cerevisiae*. Complementation assays in yeast indicate that several fungal PCS confer increased tolerance to heavy metals, including cadmium and copper, although functional efficiency varies between candidates.

Overall, our results suggest that functional PCS are more widespread in the fungal kingdom than currently appreciated. This work highlights both the evolutionary diversity and the functional variability of fungal PCS and provides a framework for linking phylogenetic diversity to detoxification potential, with implications for fungal adaptation to metal-contaminated environments and their potential use in bioremediation.

Keywords: Phytochelatin synthase, Fungi, Functional complementation, Metal tolerance

Bioaugmentation using 2,4D-degrading bacterial biocomposites: Efficiency, stability and unintended ecological impacts

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Bioaugmentation through the inoculation of pesticide-degrading microorganisms is a promising strategy for the bioremediation of pesticide-contaminated soils. However, the performance of such approaches still needs to be improved, particularly regarding inoculant efficiency and stability. To overcome these limitations, the use of carrier materials to formulate pesticide-degrading biocomposites appears to be a relevant approach to promote the establishment of microbial inoculants in soil. Here, we developed two biocomposites composed of biofilms of *Cupriavidus necator* JMP134 strain grown on two carrier materials: a raw zeolite and a zeolite modified with a layered double hydroxide (LDH). Our hypothesis was that the use of these carriers, particularly the modified one, would improve the stability and efficiency of the degrading strain.

A microcosm experiment was conducted to determine the capacity of these biocomposites, compared with free-living cell inoculation, to mineralize ^{14}C-labelled 2,4-D in three soils with contrasting physicochemical properties, and to evaluate the potential unintended impacts of both inoculation methods on native soil bacterial communities through 16S rDNA amplicon sequencing. We show that inoculation with *Cupriavidus necator* JMP134, either as free-living cells or as biocomposites, increases the mineralization of 2,4-D in soils that had not been previously exposed to this herbicide. This effect, independent of the carrier material, persisted for up to 15 or 55 days after inoculation, depending on the soil type. However, in pre-exposed soils, the effect of the inoculation is less pronounced, likely due to prior stimulation of the indigenous degrading microbial community. The assessment of the unintended effects of the biocomposites revealed changes in the bacterial community structure and a decrease in bacterial diversity, mainly related to inoculation, with no significant effect of the carrier material. Altogether, our results from the ANR-funded EPURSOL project provide a comprehensive assessment of the efficacy and ecological impacts of 2,4-D- degrading biocomposites in soils.

Keywords: Bioremediation; Pesticide; Biocomposites; Microbiome

Exploring bacterial *N*-acetylation of harmful halogenated anilines:Implications for ecotoxicology and environmental health

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In this study, a series of in-house-synthesized halogenated acetanilides (4-chloro-, 4-bromo-, 4-iodo- and 3,4-dichloroacetanilide) were used as analytical standards to identify microbial metabolites of the corresponding halogenated anilines (4-chloro-, 4-bromo-, 4-iodo- and 3,4-dichloroaniline) encountered as toxic byproducts of industry and farming that pollute the environment. Specifically, the aim of the study was to investigate bacterial *N*-acetylation as a potentially useful detoxification pathway for removal of those harmful compounds from contaminated sites. Ten representative environmental bacterial strains were incubated with each one of the aforementioned halogenated anilines, followed by removal of the microbial biomass from the culture medium and extraction of its organic content for liquid chromatography-tandem mass spectrometry (LC-MS/MS), in order to identify and quantitate possible acetylated products. The analysis detected acetylated metabolites in all relevant samples, confirming *N*-acetylation as a likely common microbial biotransformation route of potential utility in environmental protection, bioremediation and environmental health. These findings add to our knowledge about the metabolic versatility of environmental bacteria and their potential relevance to ecotoxicology and biotechnology.

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Keywords: Environmental bacteria; detoxification; halogenated anilines; synthesis of halogenated acetanilides; liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Detoxification of Effluent in Hybrid Nano-Innovative NBS Solutions

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Hybrid nature based solutions (NBS), specifically constructed wetlands (CWs) combined with various enhancement strategies, are getting increasingly recognized as a sustainable due to low energy costs requirements and nutrient removal efficiency (Waly et al., 2022; Wang et al., 2022; Fernández Ramírez et al., 2023). These systems are used as advanced NBS since they combine ecological engineering with innovative technological enhancement strategies (Fernández Ramírez et al., 2023). This study is based on a hybrid CW system that was developed at DUTH for effluent detoxification.Typha was used because of its high nutrient removal efficiency, rhizosphere oxygenation, and microbial degradation encasement (Vymazal, 2022). Caltha palustris was also incorporated to enhance removal efficiency potential. Toxicity assays performed with Daphnia magna resulted in effective effluent detoxification, while phytotoxicity analyses showed the absence of inhibitory effects on plant growth. Algal growth assays demonstrated no induced toxicity. Microbiological analyses showed effective pathogen reduction, confirming the disinfection capabilities of CW systems (Biswal & Balasubramanian, 2022; Waly et al., 2022). Chemical analyses showed effective removal of nutrients and heavy metals. These results demonstrate detoxification performance on all tested traits, which is in line with the fact that hybrid CW as NBS is an effective detoxification ecoengineering approach for advanced wastewater treatment. This work was conducted in the frame of the project, entitled “Integrated Approaches at Local Scale for Enhancing Water Reuse Efficiency and Sustainable Soil Fertilization from Wastewater’s Recovered Nutrients”, acronym ‘CIRQUA”, Grant agreement No. 2321, Call 2023 Section 1 Management of Water IA, which is part of the PRIMA programme supported by the European Union. The PRIMA programme is supported under Horizon 2020, the European Union’s Framework Programme for Research and Innovation. This content reflects only the authors’ view. The PRIMA Foundation is not responsible for any use that may be made of the information it contains.

Keywords: Constructed wetlands, Hybrid NBS, Effluent detoxification, Ecotoxicological assessment, Micropollutant removal

Functional metagenomic discovery and characterization of a pyrethroid-hydrolyzing esterase from a biobed system

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Synthetic pyrethroid insecticides are extensively used in agriculture, but their persistence and ecotoxicity have increased interest in microbial detoxification mechanisms and novel biodegradative enzymes. In this study, we explored a pesticide-exposed on-farm biobed system as a potential reservoir of pyrethroid-hydrolyzing biocatalysts using a functional metagenomic approach. A fosmid library comprising 5,170 clones was screened for esterase activity, yielding 12 positive clones, of which only one demonstrated α-cypermethrin-degrading activity. Transposon mutagenesis, heterologous expression, and biochemical characterization identified the responsible gene, estF6, encoding a BioH-family esterase belonging to the α/β-hydrolase superfamily. Purified EstF6 showed optimal activity at pH 8.0 and 50 °C and displayed a preference for short-chain substrates. The enzyme hydrolyzed the pyrethroids acrinathrin, α-cypermethrin, and deltamethrin with comparable Km values (41-63 μM), while no activity was detected against tau-fluvalinate, bifenthrin, and etofenprox, indicating substrate selectivity associated with structural features of pyrethroid molecules. To our knowledge, EstF6 represents the first functionally characterized BioH-family esterase with demonstrated direct pyrethroid-hydrolyzing activity and the first pyrethroid- hydrolyzing enzyme recovered from a biobed system through functional metagenomics.

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Black poplar root mucilage mediates plant–bacteria communication and contributes to trace metal stabilization in soil

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Trees belonging to the Populus genus (Salicaceae family) are reliable species for phytoremediation, increasing soil fertility and biodiversity, while reducing pollutant availability, especially under metallic stress. One of the mechanisms explaining heavy metal tolerance may involve mucilage production by the extracellular root cap, which can bind various metal trace elements (MTEs), favoring specific plant-microorganisms' interactions. The application of plant beneficial bacteria has been widely studied; not only to increase plant growth and pathogen resistance, but also to mitigate metal toxicity to plants, thanks to their ability to bind or accumulate MTEs and change metal speciation.

Hence, the purpose of this research is to determine the complex interactions occurring among black poplar (Populus nigra), MTEs and microorganisms through the characterization of poplar's purified-root-mucilage and bacterial isolates from the poplar rhizosphere from the Loire Valley (France). In the long term, this research aims to develop phytomanagement programs to reduce dispersion of MTEs from mining residues.

Our results show that MTEs-resistant bacterial isolates, belonging to the genera Exiguobacterium sp. and Acinetobacter sp., can metabolize poplar mucilage and grow at a faster rate than with carbon sources such as glucose or mannitol. These isolates also showed a chemotactic response favoring poplar-rhizobacterial interactions. Furthermore, these isolates demonstrated a bioaugmentation effect as well by allowing better poplar seed germination and growth under metallics stress.

Future research will focus on a deeper characterization of these complex interactions, by evaluating the effects of bacterial inoculation on plant growth and metal accumulation in mesocosms, as well as mucilage effect on bacteria in the presence of MTEs; possibly showing a bacterial-protecting plant role through the mucilage. A better understanding of these ecological interactions will allow us to develop resilient ecosystems mitigating the impacts of MTEs pollution on both human health and biodiversity.

Keywords: Phytomanagement, Bioaugmentation, Plant-bacteria interaction, Mucilage, Metal Trace Elements

Experimental evaluation of predicted PFAS defluorinating enzymes in *Microcystis aeruginosa*

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Per- and polyfluoroalkyl substances (PFAS) are persistant environmental contaminants for which few microbial degradation pathways are known. A recent comparative genomics analysis identified candidate defluorinating enzymes in the cyanobacteria Microcystis, including fluoroacetate dehalogenase (FAcD) homologs. The genomic organization suggests positive selection of these genes, reflecting an adaptive response to alleviate PFAS-driven photosynthesis inhibition. Building on this hypothesis, candidate FAcD genes were heterologously expressed in E. coli and tested for defluorination activity against perfluorooctanic acid (PFOA) and trifluoroacetic acid (TFA). Despite confirmed protein expression, no fluoride release was detected for any of the tested candidate enzymes. Furthermore, growth of Microcystis aeruginosa exposed to 0, 50, 100, and 150 µM perfluorooctanoic acid (PFOA) was monitored, but the expected dose-dependent growth inhibition reported in literature was not observed, and no fluoride release was measured. This suggests either tolerance, limited PFOA uptake or bioavailability under these conditions, or that light was not intense enough to kickstart a significant photosynthesis inhibition. Together, these negative results contrast with the comparative genomics analysis and previous reports of partial PFOA degradation by other cyanobacterial strains. We discuss several possible explanations, including the relatively high resistance of PFOA defluorination compared to smaller PFAS, suboptimal defluorination assay conditions (pH, temperature, incubation time), and the possibility that the tested compounds do not match the substrate range determined by natural selection. We propose that future work should test a broader range of PFAS structures to better align experimental compounds to those possibly encountered by cyanobacteria in contaminated environments, and to optimiz growth to the highest possible light intensity to test inhibition of photosynthesis.

Keywords: PFAS biodegradation, defluorinating enzymes, Microcystis aeruginosa

Nothing is forever: Analogue enrichment on acetate yields a microbial consortium that defluorinates the ultra-short-chain PFAS trifluoroacetate

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Trifluoroacetic acid (TFA) is a perfluoroalkyl (PFAS) pollutant of global significance; it is persistent and ubiquitous in the environment across compartments, including food, drinking water, humans, and animals. Concentrations in the environment are rising, concerns over the toxicity of the compound are increasing, and there are currently no efficient ways to remedy TFA pollution, as conventional methods are ineffective. Microorganisms possessing dehalogenating enzymes targeting the structurally analogous compound monofluoroacetate present a potential solution in the form of microbial TFA degradation. Analogue enrichment has been shown to enrich microorganisms capable of cometabolic degradation of other organic pollutants. This strategy constituted the framework of this project, which aimed at finding microbial TFA degraders through enrichment of acetate metabolizing microorganisms and screening for defluorination of monofluoroacetate. This was done to search for organisms capable of cometabolic degradation of TFA via promiscuous dehalogenase enzymes.

The experiment yielded several cultures which showed significant defluorination of TFA after 30-days. Genetic sequencing and protein-ligand modelling identified two candidate dehalogenase enzymes with capacity to degrade TFA; PAKLIA_03565 from *Achromobacter denitrificans* and KHCNEI_21275 from *Stenotrohpomonas sp.* These findings are a crucial first step towards bioremediation of TFA, and is, to the best of our knowledge, the first study to identify a TFA degrading organism

Keywords: TFA, PFAS, biodegradation, defluorinating enzymes, pollution remediation

Session 5

Microbes - Pollutants under Climate Change Scenarios

Biofilm response to stressors is determined by their thermal and hydrological history

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The ability of microbial communities to cope with disturbances and stressors, may they be chemical, physical or biological, lies in their resistance and resilience properties. Both depend on community composition and physiological state, but also on its history. For several years we have been working on how community history, and notably thermal and hydrological histories, affects the resistance and resilience of phototrophic biofilms. This is a critical question to predict the future of the essential services they provide in aquatic ecosystems exposed to numerous natural and anthropic disturbances. We mainly performed microcosms experiences with biofilms cultivated either from natural inoculum or built with microalgae strains from our collection. Biofilms were grown in contrasted conditions (e.g. high/low mean temperature, stable/fluctuating temperature, with/without a dry period) and then exposed to a disturbance. We considered either only biofilm functional response to a simple stressor (heat, salinity, copper) or biofilm structural and functional response to a complex disturbance (mix of nutrients, pesticides, heavy metal). As can be expected, we showed that thermal history determines natural biofilm tolerance to thermal stress, through both modification of algal physiology and the selection of tolerant species. A higher growth temperature increases thermal stress resistance but also salt stress resistance. Biofilm resistance to copper decreases as the amplitude of daily fluctuations of temperatures increases. Warmer growth conditions increased biofilm sensitivity to a complex contaminant, even after temperature resumed standard conditions. This legacy effect of warming decreased over time, except for the prokaryotic composition of biofilms. As a whole, our results emphasize the importance of considering past conditions when assessing the stability of microbial communities.

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Keywords: legacy effect, photosynthesis, diatoms

Bacterial contribution to nitrogen processing in the atmosphere

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The atmosphere carries diverse and metabolically active microorganisms. While microbial contributions to biogeochemical cycles are well documented in terrestrial and aquatic systems, their role in atmospheric processes remains poorly understood. Nitrogen, a key element of the biosphere, is abundant in the atmosphere in multiple organic and inorganic orms, including dinitrogen (N_2), ammonium (NH_4^+), nitrate (NO_3^-), and nitrogen oxides (NOx), present in both gaseous and aqueous phases (e.g., cloud water). These compounds originate from both natural and anthropogenic sources and may serve as substrates but also stressors for airborne microorganisms and ecosystems. In a context of increasing atmospheric nitrogen pollution, improving our understanding of interactions between atmospheric microorganisms and the nitrogen cycle is essential.

This study investigates potential microbial interactions with nitrogen compounds in the atmosphere, with a focus on inorganic forms (mainly NH + and NO -). Metagenomes and metatranscriptomes of aerosols in clear atmosphere and cloudy air masses at the mountain site of puy de Dôme (1465m asl, France) indicate that glutamate metabolism (ammonium assimilation) and denitrification (in particular nitrite reduction) contributed most (70%) of the microbial sequences of genes and transcripts linked to nitrogen utilization pathways. Other prevalent processes included assimilatory and dissimilatory nitrate reduction, and nitrogen fixation, with the latter being overexpressed in particular during clear atmospheric conditions. Complementary laboratory incubations of rainwater revealed microbial utilization of NH_4^+ and NO_3^-, at rates positively correlated with the relative abundance of Sphingomonadales and negatively with that of Burkholderiales. Overall, these results demonstrate multiple potential microbiological roles inthe processing of inorganic nitrogen in the atmosphere, in relation with atmospheric conditions and microbial diversity. This opens up new perspectives in our understanding of biogeochemical cycles and chemical processing in the atmosphere, as well as microbial Functioning in this major part of the Earth system.

Keywords: Metagenomic – Nitrogen – Atmosphere – Bacteria - Bioassimilation

Combined exposure to heat waves and antibiotics shows interacting effects in community tolerance and molecular responses in stream biofilms

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Aquatic biofilms of freshwater ecosystems are increasingly exposed to multiple anthropogenic stressors, including pharmaceutical pollution and heat waves driven by climate change. Despite growing evidence that antibiotics alter microbial community composition, trigger stress-induced community tolerance (SICT), and select for antibiotic resistance genes (ARGs), yet the combined effects of antibiotic exposure and thermal stress on periphyton structure, functioning, and ARG dynamics remain largely underexplored in ecological risk assessment. Here, we investigated whether heat waves modulate community-level responses to antibiotic stress, and aim to understand the mechanisms behind potential stressor interactions by molecular tools.

We exposed biofilm communities in microcosms to two concentrations of erythromycin (ERY) (5 µg/L and 100 µg/L) for four weeks, including a two-weeks period of combined ERY and heat exposure (+5 °C) simulating projected climate scenarios. After four weeks, we assessed a broad set of structural and functional biofilm descriptors including photosynthesis analysis for tolerance acquisition, pigment quantification, 16S/18S/rbcl amplicon sequencing, antibiotic resistance genes (ARGs) quantification, and meta- metabolome response based on UPLC-HRMS/MS based untargeted approaches.

Long-term exposure to ERY combined with a heatwave induced significant SICT (Stress- induced community tolerance), measured by short-term inhibition of photosynthetic yield. This combined exposure resulted in a higher increase in periphyton tolerance than ERY exposure alone, indicating that thermal stress amplifies antibiotic-induced community-level tolerance.

By linking molecular, biochemical and resistome response traits to physiological and community-level endpoints, our results provide experimental evidence that heat waves and antibiotic pollution synergistically disrupt the structural and functional organization of aquatic microbial communities. Meta-metabolomics analyses will be further used to characterize responsive meta-metabolome to combined stressor exposure and to identify potential mechanisms of stress adaptation.

These findings highlight the importance of multi-stressor frameworks for ecological risk assessment of antibiotics under climate change scenarios.

Keywords: Microcosms; Temperature; Erythromycin; SICT (Stress Induced Community Tolerance); Multi- omics

Freshwater periphyton meta-metabolome unravels environmental drivers of spatial microbial metabolism heterogeneity: a predictive approach

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In a multi-stress context driven by global change and chemical contamination, freshwater microbial periphyton communities are increasingly used in ecotoxicology due to their diversity and their role in key ecosystem functions. Laboratory experiments have clarified mechanistic responses to pollutants, but their findings remain difficult to transpose to real ecosystems for biomonitoring purpose, where multiple environmental drivers act simultaneously. Meta-metabolomics captures the biochemical activity and phenotype of these communities, and, when coupled with machine learning, could enable the identification of key drivers of metametabolome variability across complex environments. This study therefore aims to assess how in situ environmental factors structure the spatial heterogeneity of the periphyton metametabolome through the implementation of a predictive meta- metabolomics framework built and validated over two consecutive sampling years. In the first year, autochthonous periphyton was sampled on rocks from 100 rivers across France, selected to represent a broad gradient of physico-chemical properties and ecological status based on national water agency data. At each site, photosynthetic yield was measured in situ alongside comprehensive water quality parameters, including micropollutants, nutrients, pH, and temperature. Global biomass indicators (e.g., proteins, starch) and LC-HRMS-based untargeted metabolomics analyses were further implemented. In the second year, both periphyton were collected from glass slides after one month cultivation and from rocks at the same sites to validate the predictive framework. The diversity of physico-chemical profiles confirmed the suitability of the sites to capture strong environmental contrasts. Machine learning enabled the identification of several features predictive of environmental parameters, including pesticide concentration. Metabolite and pathway annotation are ongoing to elucidate microbial metabolic adaptations. Overall this study will support biomarker discovery for chemical stress, and define the normal operating range of the periphyton metametabolome for improved biomonitoring.

Keywords: Freshwater microbial communities, Predictive meta-metabolomics, , Chemical stress, Biomonitoring, Spatial heterogeneity

A call to integrate abiotic and biotic modifiers into ecological thresholds for antibiotic resistance selection in aquatic environments

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There is compelling evidence that antibiotic resistance selection occurs at concentrations well below lethal levels. However, widely cited ecological thresholds, such as the minimum selective concentration (MSC) and the predicted no-effect concentration for resistance ($PNEC_R$), are typically treated as fixed values despite being derived from simplified, in vitro systems that do not account for environmental complexity. This review critically examines how such thresholds should be interpreted in aquatic environments and highlights the extent to which abiotic and biotic modifiers influence their expression. Synthesized evidence demonstrates that biofilms, community composition, co-selective agents, and exposure heterogeneity substantially reshape realized selection dynamics. In contrast, the roles of pH, transformation-product bioactivity, and the transferability of thresholds across environmental contexts remain less well constrained. These findings underscore the need to evaluate threshold concepts as context-dependent constructs rather than interchangeable or universal benchmarks. The review argues that while MSC and $PNEC_R$ retain value as precautionary indicators, they are intrinsically bounded by the experimental conditions under which they are derived and should not be directly extrapolated to environmental risk assessment or regulatory decision-making. Instead, a factor-inclusive ecological threshold framework is proposed in which resistance selection thresholds are treated as conditional properties emerging from interactions among community structure, matrix chemistry, and exposure history. Importantly, the same abiotic and biotic modifiers that complicate threshold inference, including hydrodynamics, nutrient availability, temperature, co-pollutants, exposure duration, transformation processes, biodiversity, biofilm formation, and horizontal gene transfer, can also function as intervention points. Advancing a predictive environmental pharmacology of antibiotic resistance selection will require generating ecologically relevant thresholds by integrating abiotic and biotic modifiers into in-vitro experiments, or by conducting in-situ experiments across diverse aquatic environments. This, in turn, demands multi-tiered experimental approaches, systematic reporting of contextual variables, and integrative interpretation of ecological thresholds across scales.

Keywords: antibiotic resistance selection; aquatic environments; environmentally realistic thresholds; context-dependent thresholds; ecological threshold endpoints

Agricultural practices in vineyard drive periphytic biofilm structure and functioning across seasons and sites

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Intensive agriculture exerts strong pressure on aquatic ecosystems, raising concerns about its environmental impacts. Agroecology is often proposed as a less harmful alternative, yet quantitative comparisons of its effects on adjacent aquatic systems remain limited. This study addresses this gap. Periphytic biofilms, microbial communities growing on submerged surfaces, are sensitive indicators of structural and functional biodiversity due to their rapid response to environmental change. We analyzed periphyton from two rivers (Barbanne and Engranne) along a gradient of farming practices (conventional, CONV; intermediate, INT; organic, BIO) across three periods (spring 2024, spring 2025, autumn 2025). We measured structural attributes (algal composition, organic matter) and functional traits (photosynthetic activity, copper tolerance, and microbial activity via untargeted metametabolomics). PERMANOVA identified “River” as the main driver (R^2 = 13.3%), followed by a strong Treatment x Season interaction (15.75%), with Season (10.4%) and Treatment (6.8%) also significant ($p < 0.05$). Multivariate analyses clearly separated BIO from INT and CONV, which were similar. Linear discriminant analysis (LDA) indicated that BIO sites had more cyanobacteria, higher copper tolerance, greater organic matter, and higher chlorophyll a, whereas CONV and INT were dominated by green algae. Over time, algal biomass and copper tolerance declined from spring 2024 to autumn 2025. The Engranne showed higher biomass and photosynthetic efficiency, while the Barbanne had lower biomass but greater copper tolerance. Microbial activity differed between rivers. In spring, the Engranne displayed decreasing terpenoids and increasing polyketides from BIO to CONV, a pattern absent in the Barbanne. In autumn, these differences intensified in the Engranne, with BIO showing higher terpenoids, polyketides, and alkaloids than INT and CONV. No differences were observed in the Barbanne. Overall, periphytic biofilms integrate agricultural practices, seasonality, and local context, confirming their value as bioindicators of viticultural impacts on aquatic ecosystems.

Keywords: Periphyton, Agroecology, Aquatic pollution, Bioindicators, Vineyard.

Characterization of halotolerant bacteria from the Naama- Algerian sabkha: a phylogenetic approach

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Saline ecosystems such as sabkhas harbour unique microbial communities with remarkable adaptive capacities and significant biotechnological potential. This study aimed to characterize the cultivable bacterial community of the Naâma Sabkha, an endorheic saline depression in southwestern Algeria (EC up to 198 mS/cm; pH up to 8.76), and to assess the influence of adjacent urban wastewater discharge on soil microbiota. Soil samples were collected at twelve georeferenced points (0–20 cm depth) between March 2023 and March 2024. Total microbial load ranged from 1.3×10^3 to 3.4×10^4 CFU/g (mean: 1.10×10^4 CFU/g). A Student's t-test revealed a statistically significant difference ($p = 0.02$) between sites proximal and distal to the lagooning station, confirming the ecotoxicological impact of urban effluents on the local microbiota. Seventy bacterial isolates were subjected to physiological tolerance assays (NaCl: 0–20%; pH: 5–11; temperature: 35–45°C). Most isolates were neutrophilic and halotolerant, with optimal growth at pH 7 and 0–5% NaCl, and substantial thermotolerance up to 45°C. Six polyextremotolerant strains tolerating NaCl concentrations up to 20% were selected for polyphasic identification, combining classical biochemistry (API galleries), proteomic profiling (MALDI-TOF MS), and genotypic characterization (16S rRNA gene Sanger sequencing, BLAST, MEGA12 phylogenetic tree). Sequence similarities ranged from 98.52% to 99.79%, exceeding the species-level threshold. Two strains were unambiguously identified: Bacillus cereus (S01; MALDI score 2.10) and Kocuria rhizophila (S25; MALDI score 2.32), with full convergence between molecular and proteomic methods. The remaining strains belonged to the Bacillus cereus/altitudinis complex, illustrating the limitations of single- method identification in closely related taxa. These findings highlight the Naâma Sabkha as a reservoir of extremotolerant bacteria with potential applications in bioremediation and sustainable biotechnology, while underscoring the urgent need for ecotoxicological monitoring of its surrounding urban Environment.

Keywords: Naama Sabkha; halotolerant bacteria; polyphasic identification; MALDI-TOF MS; 16S rRNA; ecotoxicology; bioremediation; southwestern Algeria

Unveiling the microbial filtering capacity of sandy beaches facing climate change and chemical pollution

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Sandy Mediterranean coastlines are vast and critical interfaces between land and sea. Beneath these extensive beaches lies the subterranean estuary (STE), a highly reactive ecotone where fresh groundwater mixes with advancing saltwater. STEs are often overlooked, yet they host complex microbial communities that play a crucial role in biogeochemical cycling and potentially act as natural biological filters for contaminants of emerging concern, such as UV filters. However, this invisible ecosystem service faces unprecedented threats from anthropogenic pollution and climate change, including rising temperatures and shifting salinity. The resilience of these microbial filtering functions under combined stressors remains largely unexplored.

The multidisciplinary MED-GUARD project investigates the capacity of the Mediterranean STE to act as a natural bioreactor, filtering pollutants before they discharge into the sea. By integrating hydrogeology, analytical chemistry, and microbial ecotoxicology, we aim to unravel the complex interactions between groundwater dynamics, chemical gradients, and microbiome functioning.

Our approach combines extensive fieldwork with controlled laboratory experiments. In the field, high-resolution temporal and spatial monitoring allows us to characterize the hydrological, chemical, andmicrobiological diversity of the STE. Concurrently, microcosms experiments simulate future climate change scenarios (increased temperature and shifting salinity gradients) and contaminations by UV- filters to assess their impact on the microbial communities' functioning and depollution potential. By tracking the fate of organic and inorganic contaminants alongside microbial analyses, we evaluate the biological degradation capacities under these emerging stressors.

This presentation will introduce the MED-GUARD framework and our methodology to decipher how microbial consortia adapt to changing physicochemical conditions. Ultimately, evaluating this invisible ecosystem service is critical for predicting coastal aquifer resilience and informing sustainable groundwater management strategies in the Mediterranean basin.

Keywords:Subterranean estuary, climate change, microbial bioremediation, emerging contaminants, multidisciplinary approach

From microbes to droughtre silience: integrating SynComs and climate- risk modelling in agriculture

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Climate change increasingly threatens the sustainability of traditional crop production, highlighting the need for innovative approaches that can preserve locally important agricultural systems under drought conditions. The CLIMAGRIT project addresses drought adaptation through the development of microbiome-based biostimulants integrated with microbial ecology and climate-risk assessment. The project focuses on traditional Croatian crops of regional importance, including Varaždin cabbage, Ludbreg horseradish and pumpkin varieties cultivated in Varaždin County.

Native bacterial and fungal isolates with known plant growth-promoting traits and drought tolerance will be used for AI-assisted design of synthetic microbial consortia (SynComs) aimed at identifying keystone microbial taxa associated with improved stress resilience. Particular attention will be given to strains capable of biofilm formation and improving soil water retention capacity. SynCom efficiency under drought and heat stress will be evaluated in growth-chamber experiments through analyses of plant physiological and biochemical stress responses, including photosynthetic performance, chlorophyll content, biomass production, proline accumulation and volatile organi c compound (VOC) profiling using GC-MS. Simultaneously, shifts in soil microbiome structure and activity will be assessed using Oxford Nanopore-based metagenomics, extracellular enzyme activity measurements and BIOLOG EcoPlate functional profiling. The most effective microbial biostimulants will subsequently be validated in field experiments on local farms exposed to recurrent drought, where plant performance, soil physicochemical properties, water retention capacity and microbiome dynamics will be monitored throughout the vegetation cycle. The quality of final food products will additionally be evaluated through analyses of morphological, nutritional and crop-specific bioactive parameters.

To further connect microbial dynamics with environmental stress prediction, the project will also integrate microbiome analyses with IoT sensor networks, ERA5 climate reanalysis data and Copernicus satellite observations to develop machine-learning models for early drought-risk classification.

Acknowledgment: CLIMAGRIT project (PK.3.4.17.0002), funded by the European Regional

Development Fund through the Programme Competitiveness and Cohesion 2021–2027.

Keywords: soil microbiome; drought stress; synthetic microbial consortia; microbial biostimulants; climate change adaptation

Who recovers, Who fails? Microbial responses to drought along an urban land-use gradient

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Urbanization profoundly alters soils through changes in land use and management intensity,reshaping microbial communities and the functions they support. These modifications may influence how soils resist and recover from climatic stress. We assessed the resistance andresilience of three urban soils differing in management intensity—unused (low), roadside (intermediate), and garden soils (high)—using a controlled drought–rewetting experiment. Soils were pre-incubated under standardized conditions, exposed to severe drought (5% WHC,

40 °C) for 7 days, then rewetted to 60% WHC for a 14-day recovery period. Microbial respiration, extracellular enzyme activities, qPCR markers (16S, 18S, nitrogen-cycle genes), and Rock-Eval® carbon stability were measured across key time points.

Unused soils exhibited the strongest resistance–resilience dynamics. Fungal/eukaryotic abundance (18S rRNA) recovered after rewetting, whereas bacterial abundance (16S rRNA) did not, highlighting contrasting responses between microbial groups. Enzyme activities declined sharply under drought and showed no resilience, suggesting shifts in microbial community composition or reduced functional demand linked to drought-induced necromass accumulation. Rock-Eval® analyses revealed a transient decrease in the stable carbon fraction during drought, followed by recovery, potentially reflecting increased necromass inputs or microbial access to alternative nutrient pools.

Garden soils displayed weak resistance–resilience patterns, with minimal differences between droughted and control conditions, indicating microbial communities already adapted to frequent disturbances or resource fluctuations. Roadside soils showed intermediate and heterogeneous trajectories, sometimes resembling unused soils (e.g., resilience of communitymarkers) and sometimes garden soils (e.g., limited functional recovery), consistent with their transitional management intensity.

Overall, microbial abundance, functional activity, and carbon stability responded differently to drought–rewetting disturbance. Land-use history and management intensity strongly shaped soil recovery trajectories, underscoring the need for multi-indicator approaches to assess resilience in urban soils under increasing climatic stress. Ongoing amino-sugar profiling and metagenomic analyses will further disentangle microbial turnover, necromass dynamics, and functional reorganization during drought–rewetting cycles.

Keywords: Urban soils; Drought–rewetting; Microbial resistance; Microbial resilience; Carbon stability

Evaluating nitrate transformation in aquifers using microbiological, molecular and isotopic tools for enhanced management of key groundwater ecosystem services.

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Aquifers serve as the primary source of drinking water for Humans worldwide. This use of groundwater mainly relies on ecosystem services provided by aquifers. Among them, natural water purification is a cornerstone ecosystem service, notably mitigating the rise of the pollutants emitted by anthropogenic activities in groundwater. Up till now, this service is poorly taken into account in aquifer management and regulating policies as exemplified by the case of nitrate. The latter is a major pollutant threatening groundwater quality at a worldwide scale. However, its impact can be mitigated by in situ microbial denitrification.

On a wider angle, this project, DEEPTHOUGHT (Water4All, 2025-2028) aims to understand the mechanisms that lead to denitrification occurring in groundwater and evaluate the impacts of pressures (anthropic and climate change) on denitrification rates. To reach that goal, field samples are collected periodically from three contrasted field sites in France (agricultural pressure), Austria (urban pressure) and Denmark (agricultural pressure) to characterise the microbial denitrifying communities (activities, biomass and diversity). Physico-chemial data (NO3, pH, Eh, metalloids, pesticides…) were measured as well as nitrate reduction potentials, N and O isotopes to assess in situ nitrate reduction potential, microbial cell counts and activity and diversity through metabarcoding and shotgun sequencing.

Here we present the first results obtained on the French site from a deep karstic aquifer (north of Orléans, Beauce Aquifer), along a gradient of nitrate concentrations and pressures (pesticides, irrigation). The combination of different methods to assess the dynamics of nitrate aims to provide a pathway for the identification of new tools that consider these aquifers functionalities to further implement in the current frameworks used to manage groundwater.

Keywords: aquifers, biodiversity, nitrate, denitrification, ecosystem services

DNA-based assessment of tetracycline-driven restructuring of root-associated and bulk soil bacterial communities in a Mediterranean oregano agroecosystem

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Veterinary antibiotics entering agricultural soils through manure application and wastewater reuse constitute an emerging pressure on soil microbial communities and ecosystem functioning in Mediterranean agroecosystems. However, limited information exists regarding the differential response of bulk soil and plant-associated bacterial microbiomes to antibiotic exposure under Mediterranean conditions. In the present study, the effects of tetracycline on bacterial community composition in bulk soil and Origanum officinalis roots were investigated using Illumina sequencing of the 16S rRNA gene.

A Mediterranean alkaline agricultural soil was amended with NH4NO3 in the presence or absence of tetracycline (0.1 mg kg−1 soil), and bacterial communities were monitored over time in both bulk soil and root compartments. Alpha diversity in bulk soil remained relatively stable following tetracycline exposure, whereas root-associated bacterial communities exhibited significant temporal responses. Principal coordinate analysis and PERMANOVA demonstrated that time significantly shaped bacterial assemblages in both compartments, while tetracycline effects were more pronounced in root-associated microbiomes compared to bulk soil communities. Differential abundance analysis revealed selective enrichment and suppression of specific bacterial taxa under tetracycline exposure, particularly within Proteobacteria- and Bacilli-associated groups. Community trajectory analysis further demonstrated transient restructuring of bacterial assemblages followed by partial recovery at later sampling stages.

The findings demonstrate compartment-specific microbiome responses to tetracycline exposure and highlight the greater sensitivity of root-associated bacterial communities compared to bulk soil microbiota in Mediterranean plant–soil systems. The study further supports the potential use of DNA-based microbial indicators for monitoring ecological disturbance caused by emerging pollutants in agro-hydro systems.This work was partially funded by the Horizon Europe project Path4Med (Proposal No. 101156867), “Demonstrating Innovative Pathways Addressing Water and Soil Pollution in the Mediterranean Agro-Hydro-System”, under the call HORIZON-MISS-2023-OCEAN-SOIL-01.

Keywords: tetracycline; soil microbiome; root-associated bacteria; Mediterranean agroecosystems; DNA-based indicators

Session 6

Microbes - Pollutants and Bioprocess Engineering

Invited speaker

New rules, new opportunities – novel bioprocesses for a more sustainable urban water cycle

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Two years ago, the EU released the Revised Urban Wastewater Treatment Directive. This directive mandates among others recovery of phosphorus, removal of micropollutants, monitoring of PFAS and microplastics. The new directive requires rethinking of how wastewater treatment will be addressed in the urban context, where water can be treated on site with either biological or physicochemical processes. Onsite treatment can create better options to remove nutrients and micropollutants, particularly from separated streams such as urine that contain on average about 2/3 of humanly excreted micropollutants while also enabling recovery of nutrients. In this presentation I will discuss the key aspects of onsite treatment systems and discuss removal of micropollutants particularly in a context of electrochemical and bioelectrochemical systems.

Keywords: bioremediation, decentralized water treatment, micropollutants, wastewater treatment, resource recovery

Chemical rectification of different soil textures by the utilization of optimized biochar-digestate mixtures

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The valorisation of biochar and digestate represents the final step in the circular bioeconomy concept. Their combined application in soil is frequently advantageous; yet, their comprehensive impact across a wide range of environments is understudied. This study modeled the effects of different biochar-digestate mixtures in a range of application rates (2.5-5% w/w), mixture ratios (0-100%), and biochar types (composed-based and woody biomass-based, pyrolyzed in 500 ^{o}C and 700^{O}C) on the chemical profile of three different soil textures (coarse, medium and fine). Design of Experiments (DoE) was employed to determine the experimental pot layout. The measured chemical responses were used to construct predictive models using Response Surface Methodology (RSM) and Artificial Neural Networks (ANNs), yielding accurate fits in both modeling approaches. To identify the soil-specific best treatments that could yield the highest nutrient concentration and improve fundamental soil properties, two optimization methodologies were employed: the Desirability Index (DI) and the Genetic Algorithm (GA). Results highlighted digestate as the main micro- and macronutrient supplier, which nevertheless caused salinity spikes across all soils. The addition of biochar reduced this effect, while improving C/N ratio and retention capacity, but at the cost of increased pH, especially in the case of compost-based biochars. Woody-based biochars managed to increase CEC only in the coarse soil, while reduced Fe availability in the iron-rich medium soil. The optimization process identified distinct optimal treatments for each soil type, confirming the necessity of tailored amendment solutions for successful soil management.

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Keywords: waste valorisation; circular economy; machine learning; data modelling; sustainable soil management

Improving microbial tolerance to metal mixtures from printed circuit boards (PCB) by accelerated adaptive evolution in the laboratory

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Printed circuit boards (PCBs) account for approximately 7–9% by weight of waste electrical and electronic equipment. The metals contained in PCBs can therefore be regarded as a significant secondary resource. Their recycling through bioleaching appears to be an alternative to conventional techniques, given its reduced carbon-footprint. However, metals present in PCB waste can inhibit microbial metabolism, ultimately leading to the inactivation of the process. Our study aims to enhance microbial activity in environments rich in organic/biodegradable PCB waste. These new boards were designed as part of the DESIRE4EU project, which aims to contribute to a self-sufficient and environmentally friendly European electronics industry by developing an innovative concept: a PCB assembly (PCBA) with an eco-friendly life cycle. Iron- and sulphate-oxidizing microorganisms were cultured from selected mining waste and then exposed to increasing concentrations of PCBA-leachates. The selected consortium maintained its activity in 1/100 diluted leachate ([Cu] = 132 mg/L; [Bi] = 0.4 mg/L) and only partially at 1/10th dilution. Adaptive laboratory evolution (ALE) is currently underway using a fixed volume of 5% of the culture, a selection period of 14 days, and a selection pressure based on the progressive increase of leachate dose at each step. The microbial consortium remained active in leachates 27 times more concentrated than at the first step. Microorganisms' attachment to substrates (biofilms) is also being tested in addition to ALE, as well as random mutagenesis, to accelerate bacterial selection. The optimal culture conditions for the most resilient cultures were determined using the response surface statistical method, which permitted to simultaneously optimize several parameters. 16S rRNA sequencing permitted to identify microbial communities and their evolution throughout the adaptation process. Ultimately, these results will be used to develop new biotechnologies to anticipate the end of life of such innovative PCBAs and ensure an eco-friendly life cycle.

Keywords: adaptive evolution; printed circuit boards; bioleaching; microbial consortia.

Valorization of the Organic Fraction of Municipal Solid Waste, Poly(3 hydroxybutyrate), enzymatic degradation and upcycling for circular bio based polymers

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Major environmental issues, caused by the excessive use of petroleum-derived plastics in modern societies, have led to the need of new alternatives. Moreover, the development of sustainable biorefineries using crude renewable resources for the production of various bio-based products is essential in order to achieve a smooth transition to the circular economy era. Substitution of conventional plastics by bio-based and biodegradable ones produced via microbial bioconversion using renewable feedstocks and recycled biopolymers constitute crucial technologies towards this transition. Polyhydroxyalkanoates (PHAs) is a group of biodegradable polyesters with similar properties to conventional plastics. PHB is produced as a secondary intracellular metabolite via bacterial fermentation, and it could be used for packaging applications. Sustainable production and commercialization of PHB should be rely on renewable resources, at an optimized scale and development of downstream chemical and/or biological recycling of post-consumer PHB-based bioplastics.

The organic fraction of municipal solid waste (OFMSW) contains proteins, lipids, pectins and polysaccharides that could be used in various applications. Biorefinery development of OFMSW led to lipid, protein and pectin extraction as well as crude hydrolyzing enzyme production via solid state fermentation. The OFMSW sugar-rich hydrolysate was used for PHB production via fermentation with the bacterial strain *Paraburkholderia sacchari*. The denatured PHB was enzymatically degraded after extensive screening of PHB depolymerases. The enzymes were produced either by natural or engineered strains. A novel electrochemical membrane bioreactor was applied for the integrated degradation and extraction of PHB monomers in one unit operation. Subsequently upcycling of the degradation products led to a circular PHB production scheme.

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Keywords: Poly(3-hydroxybutyrate), Biorefineries, Enzymatic degradation, Upcycling

Application of bacterial bioluminescence (Microtox assay) as a method for evaluating the toxicity of pure compounds and complex effluents along the Moroccan Atlantic coast

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The Microtox test is a rapid, simple, and widely used test in the context of screening and comparative studies. The aim of this study is to use the Vibrio fischeri bacteria luminescence inhibition test to assess the toxicity of metals and those of industrial unit discharges. The assessment of the effects of discharges on V. fischeri luminescence reveals the great sensitivity of this bioindicator to discharges and highlights a high risk of deterioration in the quality of the marine environment, particularly in the areas where discharges, with EC50s which can reach 0.25% discharge into the test medium for petrochemical effluents after 5 minutes of exposure. The analysis of these results made it possible to classify the discharges according to their toxicity towards the bacterium V. fischeri and to draw up a profile of the impact of these discharges according to theEC50 values. The results of tests carried out on metals show that mercury is the most toxic metal (EC50 = 0.63 mg HgL-1), followed by lead (EC50 = 1.08 mg Pb L-1) and cadmium (EC50 = 1.87 mg Cd L-1) and finally copper (EC50 = 2.57mg Cu L-1). The results obtained during this study show that the Microtox test represents a good model for evaluating the toxicity of complex discharges and pure substances and that bacterial luminescence constitutes an excellent bioindicator for screening the medium quality.

Keywords: Microtox; Ecotoxicology; Global toxicity; Vibrio fisheri; Effluents; Metals

Soil microbial respiration and ecotoxicity reveal the environmental compatibility of modified levan biopolymers

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The increasing use of bio-based polymers as sustainable alternatives to synthetic and petroleum-derived materials requires a better understanding of their interactions with environmental microbiomes. In this study, levan derivatives modified with octenyl succinic anhydride (OSA) and dodecenyl succinic anhydride (DDSA) were evaluated from the perspective of microbial biodegradation and ecotoxicological safety.

Biodegradation was assessed in soil over 28 days using a Micro-Oxymax respirometric system (Columbus Instruments, Ohio, USA) by continuously monitoring oxygen consumption and carbon dioxide production as indicators of microbial respiratory activity. Ecotoxicity was evaluated using the *Aliivibrio fischeri* bioluminescence inhibition assay.

All tested levan-based materials stimulated microbial respiration compared with the soil control, indicating their utilization by the soil microbial community. Native levan showed nearly 80% biodegradation, while OSA- and DDSA-modified levans reached 63–78% biodegradation after 28 days. The cumulative O_2 consumption and CO_2 production in soils amended with native and modified levans were higher than in the control and cellulose reference, confirming active microbial degradation. Importantly, chemical modification did not inhibit the development of the soil microbial community, as bacterial and fungal numbers remained higher in levan-amended soils than in the control. Ecotoxicity testing showed no adverse effects of native levan and modified derivatives on *A. fischeri* bioluminescence, while the only derivative showing measurable inhibition was still classified as harmless.

These findings demonstrate that OSA- and DDSA-modified levans are readily biodegradable and show low ecotoxicological risk. The combined use of soil respiration monitoring and microbial bioluminescence inhibition provides a relevant approach for assessing interactions between bio-based materials and environmental microbiomes, supporting their further development as environmentally compatible alternatives to conventional synthetic products.

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Keywords: levan, biodegradation, ecotoxicity

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