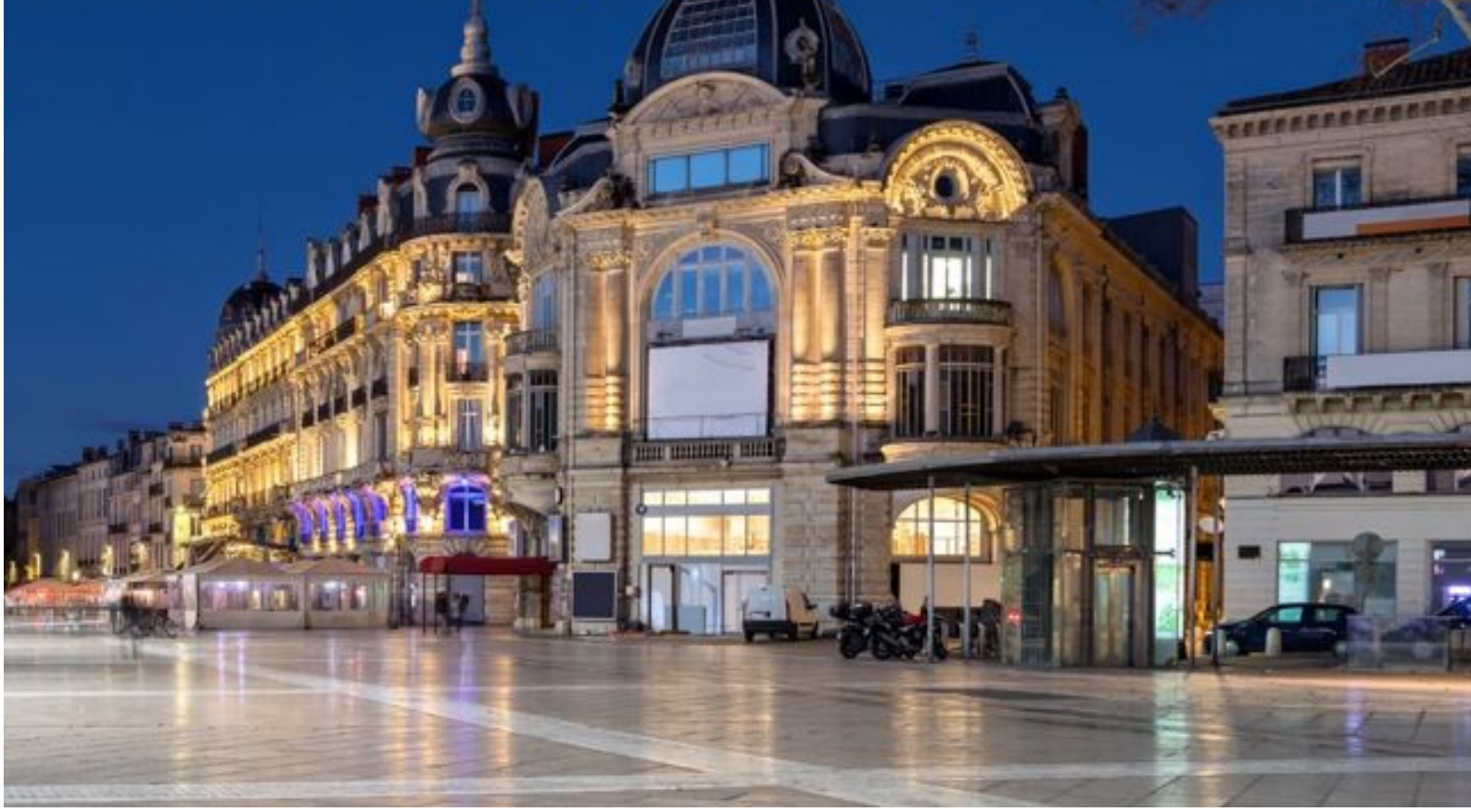

EcotoxicoMic 2022

3rd international conference on Microbial Ecotoxicology

15-18 November 2022
Montpellier, France

BOOK OF ABSTRACTS

last updated 25 November 2022

ROVALTAIN FOUNDATION
For Environmental Research

ecotoxicomic

Welcome to Ecotoxicomic 2022

On behalf of the local and scientific committees, it is a great pleasure to welcome you in Montpellier, one of the liveliest and sparkling French cities, for the 3rd Ecotoxicomic meeting, the international conference dedicated to Microbial Ecotoxicology. Organized by the Ecotoxicomic international network and the Rovaltain Foundation, this 3rd edition highlights in particular the work of young researchers through several initiatives: (1) the three laureates of the 1st Young Researchers webinar organized in 2021 will present their work as invited speakers; (2) 15 grants were provided by FEMS for Early Career Researchers to present their work at the congress; (3) prizes will be awarded to the best presentations by PhD students and post-doctoral researchers.

We are very grateful to the scientific committee, composed by international scientists, who joined their efforts to propose attractive topics and to organize the classical and special sessions. Our sincere thanks also go to the 14 persons of the local committee who have devoted a lot of time and efforts to welcome you in the best conditions. Many thanks to the Young Researchers group from the Ecotoxicomic network for their help in organizational tasks. As co-organizers, we must say that it was a pleasure to work with such dedicated and enthusiastic people, and we thank all of them for their availability and commitment.

We are grateful to the keynote speakers who prepared instructive talks to present recent advances and to expose news insights on Microbial Ecotoxicology. Do not miss them! Our thanks are also due to all colleagues who sent proposals for oral and poster presentations.

Live presentations, interactive poster and poster corner sessions, as well as informal afternoon and evening meetings, will provide an ideal setting for high-level scientific exchanges, and lively discussions. We hope that you will enjoy formal and less formal exchanges with old and new colleagues from all geographical, disciplinary and institutional horizons and wish you an exciting and fruitful meeting.

Lise Barthelmebs, *University of Perpignan*
Marina Héry, *University of Montpellier*
Chloé Bonnineau, *INRAE*
Delphine Delaunay, *Rovaltain Foundation*
Mathilde Moizo, *Rovaltain Foundation*

ORGANISERS

The International Network of Microbial Ecotoxicology

The EcotoxicoMic network aims at enhancing visibility and structuring Microbial Ecotoxicology community by developing transverse actions between studied ecosystems and researchers belonging to various research organisations. Created in 2018, following the first international conference EcotoxicoMic2017, this informal international network gathers more than 200 scientists from 42 countries.

Becoming a member of the international EcotoxicoMic network is free-of-charge. To join the EcotoxicoMic community, you only need to fill online a membership form, available on the EcotoxicoMic website (https://ecotoxicomic.org/).

The Rovaltain Foundation

Created in July 2013 by the Ministry of Higher Education and Research, the main purpose of the Rovaltain Foundation is to support high-level scientific research by promoting interdisciplinary research, particularly in the fields of environmental toxicology and ecotoxicology.

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Springer Environmental Science and Pollution Research

Local organisers

Lise Barthelmebs
University of Perpignan

Marina Héry
University of Montpellier

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Call for Papers

Thematic Issue on Microbial Ecotoxicology

"Microbial Ecotoxicology: from the lab to the field"

Guest Editors:
Barthelmebs Lise, *University of Perpignan, France* - barthelm@univ-perp.fr
Corcoll Natàlia, *University of Gothenburg, Sweden*
Héry Marina, *University of Montpellier, France*
Karpouzas Dimitrios, *University of Thessaly, Greece*
Vuilleumier Stéphane, *University of Strasbourg, France*
Wick Lukas Y., *Helmholtz Centre for Environmental Research, Leipzig, Germany*

The thematic issue will discuss recent advances in microbial ecotoxicology, including:

- the impact of chemical, environmental and biological stressors on microbial diversity, microbial processes, and species interactions, and the overall response to contamination by microorganisms at individual, population and community levels
- the role of microbial strains and communities on contaminant dynamics and bioremediation
- the use of microbial bioindicators, biomarkers, bioassays and biosensors to assess environmental risks of contaminants in various ecosystems

FEMS Microbiology Ecology will consider **full length Research Papers or Mini-reviews/Perspectives** for this issue. Authors should specify in their cover letter that their paper is submitted in the frame of the "**Thematic issue on Microbial Ecotoxicology**". For instructions on submitting a manuscript, please consult the following link:
https://academic.oup.com/femsec/pages/instructions_for_authors.

If you need some information for this special issue, please contact us.

All manuscripts will undergo regular review by members of the Editorial Board and other appropriate experts. Accepted manuscripts will be published in regular issues of the journal and the Thematic Issue will be compiled and made available online in 2023.

SUBMISSION TARGET DATE: January 2023.

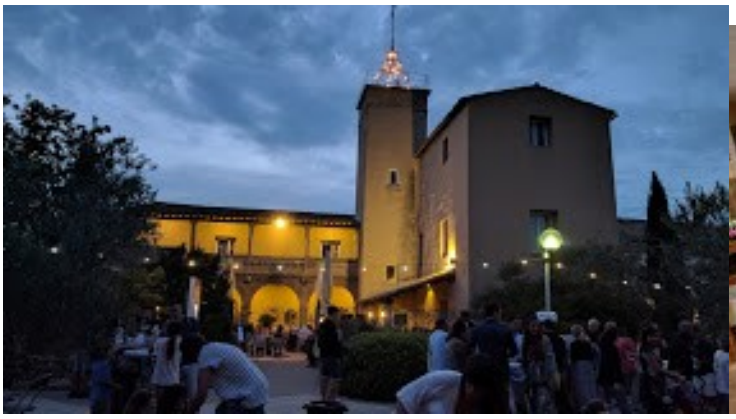
Federation of European Microbiological Societies

Conference dinner

The conference dinner will be held on Thursday evening, 17th November, at Mas Saporta, a traditional winegrower farm near from Montpellier.

Bus transfer between the Corum to the restaurant:

- **Departure** from the Corum at **19h15**
- **Return** from the restaurant to the Corum at **23h00**



Conference footprint

In order to evaluate the carbon footprint of the congress, please fill in the following questionnaire proposed by students from the master Risk and Environment (ig2e, Lyon University)

Visit Montpellier

Visit the Montpellier Tourist Office (located on the Place de la Comédie, 400 m from the Corum) and enjoy a special offer for EcotoxicoMic22 participants: 30% discount on Montpellier City Card 24h, 48h or 72h!

City Cards include a transport ticket, a guided tour of your choice (or audio guide) as well as a number of free and reduced rates for a wide range of local activities

Program overview

	Tuesday, 15 November	Wednesday, 16 November	Thursday, 17 November	Friday, 18 November	
08:30		"Microorganisms as tool to evaluate the efficiency of water treatment processes"	Keynote: **Fabrice Martin-Laurent** "Microorganisms as tool for environmental risk assessment"	Keynote: **Robert Marks** "Cell biosensors: from microorganisms to environmental applications"	08:30
09:50		Coffee break	Coffee break	Coffee break	09:50
10:40		Keynote: **Sofie Thijs** "Impact of contaminant on microbial diversity and functions"	"Microorganisms as tool for environmental risk assessment" Discussion on microbiome in ERA	"Microbial roles in contaminant fate and bioremediation" Closing session & Awards ceremony	10:40
12:20	Welcome	Poster session / Lunch	Poster session / Lunch		12:20
13:40	Introduction Opening lecture: **Balbina Nogales** Keynote: **Joakim Larsson** "Fate and impacts of antimicrobials in terrestrial and aquatic environments"	"Impact of contaminant on microbial diversity and functions" Poster corner	Keynote: **Kathrin Fenner** "Microbial roles in contaminant fate and bioremediation" Poster corner		13:40
16:00	Coffee break				16:00
16:40	"Fate and impacts of antimicrobials in terrestrial and aquatic environments"	"Impact of contaminant on microbial diversity and functions"	"Microbial roles in contaminant fate and bioremediation"		16:40
17:40	Ecotoxicomic session	Poster session	Poster session		17:40
18:00					18:00
18:20	Poster session				18:20
19:00	Welcome cocktail		19:15 - bus departure for conference diner		19:00
	Student evening		Conference dinner		

Scientific program

Classic sessions

Impact of contaminants on microbial diversity and functions

Microorganisms participate in all major functions of biogeochemical cycles, and their diversity represents a keystone for ecosystem functioning and resilience. The capacity of microorganisms for adaptation is unequalled in the living world. As other types of organisms however, microbes are increasingly threatened by multiple and pronounced environmental stresses and anthropic perturbations. This session will propose investigations of the impact of stressors on the taxonomic and functional diversity of the microbial world, and of the overall response to contamination of microorganisms at individual, population or community level. These may involve the use of 'omics' and ‘metaomics’ tools (e.g. genomics, transcriptomics, proteomics, metabolomics); approaches based on functional traits, for example to develop indicators of microbial community disturbance; as well as fundamental genetic and evolutionary engineering studies at the cellular and molecular levels.

Keywords: diversity; functions; bioindicators; omics

Microbial roles in contaminant fate and bioremediation

Microorganisms have developed a variety of metabolic adaptations and resistance mechanisms to cope with the presence of toxic elements in their environment. Microbial activity thus has considerable impact on environmental contaminants, and strongly contributes to their fate. Many microbially-driven processes may contribute to contaminant degradation, detoxification or immobilization (e.g. sequestration, precipitation) and thus to pollution mitigation, while conversely, some others may enhance toxicity of contaminants and their dissemination in the biosphere.

Evaluation and understanding of these processes is still challenging, in the field as well as in the laboratory. Thus, prediction of the fate of contaminants in ecosystems remains difficult. The bioavailability and mobility of chemical contaminants in the environment, as key factors of microbial transformation of chemicals, require particular attention today. Moreover, linking available evidence of contaminant biotransformation to key microbial players involved in these processes remains mostly indirect, especially in complex ecosystems. Finally, understanding the environmental factors governing microbial activity is an essential step to develop efficient bioremediation strategies. In this session, we welcome original reports on all aspects of these challenging research areas, and especially those involving interdisciplinary approaches at the interface of chemistry, physics and microbiology, including modelling studies.

Keywords: bioremediation, biodegradation, biotransformation, bioavailability, immobilization, dissipation, ecological engineering

Scientific program

Classic sessions

Microorganisms as tool for environmental risk assessment

Microorganisms are key players of earth life: they drive important ecosystem processes and contribute substantially to global biogeochemical cycles. They exhibit a broad range of sensitivity to many toxicants and are known to be natural early warning systems that detect acute and long-term effects produced by toxic pollutants.

Until now chemical analysis remains essential to evidence contamination, however biological indicators can provide valuable complementary information on both the impact and fate of contaminants. Microorganisms are good candidates as a tool for environmental risk assessment (ERA) since they can be found in all types of environments (water, soil…) and have a large range of sensitivity to a wide range of chemical pollutants. Innovative methods and tools for environmental risk assessment based on microorganisms have been developed in order to i/predict hazard and assess risk before the release on the market of a new active compound (a priori ERA) as well as ii/ assess, the ecotoxicological impacts of chemical residues in the environment (a posteriori ERA).

Despite the recognized importance of microbial communities in supporting a range of functions and ecosystem services, microorganisms are only barely not considered in both a priori and a posteriori environmental risk assessment and they are typically not yet implemented in the current regulations or legislations. This session will focus on the use of microbial bioindicators/biomarkers/bioassays as well as of microbial biosensors to assess environmental risk of contaminants in various ecosystems. In particular, communications on the interest of monitoring in response to pollutant exposure the diversity and function of microbial communities using omic approaches for ERA in various ecosystems are welcome. In this session, we also intend to discuss the possible strategies to promote the use of microorganisms (at different levels of biological organization) in both a priori and a posteriori environmental risk assessment.

Keywords : bioindicators/biomarkers, biosensors, risk assessment, environmental managers, biomonitoring

Scientific program

Special sessions

Fate and impacts of antimicrobials in terrestrial and aquatic environments

organised by Kristian Koefoed Brandt (University of Copenhagen, Denmark) and Ed Topp (Agrifoodn Canada)

National and international authorities are now recognizing that the environmental dimension of antimicrobial resistance must be taken into consideration when developing strategies to combat this public health menace. Antimicrobial chemicals enter the environment indirectly through human and animal waste streams and manufacturing effluents, and directly through their use in aquaculture and as pesticides in plant-based food production systems. Since these chemicals are designed to kill microorganisms (viruses, bacteria, fungi, parasites) they have potent antimicrobial activity.

Thus, there are concerns about their impacts on microorganisms undertaking key ecosystem services. Furthermore, microorganisms in anthropogenically impacted environments may adapt by evolving or acquiring genes that confer antimicrobial resistance, increasing the reservoir of resistance genes that ultimately can be transmitted to microorganisms of human health concern. Presentations concerning any aspects of environmental development and transfer of antimicrobial resistance or antimicrobial impacts on environmental microorganisms and the functions that they carry are welcome.

Keywords: antibiotic; fungicide; antiviral, impacts, fate

Microorganisms as a tool to evaluate the efficiency of water treatment processes

organised by Marlen I Vasquez (Department of Chemical Engineering, Cyprus University of Technology, Cyprus) and Christina Pavloudi (The George Washington University, USA - Hellenic Centre for Marine Research, Greece)

Microbial communities are in the core of many water treatment applications at the lab, pilot and full scale. Despite their significant role in these processes their study has only recently been achieved through the development of the -omics molecular techniques. Only recently, special focus has been given on how microbial communities of engineered environments can act as carriers of genetic elements such as antibiotic resistant genes (Rizzo et al 2016). The microbial interaction of engineered and receiving environments is an untapped transdisciplinary research field that can significantly help us increase our understanding of the role of microbes to act as carriers or as units being transferred between compartments.

At the same time, new microbial metrices and indices are being proposed to decipher impacts of engineered environments on receiving environments (Sagova-Mareckova et al 2020). These impacts relate to the microbial community structure and function. The ultimate goal would be to use microbial bioindices to assess the efficiency of treatment processes and link them to the ecological status of receiving environments. Investigating the microbial community continuum can increase understanding of this dynamic microbiome and provide insights of its behavior under multiple stressors scenarios. Deciphering this dynamic microbiome can ultimately expand our knowledge about improving existing and upcoming treatment technologies and developing tools to better control these systems.

Keywords: water treatment process, aquatic microbiology, biofilm structure, microbiology of engineered systems, multiple stressor

Scientific program

Special sessions

Cell biosensors: from microorganisms to the systems applied to environmental issues

organised by Gerald Thouand, Marie José Durand, Sulivan Jouanneau , Ali Assaf (UMR GEPEA, CNRS 6144, IUT de la Roche sur Yon, France)

The exposome concept has been established for human health and tends to be extended to environmental pollutions leading to the concept of ecoexposome. In both cases, the concept depends to the capacity to collect datas and monitor the effect of pollution. Alongside specific and standard chemical methods, biological methods, like biosensors, are certainly the best way to detect the global effect of either a pollutant or a complex mixture. Cell biosensors, whose concept was born 55 years ago, have never ceased to be enriched by the scientific ambitions and ingenuity of researchers, and societal needs in terms of applications. A cell biosensor is, above all, a hybrid system associating biology, electronics, materials and digital science. It is a measuring instrument with multiple applications that combines a biological recognition element (here a cell usually called bioelement) with an electronic part.

The majority of applications is certainly on the environmental part with plenty of example for metal, organometallic, organic compounds but also considering global ecotoxic effect. In this session, this complete scientific field will be considered in all its complexity by integrating four fields which are microorganisms (their environmental resourcing, their genetic modifications), systems engineering, field application and industrial constraints with a view to the commercialization of biosensors.

Keywords: cell biosensor, engineering, field application, environment

Poster corners

Wednesday, 16 November 15:20 – 16:00

Ecotoxicology of pesticides

- Assessment of the impact of pesticides on microbial communities with different trophic complexity and predator-prey interactions. ***M. E. Pérez Villanueva*** *et al.* – P13
- Impact and fate of the fungicide tebuconazole in a biofilm/water system: Chemical transformation, fatty acids and taxonomic-related changes. ***D. Gómez Martínez*** *et al.* – P10
- Toxicokinetic of a pesticides cocktail pulse on stream biofilms with different hydrological histories. ***L. Bertrans-Tubau*** *et al.* – P37
- Preventive bioremediation for agricultural soils to reduce pesticide contamination. ***R. Dhommée*** *et al.* – P38

Innovative tools & methods in microbial ecotoxicology

- Development of a collaborative web platform documenting the diversity and extent of diatom deformities. ***I. Vallanzasca*** *et al.* – P58
- Harmonizing bioinformatics procedures in microbiome amplicon high-throughput sequencing. ***V. Gelhay*** *et al.* – P34
- DIMITRA: an upcoming database on effects of pesticides on the soil microbiome. **M. *Swaine*** *et al.* – P4

Thursday, 17 November 15:20 – 16:00

Metals in the environment

- Heavy metal contamination of sediments along Nigerian Coastal waters and its influence on the bacterial diversity. ***J. Idomeh*** *et al.* – P26
- Impact of Copper oxide nanoparticles on *Bacillus megaterium*. ***G. D. Tripathi*** *et al.* – P31
- Drying effects on resistance and resilience of hyporheic microbial communities exposed to copper contamination. ***L. Kergoat*** *et al.* – P27
- Interactive effects of temperature and bismuth exposure on fatty acid composition, antioxidant enzymes and lipid peroxidation in snails fed on bismuth-contaminated algal biofilms. ***M. Fadhlaoui*** *et al.* – P30

Effects of contaminants on microbiota associated with plants & animals

- Use of metaproteomics for the taxonomical and functional analysis of poplar roots and endophytes. ***B. Alpha-Bazin*** *et al.* – P5
- Effect of glyphosate on the microbiota of rainbow trout, *Oncorhynchus mykiss*. ***L. Bellec*** *et al.* – P14
- Gut microbiota impairment is associated to physiological alterations in *Xenopus laevis* tadpoles exposed to graphene oxide. ***L. Evariste*** *et al.* – P15
- Chronic exposure to antiseizure drugs impacts the gut microbiota community and function. ***C. Dop*** *et al.* – P17

Scientific program

FEMS grantee *IRD grantee* *best oral presentation at Ecotoxicomic YR2021*

Tuesday, 15 November

12h20 – 13h40	Welcome
13h40 – 14h00	Introduction
14h00 – 14h40	Opening lecture: **Balbina Nogales**
Fate and impacts of antimicrobials in terrestrial and aquatic environments	
14h40 – 15h20	Keynote : **Joakim Larsson**
15h20 – 15h40	Exposure of benthic microbial communities to pharmaceuticals and resulting adaptation including tolerance, biodegradation and antibiotic resistance: advances and challenges. ***C. Bonnineau***, *J. Artigas, A. Bouchez, C. Dagot, M. Devers-Lamrani, J. Labanowski, E. Lyautey, F. Martin-Laurent, C. Miège, L. Mondamert, S. Pesce.*
15h40 – 16h00	Uncover the microbial diversity as barrier to the antibiotic resistance diffusion in freshwater biofilms. ***G. Gionchetta***, *J. Lee, O. Hansen, H. Bürgmann.*
16h00 – 16h40	COFFEE BREAK
16h40 – 17h00	Biodiversity in freshwater biofilms as a barrier to antibiotic resistance genes dissemination. ***E. Catão***, *G. Gionchetta, U. Kluemper, H. Bürgmann, T. Berendonk, X. Bellanger, C. Merlin.*
17h00 – 17h20	Environmental fate of three antibiotics, sulfomethoxazole, tiamulin and tilmicosin, and their impact on the microbial community composition, resistome and mobilome of two different soils. *E. Katsivelou, C. Perruchon, A.P. Karas, A. Sarantidou, S. Sotiraki,* ***S. Vasileiadis****, G. D. Karpouzas.*
17h20 – 17h40	Sludge management optimization for mitigating the antimicrobial resistance dissemination. *A. Ezzariai, S. Sire, L. Sauvadet, J. Gnaneswaran, N. Arpaillange, D. Bibbal, V. Bru-Adan, V. Dupouy, M. Guiresse, M. Hafidi, J. Jimenez, M. Lacroix, D. Riboul, E. Pinelli, N. Wery,* ***M. Barret,*** *D. Patureau.*
17h40 – 18h20	**Ecotoxicomic session**
18h20 – 19h40	**Poster session**
19h00 – 20h30	WELCOME COCKTAIL
20h30…	Student evening

Scientific program

Wednesday, 16 November

Microorganisms as tool to evaluate the efficiency of water treatment processes	
08h45 – 09h05	Freshwater microbial communities as a tool to evaluate the efficiency of an innovative treatment for abandoned mines effluents. *M Abril, L. Vendrell, C. Espinosa, L. Bertrans, L. Llenas, J. Lopez,* ***L. Proia.***
09h05 – 09h25	TOXLAB: Multidimensional biosensor to assess toxicity of wastewaters. ***S. Jouanneau****, M.J. Durand-Thouand, T. Louineau, G. Thouand.*
09h25 – 09h45	Contaminants of emerging concern in a constructed wetland system: microbial ecotoxicity and antibiotic resistance genes spread. *M.Andronikou, P. Pissaridou, I. Vyrides,* ***M. I. Vasquez****.*
09h45 – 10h40	COFFEE BREAK
Impact of contaminant on microbial diversity and functions	
10h40 – 11h20	Keynote : **Sofie Thijs**
11h20 – 11h40	Determining *in situ* periphyton quality and microbial biodiversity responses to nutrients and pesticides. *A. Håkansson, H. Nilsson, E. Kristiansson, T. Backhaus, K.S.L. Johansson, A. Nilsson,* ***N. Corcoll.***
11h40 – 12h00	Exploring the toxicity of the new emerging toxicant 6PPD-Quinone to environmental microorganisms. *A. Martins, T. Mhadhabi, A. Chauviat, R. Caracciolo, S. Favre-Bonté, C. Baduel, T. Meyer, G. Uzu, S. Nazaret, M. Pirrung,* ***J.M.F. Martins.***
12h00 – 12h20	Effect of simulated cyanobacterial blooms on the gut microbiota and metabolome of the medaka fish *Oryzias latipes*: a case study of microbiome-aware ecotoxicology. ***S. Duperron****, A. Gallet, S. Halary, P. Foucault, C. Duval, H. Huet, B. Marie.*
12h20 – 13h40	LUNCH
13h00 – 13h40	**Poster session**
13h40 – 14h00	Response of soil bacterial and hppd communities to tembotrione herbicide. ***H. Terol****, C. Thiour-Mauprivez, M. Devers-Lamrani, F. Martin-Laurent, C. Calvayrac, L. Barthelmebs.*
14h00 – 14h20	Compositional, genetic and functional characterization of culturable microbial communities isolated from polychlorinated dibenzo-p-dioxins/furans contaminated soil. *S. Mahfouz, G. Mansour,* ***A. Hanano.***
14h20 – 14h40	Phenanthrene impacts poplar physiology and affects differently fungal versus bacterial communities in the rhizosphere and the root endosphere. ***L. Gréau****, M. Le Jean, T. Beguiristain, D. Billet, D. Heintz, J. Zumsteg, D. Blaude, A. Cébron.*

Scientific program

Wednesday, 16 November

Impact of contaminant on microbial diversity and functions	
14h40 – 15h00	Impact of graphene oxide on *Nitzschia palea*: approach in flow cytometry. ***P. Braylé,*** *M. Barret, F. Mouchet, L. Gauthier, L. Evariste, E. Pinelli.*
15h00 – 15h20	Metal resistance genes enrichment in marine biofilm communities selected by biocide-containing surfaces in temperate and tropical coastal environments. *E. Catao, N. Gallois, B. Misson, F. Fay, K. Rehel, D. Copin, I. Linossier, C. Garnier, A. Tunin-Ley, J. Turquet, T. Pollet, R. Barry-Martinet, C. Bressy,* ***J. F. Briand.***
15h20 – 16h00	**Poster corner**
16h00 – 16h40	COFFEE BREAK
16h40 – 17h00	Metabolomic responses of freshwater periphytic microbiome to combined stress of artificial light at night (ALAN) and benzalkonium chloride. ***N. Creusot****, R. Vrba, M. Eon, N. Mazzella, A. Moreira, D. Millan-Navarro, S. Morin.*
17h00 – 17h20	Multi-omics applications reveal stress adaptation processes in microbial communities differing in exposure history. *F. Larras, S. Lips, P. Veber, S. Schreiber, E. Billoir, M. L. Delignette-Muller,* ***M. Schmitt-Jansen.***
17h20 – 17h40	Structural and functional impact of wastewater microorganisms in stream biofilms. ***L. Carles****, S. Wullschleger, A. Joss, R.I.L. Eggen, K. Schirmer, N. Schuwirth, C. Stamm, A. Tlili.*
17h40 – 18h40	**Poster Session**

Scientific program

Thursday, 17 November

Microorganisms as tool for environmental risk assessment	
08h30 – 09h10	Keynote : **Fabrice Martin-Laurent**
09h10 – 09h30	Microbial indicators and Quality Ratio Index for risk assessment in oil-contaminated tropical coastal sediments. *A. Carvalho, C. Cravo-Laureau,* ***V. Moreira****, A. Baldy, M. Vicente, E. Bidone, M. Bernardes, E. Sabadini-Santos, R. Duran.*
09h30 – 09h50	Extended spectrum beta-lactamase-producing *Escherichia coli* in surface water and groundwater of a French karst system. ***C.P. Henriot****, V. Klaba, P. Amiotte-Suchet, J.C. Beugnot, K. Phan Huy, T. Karbowiak, X. Bertrand, H. Celle.*
09h50 – 10h40	COFFEE BREAK
10h40 – 11h00	Marine biofilms as biological indicators of the seawater chemical quality of Mediterranean French coasts. ***A. Barré****, M. Bouchoucha, R. Barry-Martinet, M. Briand, N. Briant, B. Dormoy, P. Marchand, A. Ortalo-Magné, J.F. Briand.*
11h00 – 11h20	Down under the surface of the Adriatic Sea: benthic microbial communities and how anthropogenically-induced pollution affects them? ***A. Ramljak****, J. Žučko, I. Babić, M. Lučić, M. Furdek Turk, M. Fafanđel, S. Matijević, N. Udiković-Kolić, I. Sviličić Petrić.*
11h20 – 11h40	Bio-indication of trophic and mining alterations and determination of New-Caledonian rivers' ecological state, using a new multimetric diatomic index (IDNC). ***F. Delmas****, J. Marquie, S. Boutry, E. Lefrançois, Y. Dominique.*
11h40 – 12h20	**Discussion on microbiome in ERA**
12h20 – 13h40	LUNCH
13h00 – 13h40	**Poster session**
Microbial roles in contaminant fate and bioremediation	
13h40 – 14h20	Keynote: **Kathrin Fenner**
14h20 – 14h40	Dynamics of the total and active bacterial communities in a bioremediation field-trial of As-rich acid mine drainage. ***C. Diaz-Vanegas****, C. Casiot, A. Desoeuvre, L. Lin, O. Bruneel, F. Battaglia-Brunet, C. Joulian, J. Jacob, M. Héry.*
14h40 – 15h00	Role of micro-organisms in the leaching of critical metals from tungsten mine wastes: from the microscale to the field scale. ***E. Laroche****, L. Spadini, L. Oxarango, A. Crouzet, C. Duwig, Y. Rossier, G. Boujut, L. Cand, T. Maret, J.M.F. Martins.*
15h00 – 15h20	Microbial role in plastic biodegradation in the marine environment: the case of polyhydroxyalkanoates. ***L. Philip****, C. Odobel, J. Jacquin, G. Derippe, É. Villar, V. Barbe, S. Bruzaud, A.L. Meistertzheim, J.F. Ghiglione .*

Scientific program

Thursday, 17 November

Microbial roles in contaminant fate and bioremediation	
15h20 – 16h00	**Poster Corner Session**
16h00 – 16h40	COFFEE BREAK
16h40 – 17h00	Identifying potential blockbuster trifluoroacetate precursors. ***S. Karakurt-Fischer****, S. L. Robinson, D. R. Johnson, K. Fenner.*
17h00 – 17h20	How can multi-contamination and redox variation affect metformin biodegradation in continental waters at the sediment-water interface ? ***A. Borreca****, S. Vuilleumier, G. Imfeld.*
17h20 – 17h40	Metagenomics, metatranscriptomics and statistical learning to enhance the remediation of oil sand processed water in Northern environments. ***S. Correa-Garcia****, J. Tremblay, M.J. Bergeron, K. Trepanier, D. Degenhardt, C. Martineau, E. Yergeau.*
17h40 – 18h20	**Poster Session**
19h15	Bus departure to the conference dinner
20h00…	Conference Dinner

Friday, 18 November

Cell biosensors: from microorganisms to environmental applications	
08h30 – 09h10	Keynote : **Robert Marks**
09h10 – 09h30	Sensing the environment by distributed microbial sensors: detection of buried landmines as an example. ***S. Belkin,*** *B. Shemer, T. Elad, E. Shpigel.*
09h30 – 09h50	Development of biosensors for the assessment of seawater toxicity: Choice of the bioreporter. ***E. Delaunay****, S. Jouanneau, M.J. Durand-Thouand, G. Thouand.*
09h50 – 10h40	COFFEE BREAK
Microbial roles in contaminant fate and bioremediation	
10h40 – 11h00	Growth condition dependant glyphosate degradation in *Ochrobactrum pituitosum* strains. ***K.J. Thompson****, P. Hernandez, A. Langarica-Fuentes, S. Kleindienst.*
11h00 – 11h20	Increased temperature and light availability accelerate the decomposition of glyphosate by stream biofilms. *S. Abdelhak, Y. Menard,* ***J. Artigas.***
11h20 – 12h00	**Closing Session & Awards**

Communication abstracts

Talk

Opening lecture

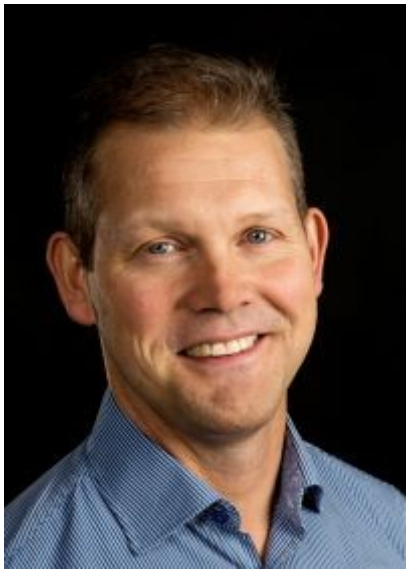
Dr. Balbina Nogales, *University of the Balearic Islands, Spain*

Balbina Nogales is a Lecturer in Microbiology at the University of the Balearic Islands. Since her PhD she has done research in the field of environmental microbiology, mainly in the study in microbial communities of polluted environments. The main research focus of her group is the study of coastal microbial communities with different levels of anthropogenic impact, mainly by hydrocarbon pollution, and the role of marine bacterial groups in the degradation of these pollutants. More recently, the group has extended their research to the topic of plastic degradation by marine bacteria. The group combines global approaches of community analysis (metagenomics) with studies on the genomics, biochemistry and physiology of cultivated pollutant degrading bacteria by proteomics and metabolomics.

Keynote conference - "Pollutant degradation in the environment: who is doing the job?"

It is not new, and often not noticeable, that human-derived activities cause severe pollution problems to the environment. The magnitude of pollution is such that most likely we cannot find pristine environments in our planet any more. The scientific community has been addressing the problem of environmental pollution for at least sixty years. Enormous efforts have been, and are currently being, dedicated to the discovery of microorganisms degrading pollutants and the elucidation of the genetics and biochemistry of degradation pathways of model bacteria. The paradox is that in environmental microbial communities these "professional" degraders usually belong to the so-called rare biosphere. Yet pollutants are degraded or transformed in the environment meaning that unrecognized members of microbial communities are involved in pollutant, or pollutant intermediates, degradation or transformation. This has been the hypothesis of our work on hydrocarbon pollution in marine environments and motivates also our most recent research on marine plastic-degrading bacteria. Our analysis of genomic and metagenomics data show that we should not make straightforward assumptions on the role of marine bacteria in pollutant degradation. The metabolite landscape generated when pollutants are transformed in the environment is complex and unsolved, but it probably determines which bacteria participate in pollutant degradation and contributes to the fate of the pollutant in the environment.

Keynote



Pr. Joakim Larsson, *Department of Infectious Disease, University of Gothenburg, Sweden*

Joakim Larsson is a Professor in Environmental Pharmacology at the Department of Infectious Disease, University of Gothenburg, Sweden. He received his PhD in animal physiology in 2000 in Gothenburg, and after two years of guest research in Canada and USA, he decided to combine his interest for the environment with medicine. He became associate professor in human physiology in 2007 and full professor in 2013. From 2016 he is director for the multidisciplinary Centre for Antibiotic Resistance Research (CARe) at University of Gothenburg, involving +10 0 researchers from six faculties. Larsson has (co)-authored more than 185 papers, and he is among the 1% most highly cited researchers on Web of Science according to Clarivate Analytics.

His earlier work on environmental pollution from drug manufacturing, and his research on selective concentrations of antibiotics has contributed various management initiatives across the world. His current research focus on the environmental dimensions of antibiotic resistance. Ongoing projects include *e.g.* research on: the role of antibiotics and biocides in the evolution of antibiotic resistance; understanding the evolutionary history of antibiotic resistance acquisition in pathogens; exploration of the environmental resistome for novel resistance genes; surveillance of resistance in the human population using sewage bacteria; environmental transmission of resistant pathogens; as well as both technical and societal measures to reduce environmental pollution with antibiotics and antibiotic resistant bacteria.

Keynote conference - On the environment's role in evolution, transmission and surveillance of antibiotic resistance

The role of the environment in antibiotic resistance development has become more and more recognised among both the scientific community and stakeholders. Thousands of scientific papers describe widespread occurrence of antibiotic residues, resistant bacteria and resistance genes in various environments. Accordingly, policy makers call for actions to reduce exposures. Still, perspectives on exactly how or to what extent observations of antibiotics, resistant bacteria or resistance genes in the environment indeed reflect an impact on or a risk for human and animal health are often lacking.

The environment plays at least three conceptually different roles in antibiotic resistance. The first is as an arena for the evolution of resistance in pathogens. Indeed, the most striking feature of the environmental microbiome is its immense diversity, providing an almost endless palette of genetic mechanisms that potentially could be acquired and used by pathogens to counteract the effect of antibiotics. The second role of the environment is not related to changes in DNA but merely as a transmission route for certain resistant pathogens that are already circulating widely among people and/or domestic animals. These two, evolution and transmission, could be seen as "active roles" where processes in the environment will affect the risks for the development and spread of resistant infections. More recently, it has been acknowledged that the abundance and pattern of resistance in environmental microbiota, particularly sewage, could serves as a passive reflection of the resistance situation in the local human, or animal, populations. A fourth role, not covered in this talk, is as a source for novel antibiotic molecules and thus drug development.

Our understanding of, and the evidence base for the three first roles, is fragmented. In my talk, I will attempt to structure the different risk scenarios, and present and discuss the evidence for each of the steps involved, highlighting some of our recent contributions to the field.

Exposure of benthic microbial communities to pharmaceuticals and resulting adaptation including tolerance, biodegradation and antibiotic resistance: advances and challenges

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Since the early 1920's, pharmaceuticals, including antibiotics, have been massively produced and consumed for the benefit of both human and animal health. Pharmaceuticals residues have then reached the aquatic environment through diffuse and point (wastewater) sources. Among the pharmaceutical residues, the ubiquitous presence of antibiotics could exert a selective pressure on microbial communities leading to the acquisition and dissemination of antibiotic resistance in the environment.

We present here the synthesis of recent research projects (*e.g.* PANDORE, Antibio-tools, Antibiotox, PharmaTox...) investigating the dissemination of pharmaceuticals, including antibiotics, in the different aquatic compartments (surface water, periphyton, sediment) and its impact on periphyton and sediment microbial communities. These projects mainly focused on antibiotic resistance genes (ARG), community tolerance to pharmaceuticals (PICT) and antibiotic biodegradation capacity by combining field studies on different lake and lotic ecosystem (*e.g.* rivers Arve, Tillet and Le Clain; lake Geneva) and experimental approaches in microcosms. Our results highlight the ubiquitous presence of pharmaceuticals in the studied ecosystems and the specific distribution of pharmaceuticals in the different investigated compartments. The field surveys generated an important antibiotic resistance database including relative abundance of ARG and genetic mobile elements as well as functional measurements of microbial tolerance (PICT approach) to selected pharmaceuticals and biodegradation potential of sulfonamide antibiotics. Antibiotic resistance was found to be positively correlated with the presence of wastewater treatment plant effluents, but we confirm that the links between exposure levels, *i.e.* antibiotics concentrations, and antibiotic resistance must be considered in all ecosystem complexity. Further analyses are currently on-going to better take into account potential confounding factors.

These research projects generated advances in our knowledge on pharmaceuticals and antibiotic resistance dissemination within the aquatic environment but also reveal the current challenges to better understand the drivers of antibiotic resistance in such complex environments.

Keywords: antimicrobial, periphyton, sediments, river, lake

Uncover the microbial diversity as barrier to the antibiotic resistance diffusion in freshwater biofilms

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Microbiomes of aquatic biofilms could experience increased levels of exchange of genetic information between bacteria, due to the high density of cells and their close proximity. Conjugation consists of the transmission of mobile plasmids between bacteria through horizontal gene transfer (HGT) and it is one of the most common mechanisms for the acquisition of antibiotic resistance. Aquatic environments receiving episodic or chronic pollution from wastewater discharges are vulnerable to invasion by antibiotic resistant bacteria (ARB). While an ARB invader may or may not be successful in establishing a lasting presence in an environmental biofilm, conjugative transfer could provide a mechanism by which a microbial invader, spreads its resistant plasmids into a new environment or community. According to the diversity-invasion effect, the diversity of the natural community may influence the success of the invasion, and ultimately of HGT. Here we experimentally assessed the microbial invasion on river-grown biofilms communities of contrasting diversity. Biofilms were exposed to invasion by a previously constructed donor strain of non-pathogenic *E. coli* MG1655 Δ*lacZY* possessing a native conjugative IncPα plasmid donor of *nptII* resistant gene. Specific molecular methods allowed quantifying the spread of the resistant invader and its plasmid. Although the general trend is similar between low and high diversity sites, biofilm communities shown different responses depending on the original diversity. As hypothesized, the relative abundances of invaders and plasmids were higher in the low diversity biofilms. On the other hand, the transfer ratio decreased over time, indicating a low potential conjugation rate to the native community in the biofilms. Aquatic biofilm communities with high diversity could be less susceptible to invasion by resistant bacteria and resistance gene transfer, since fewer niches are available, indicating that healthy, diverse biofilm communities could have a natural function as barriers against the spread of antibiotic resistance in the environment.

Keywords: antibiotic resistance; microbial invasion; gene transfer

Biodiversity in freshwater biofilms as a barrier to antibiotic resistance genes dissemination

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Antibiotic resistant bacteria (ARB) and resistance genes (ARGs) are frequently discharged into the environment by human activities**.** We hypothesized that to persist, invading ARGs should disseminate by horizontal transfer into native communities following an invasion process by either ARB, bacteriophages, or naked DNA. Here, we present a pan-European study investigating the persistence and dissemination of ARB and ARGs in aquatic ecosystems with a focus on the role played by the biodiversity of native microbial communities. First, natural epilithic biofilms were sampled in 7 countries before determining their taxonomic biodiversity and ARG content by 16S rDNA sequencing and high-throughput chip-based qPCR, respectively. Although bacterial diversity was relatively homogenous, the ARG patterns displayed site-specific profiles, indicating that biofilms serve as integrative indicators of local anthropogenic pressures. Similarly, correlations between the alpha diversity indexes and the ARG relative abundance were also site-specific. The invasion hypothesis was further explored using river biofilms formed on immerged glass slides and placed in closed-circuit river water microcosms before to be exposed to either (i) genetically tagged *Escherichia coli* carrying an IncP1α conjugative plasmid, (ii) nacked DNA of the donor strain, or (iii) an enrichment of natural transducing phages hosting the monitored markers. Whatever the form of the inoculum, the plasmid and chromosomal DNAs were independently monitored by qPCR to assess their persistence and dissemination in the biofilm communities. The inoculated bacteria, when used as invader, showed differing persistence depending on the biofilm diversities, suggesting that high diversity of the native community may present a barrier to invasion. However, the spread of the plasmid in communities appeared rather sporadic, indicating that conjugation of the plasmid is controlled by additional factors than the bacterial donor invasion. Inoculation with naked DNA or phages resulted in different persistence patterns of the markers, thus implying different contributions/efficiencies in the ARG horizontal transfer.

Keywords: Environmental biofilms, antimicrobial resistance, invasion, gene dispersion

Environmental fate of three antibiotics, sulfomethoxazole, tiamulin and tilmicosin, and their impact on the microbial community composition, resistome and mobilome of two different soils

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+ Equal contribution

Use of animal manure for soil fertilization may facilitate veterinary antibiotic (VA) release, and antibiotic resistance gene (ARG) and pathogen dispersal, threatening environmental quality and human health. We studied, in two agricultural soils, the persistence and the effects of three VAs, sulfomethoxazole (SUL), tiamulin (TIA), and tilmicosin (TLM), on the soil microbial community. VAs were repeatedly applied in soils (3 cycles) either directly or through application of pig feces containing VAs in environmentally relevant levels. We measured antibiotic dissipation, inorganic N soil pools, potential nitrification (PN); and the abundance of total bacteria, antibiotic resistance genes, mobile genetic elements and ammonia oxidizing bacteria and archaea (AOB/AOA) (with qPCR). We further analyzed the community composition of total prokaryotes, fungi and eukaryotes (focusing on protists) through high throughput amplicon sequencing. Out of the three antibiotics, only TIA showed accelerated biodegradation upon repeated application and only in the high pH soil (DT_{50} = 14 and 7.5 days in the first and third cycle respectively) and only under direct soil application. TLM accumulated upon repeated application and SUL had a relatively stable dissipation pattern in both soils and application regimes. SUL negatively impacted PN, increasing ammonia as the application cycles progressed. TLM did not show any adverse effects on PN, while TIA showed an adverse effect in the end of the last application cycle with varying responses between the two application regimes. Molecular analysis and high throughput amplicon sequencing analyses are on the way and will be presented in the conference.

Acknowledgements: This research was funded by the project INVERT (**IN**teractions of **V**eterinary antibiotics with soil microorganisms: exploiting microbial degradation to avert **E**nvironmental contamination and **R**esis**T**ance dispersal; HFRI project number 01183).

Keywords: veterinary antibiotics, feces, soil, resistome, microbial community

Sludge management optimization for mitigating the antimicrobial resistance dissemination

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Antimicrobial resistance (AMR) is a major concern with respect to global public health. The persistence and dissemination of AMR concern encompasses human, animal and environment issues and thus fits into the One Health framework. AMR has been quantified in multiple compartments of aquatic and terrestrial ecosystems and wastewater treatment plants were pointed out as a major source of AMR dissemination into the environment, including through sludge spreading as a soil fertilization practice. In this study, the dynamics of antibiotics and antimicrobial resistance were investigated along the sludge – sludge treatment process – soil continuum. For this purpose, 5 sludge treatments were carried out in the laboratory on the same raw sludge: drying, liming, composting, anaerobic digestion, and anaerobic digestion followed by composting. Soil microcosms were then conducted for mimicking the spreading of the raw and 5 treated sludges in agricultural soil. To better constrain the driving processes, sludge samples were placed into litterbags, between two soil layers. Antibiotics were quantified all along the sludge treatments and during the soil incubation. Antimicrobial resistance was captured using culture-dependent as well as culture-independent methodologies (e.g. highthroughput quantitative PCR) for characterizing the total microbial communities, the abundance and diversity of the resistant bacteria and genes, but also the mobile genetic elements. The most abundant AMR genes in raw sludge were those encoding for resistance to aminoglycosides (aadA and strB), sulfonamides (sul1), macrolides (ermB) and transposases. The higher the overall biological activity, the higher the AMR removal and the changes in microbial community structure, during sludge treatment as well as during soil incubation. The main outputs of this work are thus the hierarchisation of the sludge treatment according to their ability to mitigate the antimicrobial resistance dissemination, but also the identification of the main factors triggering antibiotic resistance dissemination.

Keywords: antibiotics, resistance gene, anaerobic digestion, compost, soil

Freshwater microbial communities as a tool to evaluate the efficiency of an innovative treatment for abandoned mines effluents

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Abandoned mines cause serious environmental damage to their surroundings with considerable impacts on freshwater ecosystems. These impacts occur mainly due to the uncontrolled discharge of polluted effluents with different composition depending on the characteristics of the abandoned mine. The LIFE DEMINE project aimed to demonstrate and disseminate the technical and economic feasibility of decreasing the overall environmental impact caused by abandoned mine effluents on water bodies, by adopting an innovative and versatile treatment process with the global aim of obtaining a non-polluting final effluent. Freshwater microbial attached communities (biofilms) were used as an indicator to quantify the overall efficiency of different innovative treatment technologies in reducing the ecological impacts caused by mining effluents of the developed. The main important results of different experiments carried out exposing biofilms to treated and untreated water from real effluents of two abandoned mines will be presented. A multi-biomarker approach was used by measuring a wide range of structural and functional biofilm responses (i.e. diatoms community structure, algal biomass and community composition, photosynthetic efficiency, nutrients uptake capacities) as well as potential pollutants bioaccumulation. The results of the different experiments evidenced a significant reduction of the ecological impacts achieved by treating the mines effluents with the DEMINE technologies. In fact, biofilm exposed to untreated mines effluents always showed significant structural and/or functional responses respect to microbial communities exposed to the DEMINE-treated mine wastewater which normally did not differ from control. Overall theses studies confirmed the relevance of using microbial communities as a tool to evaluate the efficiency of water treatment processes.

Keywords: biofilms, bioindicator, mining effluents, water treatment, ecological impacts

TOXLAB : Multidimensional biosensor to assess toxicity of wastewaters

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The treatment of domestic wastewater is a major challenge for our modern societies in terms of public health and environmental protection. According to Jones et al., nearly 188.1 km^3 of domestic wastewater is treated each year in domestic wastewater treatment plants (WWTP). Consequently, these facilities are guarantors of collective health. It is essential to ensure the optimal functioning of these installations in order to prevent health and/or environmental risks linked to the discharge of such effluents into environment.
Currently, the management of domestic wastewater treatment plants is based on physico-chemical indicators relating to the pollutant load such as the total organic carbon, the chemical/biological oxygen demand (COD/BOD). These parameters remain insufficient to predict a potential toxic effect of the inputs on the biomass involved in the depollution process (activated sludge). Consequently, complementary methods are needed to assess the quality of domestic wastewater.
The issue of our project is to propose an assessment approach of the toxicological level of wastewaters in order to predict their effects on the depollution bioprocesses. For this, the proposed strategy is based on two bottlenecks. The first relates to the number of bioreporters involved in the assessment of toxicity. The reference methods are generally based on monospecific approaches (a single bioreporter). Although reproducible, these approaches are however limited in terms of representativeness; how to transpose the toxicological provided data in the context of the protection of a bioprocess? Indeed, the results obtained are based on the toxicological resistance capacities inherent to the bioreporter used. In order to overcome this first limitation, the approach proposed here uses a set of microorganisms (n=10). The second bottleneck is based on the choice of bioreporters. Most of recommended tests were developed in a context of environmental preservation. Consequently, the used organisms (bioreporters) were chosen for their representativeness of environment (river, lake, soil) and are, by nature, not suitable to represent the ecosystems implied in the wastewater treatment. To solve this second limitation, several bioreporters were selected (resulting from the study of the microbial communities involved in these processes) in order to best reflect the biological diversity of the WWTP ecosystems, and thus to increase the representativeness of the bioreporters.

Keywords: Multidimensional approach; biosensor; bioreporter; toxicity assessment; WWTP monitoring.

Contaminants of emerging concern in a constructed wetland system: microbial ecotoxicity and antibiotic resistance genes spread

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Antibiotics are well-known pharmaceutical compounds that are mainly used to target either inhibit bacteria growth (bacteriostatic) or exterminate them (bactericidal) and are important for the treatment of bacterial infections. In the last two decades, the consumption of antibiotics has increased rapidly, which has been cited as one of the world's worst abusers of antibiotics. Studies show that a large part of the antibiotics can not be efficiently removed in conventional wastewater treatments and can be detected at low concentrations even after the treatment. The impact of antibiotic residues on microbial diversity is widely studied over the last decade. Exposure can lead specific members of the microbial community sensitive to these compounds to disappear and allow others to thrive changing the microbial communities' balance. Hence, contamination of the aquatic environment with antibiotics is becoming a rising concern for global health. The persistence of antibiotics in the environment can vary depending on the type of antibiotic. For example, penicillins are easily degraded but fluoroquinolones (e.g. ciprofloxacin), macrolides (e.g. tylosin) and tetracyclines are considerably more persistent and therefore remain and accumulate in higher concentrations. This perseverance in the environment leads to a constant selection of bacteria to maintain antibiotic resistance genes (ARG). This increases the antibiotic-resistant bacteria that we encounter in the environment and therefore those detected in patients. Thankfully, in recent years technology allows the quick detection of ARG by using RT-qPCR techniques. Developed countries have introduced several methods for antibiotic removal, and constructed wetlands (CWs) have been found low cost and easily maintainable technology and have been proven as a reliable alternative to traditional wastewater treatment technologies. A free water surface (FWS) CW system of Choletria has been the object of study to investigate where we can detect ARGs in the FWS. Remarkably, the results of the RT-qPCR technique show that three types of AGRs appear to be prominent in different areas of the FWS system.

Keywords: Antibiotics, microbial ecotoxicity, ARG, constructed wetlands

Keynote

Dr. Sofie Thijs, *Center for Environmental Sciences, Hasselt University, Belgium*

Sofie Thijs is postdoctoral researcher at the Center for Environmental Sciences (University of Hasselt). Her main research focus is on utilising and understanding plant-microbe interactions for soil remediation and sustainable crop growth. She uses both cultivation-based approaches, and metagenomics to disentangle the multitude of signals and interaction pathways between plant-microbe and pollutants.

Her current work is on implementing and merging molecular biology tools in phytoremediation feasibility and monitoring studies, to inform and predict biodegradation processes, and suggest bioaugmentation strategies to speed up pollution degradation.

Keynote conference - Expanding the phytoremediation toolbox: multiple line of evidence approach

Contaminated sites pose a significant threat to human health when left untreated. In search of sustainable clean-up technologies, ‘nature-based solutions’ including phytoremediation are gaining increased popularity. Classical site-assessment tools to evaluate the feasibility of these nature-based solutions are however not always sufficient, or straightforward. Therefore, we addressed this issue in a recently compiled ‘Code of Good Practice Phytoremediation’, in which we reviewed and summarized the necessary steps to conduct a feasibility and monitoring study tailored for phytoremediation, utilizing a multiple-line-of-evidence approach.

In my talk, I will provide an overview of these recommendations, tips and tricks to implement phytoremediation in a more qualitative way. I will discuss how to perform a feasibility study, balancing the various phytoremediation options, including state-of-the art analytical and genetic tools, and their practical implementation. It will be illustrated for a chlorinated solvent groundwater pollution case, in Belgium. The benefit of creating added-value products, and the need for good solutions to destruct polluted biomass is briefly touched upon as well. With this talk we hope you have a new backpack of tools, that you can use to gain a deeper understanding of the biodegradation processes taking place in the field, and to utilize them in a smarter way to speed up bio- and phytoremediation.

Determining *in situ* periphyton quality and microbial biodiversity responses to nutrients and pesticides

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Agrochemicals, including fertilizers and pesticides, are significant contributors to surface water pollution and have the potential to negatively impact periphyton. Periphyton provides many of the essential polyunsaturated fatty acids (PUFA) that are needed for organisms at higher trophic levels in river food webs. This study aims to assess the effects of agrochemicals on periphyton quality and microbial biodiversity *in situ*. Three streams (Höje å, Skivarpån and M42) located along an agrochemical gradient in the south of Sweden were sampled. The impacts of agrochemical pollution were assessed by linking chemical profiles (nutrient and pesticide levels in surface water) with GC-MS fatty acid profiles, pigment content and microbial biodiversity. We investigated bacterial, fungal and algal diversity by Illumina sequencing targeting 16S, ITS2 and LSU 23S regions of ribosomal RNA, respectively Results from water chemical analyses clearly showed higher levels of nutrients and pesticide pollution in Skivarpsån and M42 than in Höje å. Ecotoxicity tests using the passive sampler extracts demonstrated that the pesticide mixtures occurring at Skivarpsån and M42 were toxic for periphyton communities. Cluster and principal component analyses based on pigments content and microbial biodiversity profiles, clearly separated the periphyton from the three river sites studied. The nutritive quality of the periphyton differed among streams, and fatty acids considered high-quality feed such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were also more abundant in pesticide polluted streams (Skivarpsån and M42). Overall, results from the lab show that the mixture of pesticides pollution in the studied streams might be toxic for periphyton (i.e. inhibiting the photosynthetic activity). However, results from the field, indicate that when the levels of pesticide pollution are low and co-occur with high levels of nutrients pollution, nutrients might mask pesticides´ effects on periphyton quantity and quality due to compensatory effects from nutrients.

Keywords: pesticides, microbial ecology, ecotoxicity, fatty acids

Exploring the toxicity of the new emerging toxicant 6PPD-Quinone to environmental microorganisms

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This study examines the toxicity of the newly discovered molecule 6PPD-Quinone (6PPD-Q) to selected environmental microorganisms. After decades of intense research, this molecule, derived from a very common antioxidant (6PPD) used as a car tyre protector, has recently been identified as the main cause of the death of Coho salmon in the north-western United States, and is suspected to be a common toxicant worldwide through tyre treading. 6PPD-Q is produced by the ozone oxidation of 6PPD during the ageing of tyre particles after deposition on roads. The 6PPD-Q produced in this way at low concentrations can accumulate in surface waters after storm water runoff, resulting in acute mortality of salmon migrating to urban streams. In this context, we assessed the toxicity of a wide range of 6PPD-Q concentrations through its intrinsic oxidative potential, and through the response of single-cell microorganisms commonly found in the environment (3 bacterial species, a yeast, an algal strain and an amoeba) by cell tracking with various modern techniques. To assess the mechanisms involved in 6PPD-Q toxicity, particular attention was paid to model bacteria and their capacity for resistance (role of efflux pumps in the response to the toxicant) and degradation of the molecule, monitored by LC-MS/MS. Overall, our results showed that 6PPD-quinone had no or limited impact on all microorganisms tested, at the realistic concentrations tested. For some bacteria and yeasts, a slight inhibition of growth was observed with some concentrations, while other organisms were not impacted at all or were even stimulated by the presence of 6PPD-quinone, probably used as a carbon source by these organisms. Further investigations are needed to assess the potential chronic effects of 6PPD-Q on microbial communities in natural waters and on their ecosystem services.

Keywords: 6PPD-Quinone, ecotoxicity, environmental microorganisms, growth inhibition, efflux pump

Effect of simulated cyanobacterial blooms on the gut microbiota and metabolome of the medaka fish *Oryzias latipes*: a case study of microbiome-aware ecotoxicology

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Cyanobacterial blooms are one of the most common stress encountered by fish living in lakes and ponds. The impact of blooms on fish health, notably through oxygen depletion and production of compounds such as cyanotoxins is well documented, yet little is known regarding their influence on associated microbiota. Sitting at the interface between a host and its environment, the microbiome is a relevant yet understudied compartment for ecotoxicology research, known to react to and interact with contaminants. To address the impact of blooms on fish gut microbiota and holobionts metabolism, we conducted microcosm-based experiments on the model fish *Oryzias latipes* exposed to the cyanobacterium *Microcystis aeruginosa*. Results demonstrate that blooms affect fish microbiome composition and genome-encoded functions, as well as the metabolome of holobionts organs, in particular in the more intense bloom conditions. Differential effects on the dominant members of the microbiota are observed. After the end of the bloom, bacterial communities tend to return to original composition, yet they remain sensitive in case of a second bloom, reflecting a highly reactive gut community. In these days of the "microbiome revolution", this work demonstrates the relevance of gut microbiota to ecotoxicology and stress physiology, and advocates for a microbiome-aware ecotoxicology. The significance of bloom events to fish health and fitness through microbiome-related effects should be further explored in the context of increasingly frequent and intense blooms worldwide, in particular because of potential outcomes relevant to conservation biology as well as aquaculture.

Keywords: symbiosis, microcystin, holobiont, resilience

Response of soil bacterial and hppd communities to tembotrione herbicide

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Herbicides used in agriculture aim to prevent weed growth but are known to end up in contact with soil microorganisms, thus defined as non-target organisms. Tembotrione, a recently marketed β-triketone herbicide, is known to inhibit the 4-HydroxyPhenylPyruvateDioxygenase (4-HPPD) in weeds. This enzyme is also found in numerous soil microorganisms, such as some PGPR and symbiotic bacteria, that play a key role in maintenance of ecosystem services.

In this study, one of the major concerns is to assess whether tembotrione could have toxic effects on soil microorganisms and could disturb soil microbial community dynamic and structure. To investigate the possible impacts of this herbicide on these communities, a soil microcosm approach was performed using 1-fold or 10-fold the recommended tembotrione agronomical dose (RAD).

Soil samples were collected at day 0, 3, 7, 14, 24, 40, and 55 to determine the following endpoints: (i) dissipation of the active ingredient in soil, (ii) bacterial diversity with high throughput sequencing of the 16S rDNA, (iii) *hppd* community diversity with high throughput sequencing of the *hppd* gene using degenerated primers designed by our own. Moreover, a culturable approach was applied to soil microcosm samples to study antibiotic resistance (ABR) of isolated soil bacteria, and to screen for $HPPD^+$ strains.

Whatever the treatment applied, tembotrione was fully dissipated from soil after 24 days at 1*RAD with a DT_{50} of 7 days, and after 55 days at 10*RAD with a DT_{50} of 15 days. Interestingly, among the culturable strains isolated from the microcosms, 29 presenting ABR, were also $HPPD^+$. Further studies are currently performed to characterize their sensitivity to β-triketones herbicides, and their resistance to antibiotics. Finally, microbial and *hppd* diversity analyses with sequencing data are still ongoing at the time of writing this abstract, but results should be available by October 2022.

Keywords: Microbial ecotoxicology, Herbicide, Soil Bacterial Communities, Antibiotic Resistance, Tembotrione.

Compositional, genetic and functional characterization of culturable microbial communities isolated from polychlorinated dibenzo-p-dioxins/furans contaminated soil

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Dioxins (PCDD/Fs) are one of the most toxic environmental pollutants known to date. Due to their structural stability and extreme hydrophobicity dioxins persist in the ecosystems and can be bioaccumulated to critical levels in both human and animal food chains. Soils are the most important reservoirs of dioxins, thus soil microbes are highly exposed to dioxins, impacting their diversity, genetics and functional characteristics. To experimentally evaluate these effects, the diversity and functionality of soil microbes were assessed in seven local sites potentially exposed to PCDD/Fs.

Concentration of dioxins in soils samples was firstly determined and the soils cultivable microbes were identified and molecularly characterized as a function of their *in vitro* ability to degrade the TCDD. Our results revealed that the diversity of microbial communities largely varied among the sites and was likely inversely proportional to their level of contamination with PCDD/Fs. Furthermore, the genetics profiling of dioxin-degrading bacteria revealed that the Cytochrome P450 CYPBM3-positive species largely belong to the genus Bacillus and were randomly distributed among the soils samples, while the angular dioxygenase (AD)-positive species were mainly found in highly polluted soils with a major presence of the genus Pseudomonas. Finally, the functionality of dioxin-biodegrading genes (AD or CYPBM3), was confirmed by the ability of bacteria to consume 2,3,7,8-TCDD, and this was synchronized with an induced level of both pathways. Our results suggest that different dioxin-metabolizing pathways exist under the same environmental conditions and work differentially for an effective removal of PCDD/Fs.

Keywords: polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), angular dioxygenase (AD), biodiversity, cytochrome P450 (CYPBM3), biodegradation.

Phenanthrene impacts poplar physiology and affects differently fungal versus bacterial communities in the rhizosphere and the root endosphere

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Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants of anthropogenic origin that pose major environmental health problems. The use of plants and their associated microorganisms can provide a gentle and cost-effective remediation of large areas of contaminated soils. Indeed, plants can be colonised by a wide diversity of beneficial microorganisms in their rhizosphere and endosphere. Rhizosphere and root colonizing endophytes could play a major role in the fate of PAHs and on the stress response of plants to PAH-mediated toxicity.

Our study aimed to investigate the response of *Populus canadensis* and its associated microbial communities (both bacteria and fungi) when subjected to a PAH contamination gradient. Poplar cuttings were grown for 4 weeks in a multi-contaminated soil from a former coking plant. It was spiked with increasing concentrations of phenanthrene (PHE) up to 2000 mg/kg. The degradation of PHE in the soil and the uptake of PHE by the plant were monitored. Amplicon-sequencing diversity based on fungal ITS and bacterial 16S rDNA was assessed from rhizospheric soil and plant roots. The plant response to the contamination was explored through plant growth monitoring and transcriptomic analysis.

Phenanthrene analyses indicated that the concentrations of PHE of the soil and plant tissues were proportional. High concentrations of PHE (above 400 mg/kg) reduced plant biomass and induced numerous cellular responses. The structure of fungal communities was impacted by the PHE gradient in both soil and roots whereas the structure of bacterial communities was only impacted in the soil. Sensitive and tolerant microbial indicator species were identified. Their relative proportion was correlated with the PHE gradient. Altogether, our findings provide a global overview of dose-response effects on complex plant-microorganism interactions in the case of a soil PAH contamination.

Keywords: Phytotoxicity; Microbial communities; Soil pollution; Phenanthrene; Endophytes

Impact of graphene oxide on *Nitzschia palea*: approach in flow cytometry

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Graphene oxide (GO) is a carbon nanoparticle discovered in 2004. Its properties have made it a very coveted material, leading to an increase of its production and subsequently its release in the environment. One of the main receptacles of graphene are surface waters. At their basis, biofilms are a key biological compartment for the aquatic ecosystem health as they represent the first carbon source for higher trophic levels. One of the main components of biofilms are diatoms, responsible for primary production. Therefore, in order to evaluate the potential harm of GO on the aquatic ecosystem, it is crucial to understand the interactions between GO and diatoms. In this study, the diatom *Nitzschia palea* was exposed to 0.1 -1 and 10 mg/L of GO for 144h. Flow cytometry, coupled with fluorescent markers, allows a high throughput, quantitative and qualitative analysis of cells. It was used in order to enumerate cells and quantify lipid and chlorophyll content in order to underline physiological changes. Limits to the use of flow cytometry with graphene oxide were underlined and discussed such as nanoparticle concentration. Results show an interesting increase of cell growth, whereas interestingly, by monitoring the oxygen content throughout the 144h of exposition, it was revealed that the photosynthesis and respiration cycle was reduced by 10 mg/L of GO. To understand the interaction between the diatom and GO, electronic microscopy and RAMAN analyses were done in order to investigate the bonding of GO by the exopolymeric substances produced by *Nitzschia palea* and the potential change of structure of GO after exposition.

Keywords: primary producer, physiological response, analytical limits

Metal resistance genes enrichment in marine biofilm communities selected by biocide-containing surfaces in temperate and tropical coastal environments

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Microorganisms able to form biofilms in marine ecosystems are selected depending on immersed surfaces and environmental conditions. Cell attachment directly on toxic surfaces like antifouling coatings suggests a selection of tolerant (or resistant) organisms with characteristics conferring adaptive advantages. We investigated if environment would drive metal resistance gene abundance in biofilms on artificial surfaces. Biofilms were sampled from three surfaces (a PVC reference and two antifouling coatings) deployed in three coastal waters with dissimilar characteristics: The North-Western Mediterranean Sea (Toulon) and Atlantic (Lorient) and Indian (La Reunion) Oceans. The two coatings differed in metals composition, either Cu thiocyanate and Zn pyrithione (A3) or Cu2O (Hy). Metal resistance genes (MRG) specific to copper *(cusA, copA, cueO*) or other metals (*czcA* and *pbrT*) were monitored with qPCR in parallel to the microbial community using 16S rRNA gene metabarcoding. A lower α-diversity on A3 or Hy than on PVC was observed independent on the site. Weighted Unifrac suggested segregation of communities primarily by surface, with lower site effect. Metacoder log2 fold change ratio and LeFSe discrimination suggested *Marinobacter* to be specific of Hy and *Altererythrobacter*, *Erythrobacter* and *Sphingorhabdus* of A3. Likewise, the relative abundance of MRG (MRG/bacterial 16S rRNA) varied between surfaces and sites. A3 presented the greatest relative abundances for *cusA, cueO* and *czcA*. The latter could only be amplified from A3 communities, except at Toulon. Hy surface presented the highest relative abundance for *copA*, specifically at Lorient. *Dasania* correlated positively with all MRG except *cueO*. *Marinobacter* found in greater abundance in Hy biofilm communities correlated with the highest abundances of *copA* and *Roseovarius* with *czcA*. These results prove the selection of specific communities with abilities to tolerate metallic biocides forming biofilms over antifouling surfaces, and the secondary but significant influence of local environmental factors.

Keywords: Biofilm community, antifouling surface, copper tolerance, metal resistance genes, anthropogenic effect

Metabolomic responses of freshwater periphytic microbiome to combined stress of artificial light at night (ALAN) and benzalkonium chloride

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Urban activities can be a threat for ecosystem sustainability. For instance, there are numerous findings about the adverse effects of anthropogenic chemicals released from urban treatment plants on freshwater periphytic communities, which play key role in ecosystems function and associated services. Despite this increasing evidence, there a still a paucity of knowledge on the effect of chemicals combined to other anthropogenic stress. Among them, of particular concern is the Artificial Light At Night (ALAN). Thus, through ten days exposure of freshwater periphyton in controlled conditions, we have recently highlighted the combined effect of ALAN and benzalkonium chloride (i.e. main component of alcohol-based hand sanitizers) on photosynthetic function, diatoms morphology and pigment composition whereas the associated molecular/biochemical mechanisms/responses remained unknown. In this context, the present study aims to fill this gap of knowledge by providing metabolomic insights on this combined effect. To this end, following the same experiment the metabolome and the lipidome were characterized. In particular, high-resolution mass spectrometry based-untargeted metabolomics was implemented in order to provide a comprehensive picture of the microbial activities (i.e. molecular phenotype) and identify biochemical pathways involved in the physiological and morphological impairments. In addition, targeted analysis of key class of lipids (phospholipids, glycerolipids, fatty acids) was performed to highlight potential effect on energy storage and chloroplast/thylakoid membranes. Preliminary results show clear effect of both individual factors and their combination through the discovery of specific metabolome fingerprints and associated pathways that change over exposure time. Complementary analyses are still ongoing. The results highlight as well a shift in the composition of targeted lipid classes following exposure to (e.g. unsaturation rates of polar lipids). Overall, this study confirms the relevance of metabolomics/lipidomics approaches to provide mechanistic understanding of the response of environmental microbiomes to multiple stress, further supporting the discovery of biomarkers of ecosystem function impairment along the adverse outcomes pathway framework.

Keywords: periphyton, combined anthropogenic stress, photosynthesis, metabolomics

Multi-omics applications reveal stress adaptation processes in microbial communities differing in exposure history

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In stress ecology, characterizing and understanding the adaptive processes rendering a community more tolerant to a stressor is a crucial challenge. Unbiased molecular methods like metabolomics and transcriptomics have the potential to tackle this challenge because they provide comprehensive functional information at molecular level. However, they are rarely applied to identify adaptive processes in communities with different exposure history and community tolerance. Our study aimed to understand acute stress responses of microbial communities differing in chronic pre-exposure to the photosynthesis inhibitor diuron, combining untargeted metatranscriptomics (RNA-seq) and meta-metabolomics in a dose-response experimental design with phenotypic functional observations. Aquatic microbial communities were incubated for 5-weeks in microcosms 1/ under constant exposure to 4µg/L of diuron (stressed community) or 2/ without contamination (non-stressed community). Then, both communities were exposed for 1 hour to a gradient of diuron concentrations to investigate differences in stress responses after chronic exposure. Clear differences in the metabolome and gene expression profiles displayed stress adaptation. Phenotypic observations indicated propagation of molecular differences to community tolerance but an impairment of community functioning. The experimental design of the acute exposure experiment combined with the DRomics workflow for RNA-seq and metabolomics data analysis enabled the determination of response trends as well as sensitivity thresholds for thousands of molecular responses pointing to adaptation processes in communities with exposure history. While non-stressed communities displayed mostly impairment on metabolism and photosynthesis, the stressed communities exhibited mostly an increasing response trend and a lower sensitivity for photosynthesis, epigenetic phenomena, translation and traduction-related processes after acute exposure. In conclusion, multi-omics – technologies applied in a gradient design provide key mechanistic insights for the understanding of adaptive processes and tolerance acquisition on environmental communities.

Keywords: Ecosystem Functioning, Pollution-induced Community Tolerance (PICT), dose-response modelling, DRomics-tool

Structural and functional impact of wastewater microorganisms in stream biofilms

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Effluents from wastewater treatment plants contain several constituents, such as microorganisms, nutrients and micropollutants. These can potentially interact with microbial communities in the receiving stream and alter their diversity and the ecological functions they provide. While a great deal of research has been done to evaluate the effects of micropollutants or nutrients, little is known about the role of microorganisms contained in the effluents. The aim of this study was therefore to determine the impact of wastewater microorganisms on microbial diversity and functions of stream periphyton, key component in stream. To reach this goal, periphyton was grown in flow-through channels that were continuously alimented with a mixture of stream water mixed with unfiltered or ultra-filtered urban wastewater. Impacts of wastewater and wastewater microorganisms were assessed on periphyton biomass, activities and tolerance to micropollutants, as well as on microbial diversity. Moreover, micropollutants in water and in periphyton were comprehensively quantified. Our results showed that microorganisms from the wastewater colonized periphyton communities, leading to a shift in the final community composition, either directly or indirectly via species interactions. For instance, the abundance the phylum Chloroflexi that originated exclusively from the effluent increased in periphyton, whereas those of diatoms and green algae decreased in the presence of wastewater microorganisms. These structural alterations affected nutrient stoichiometry in periphyton and increased the capacity of heterotrophs to mineralize carbon substrates, suggesting a shift towards heterotrophy. Importantly, an increased tolerance towards micropollutants was found for periphyton exposed to unfiltered wastewater, but not to the ultra-filtered wastewater. This indicates that wastewater microorganisms were the main contributor to this increased tolerance. Overall, the contribution of wastewater microorganisms to the periphyton community structure and functions highlights the need to consider their role when studying potential impacts of wastewater on the receiving water bodies.

Keywords: periphyton; prokaryotes; eukaryotes; DNA metabarcoding; pollution-induced community tolerance

Keynote

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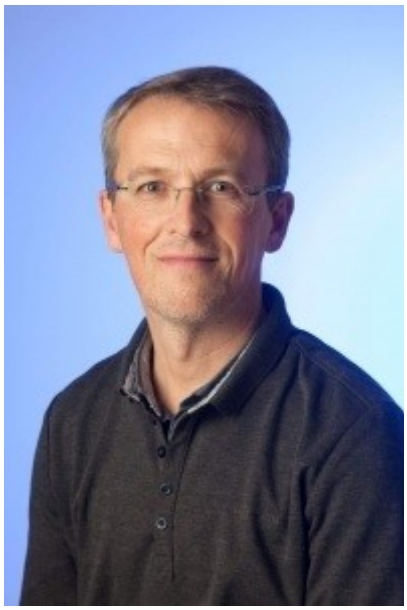

F Martin-Laurent was trained as a biochemist and molecular biologist at the University of Burgundy (France). During his PhD he studied genes induced in plants at early stages of endomycorrhizal symbiosis establishment (INRA Dijon, France and Max Plant Institut Marburg, Germany). He worked for CIRAD Forêt at Nanyang University of Technology (Singapore) on microbial inoculation of *Accacia mangium* for reforestation of degraded soils in South-East Asia. He then worked as a post-doc for the CNRS in Gif-sur-Yvette to study the expression of aquaporins in planta. He was recruited in 1999 as a research fellow in the soil microbiology laboratory of INRA in Dijon to deploy the use of molecular methods to analyse soil microbial communities. Since then he has been developing his research on the ecotoxicological impact of pesticides on soil microorganisms. For 7 years he was the scientific director of a regional center for innovation and technology transfer in the agri-environment of the University of Burgundy. He is co-creator and co-leader of the microbial ecotoxicology network (EcotoxicoMic, https://ecotoxicomic.org/) and also co-leader of the Ecotox network (https://www6.inrae.fr/ecotox/). He is a scientific expert in terrestrial ecotoxicology for standardization bodies (AFNOR, ISO) and regulatory bodies (ANSES and EFSA). He is the head of the Agroecologie department (https://www6.dijon.inrae.fr/umragroecologie_eng/.)

Keynote conference - Soil microorganisms for the environmental risk assessment of pesticides: where are we and where are we going?

Soils host a tremendous biodiversity among which microorganisms which are accomplishing a wide range of functions supporting soil ecosystem services. Arable soils are threatened by agricultural practices which are still highly dependent on pesticide use. Within this context this talk will exposed how microorganisms can be better implemented in the environmental risk assessment of pesticides to better protect soils.

Microbial indicators and Quality Ratio Index for risk assessment in oil-contaminated tropical coastal sediments

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Due to anthropogenic activities, coastal sediments are prone to hydrocarbon contamination, which can be reflected in the microbial community structure and diversity. In order to determine the impact of oil contamination on the microbial assemblages inhabiting the sediment of several stations located around the Sepetiba Bay (Brazil), highly impacted by human activities, several approaches were combined. Chemical contamination factor (CF, *n*-alkanes [$CF_{n\text{-alkanes}}$] and 16PAHs [CF_{16PAHs}]) and Quality Ratio (QR) index for ecological risk assessment were combined with microbial community characterization (16S rRNA gene sequencing). Oil pollution ($CF_{n\text{-alkanes}}$ and CF_{16PAHs}) present a general decreasing trend from the internal to the external sector. The QR, a function of the microbial term (dehydrogenase/esterase enzymatic activities) and the geochemical term (TOC × CF_{16PAHs}/fine-grained content), ranked most of the internal sector stations as moderate and high risk to the local biota. The canonical correspondence analysis, explaining 55% of the microbial community composition variation (ASV level), exhibited the CF_{16PAHs} as the main factor driving the microbial community structure. Phylogenetic analysis revealed predominance of Proteobacteria (54%) phylum with a high abundance of the gamma-delta- and alpha-proteobacterial classes (29%, 21%, and 3% respectively), among which diverse members have been described as capable of degrading hydrocarbons. Among the potential hydrocarbon-degrading bacterial taxa, the most prevalent genera were *Vibrio* (12%), *Desulfatiglans* (11%), and *Bacillus* (8%). The cluster correlation analysis revealed prokaryotic taxa resistant in the internal sector, which mainly present anaerobic metabolisms linked to sulfate-reduction, methanogenesis, and hydrocarbon-degradation. The Linear discriminant analysis effect size revealed habitat-specific bioindicators according to the QR risk classification: (a) *JTB255 marine benthic group* (low risk); (b) *Bacillus* (moderate risk); and (c) sulfate-reduction *bacteria* (high risk). Overall, the microbiota structure validated the QR risk classification, revealing both useful and efficient for monitoring the sediment quality status and risk of coastal sediments.

Keywords: Risk assessment, hydrocarbon pollution, microbial ecology, Quality Ratio index

Extended spectrum beta-lactamase-producing *Escherichia coli* in surface water and groundwater of a French karst system

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Karstic aquifers represent a major drinking water reserve as 25% of the world's population uses them. Their vulnerability, due to a complex structure, combined with the diversity of anthropogenic pressures and the issue of climate change, strongly affects water quality. Their study is therefore crucial to address these societal, economic, and ecological challenges, both on a large and small scales.

TRANSKARST (TRANSdisciplinary research on KARST, SNO Karst, IR OZCAR, ZAAJ, IR RZA) and SENSAAS (SENSors and Analyses for AquiferS, Isite UBFC) are two transdisciplinary consortia for research on karst, gathering scientists and water resource managers around the functioning of the Arcier hydrosystem (Eastern France) that supplies about half of the drinking water of Besançon (117 000 inhabitants), and the development of innovative sensors to improve the knowledge of the fate of emerging contaminants in karst hydrosystems, respectively.

A holistic approach has been established to better understand *i)* the origin, dispersal, pathway, and fate of antibiotic resistance determinants in karstic aquifers and *ii)* the impact of karst on antibiotic resistance determinants. During two years (February 2020 to February 2022), numerous data have been collected on the bacteriology (extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli*), pharmaceuticals, hydrodynamics (rainfall, water level/flow rate) and physico-chemistry (electrical conductivity, temperature, turbidity, major and traces ions, isotopes) of the Arcier hydrosystem. Longitudinal monitoring of bacterial resistance and antibiotic pollution combined with whole-genome sequencing of multi-drug resistant bacteria will make it possible 1) to characterize the dynamic of multi-resistant bacterial clones, 2) to understand the fate of antibiotic resistance determinants, and 3) to establish a correlation between antibiotic pollution and the presence of multi-resistant bacteria in karst hydrosystems.

Keywords: karst, antibiotic-resistance, bacteria, antibiotic

Marine biofilms as biological indicators of the seawater chemical quality of Mediterranean French coasts

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Marine biofilms are heterogeneous biological complexes colonizing immersed biotic and abiotic surfaces. They consist in highly diversified prokaryotic and eukaryotic microbial communities embedded in a matrix of extracellular polymeric substances (EPS). Among these microbial communities, the autotrophic component is mostly represented by diatoms, which are unicellular photosynthetic micro-organisms with specific tolerance and preferences towards environmental parameters.
Because both their taxonomic diversity and pollutant sensitivity result in modulation of communities' composition in response to their environment, diatoms are spearheading the routine assessment of freshwater quality worldwide through their integration as bioindicators in biological indexes (e.g.: the Biological Diatom Index (IBD), the Specific Polluosensibility Index (IPS)). However, the potential for diatom communities in marine biofilms to be used as bio-indicators, remains unclear. This work aimed to investigate the extent to which diatom communities in biofilms could be used as a bioindicator tool for seawater quality of the Mediterranean coast. 150 polyethylene A4 plates were immersed during 3 months between March and July 2021 along the 1800 km of the French Mediterranean coast, at 50 sites selected to comprehensively represent the diversity of Mediterranean coastal contexts. Biofilms were collected from each plate by scraping and stored for further analysis.
Several analyses were conducted, including (i) Diatom communities metabarcoding, targeting the *rbcL* gene, (ii) biofilms visualization using Scanning Electron Microscopy (SEM), (iii) quantification of inorganic and organic contaminants adsorbed onto the biofilms as well as (iv) proteins, carbohydrates and uronic acids of the EPS matrix. Preliminary results demonstrate both an adsorption of metallic and organic contaminants within biofilms at environmentally relevant concentrations and morphologically distinct biofilms between ecoregions. Ongoing analyses will provide additional key elements to assess biofilms community selection and pollutant interactions to better assess the reliability of bio-indication based upon marine biofilms monitoring in coastal environments.

Keywords: Diatom, biofilm, bio-indication, pollution, marine environment

Down under the surface of the Adriatic Sea: benthic microbial communities and how anthropogenically-induced pollution affects them?

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Abstract:

Recognizing multiple anthropogenic pressures in marine coastal zones, countries of the Mediterranean region have implemented several directives focused on sustainable management of the Mediterranean Sea coastal zones. One of the essential directives is the Marine Strategy Framework Directive (MSFD), challenging countries, including Croatia, to achieve and maintain Good Environmental Status of their marine environment. The health of the marine environment is undeniably influenced by microorganisms which play a key role in functioning of marine food webs and biogeochemical cycling. The MSFD neglects the importance of microbial communities and possible changes in their function and structure under the anthropogenic influence influencing stability of the whole marine ecosystem. Project MicroLink, funded by the Croatian Science Foundation, aims to highlight the significance of microbial communities in monitoring and consequently preserving the quality of marine environment with the final goal to propose incorporation of the selected microbial attributes among MSFD Quality Descriptors. Sampling of 67 sediments was performed in seven harbors along the eastern Adriatic coast, which were previously identified as pollution “hot spots”. Sediments were comprehensively analyzed and parameters such as metal concentrations, Hg, TP, TN, TC, TBT, toxicity testing etc. were measured. To find the correlation between anthropogenic pollution and changes in the microbial communities, the project MicroLink integrates different approaches: i) multi-domain approach (analyzing changes in Bacteria, Archaea, Protists, and Fungi) and ii) multi-layer approach (changes in structure, microbial networks, abundance of functional genes and biodegradation potential). Due to continuous accumulation of pollutants, sediments and benthic microbial communities could be reliable indicators of the persisting exposure of the marine environment to anthropogenic pressures. Microbial communities should not be neglected in future strategies and measures for conservation of coastal and marine environments.

Keywords: benthic microbial community, marine sediments, anthropogenic pollution, Adriatic Sea, Good Environmental Status (GES)

Bio-indication of trophic and mining alterations and determination of New-Caledonian rivers' ecological state, using a new multimetric diatomic index (IDNC)

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New Caledonia, French local authority located in Pacific South-West, hosts various human activities that could alter rivers and littoral lagoons ecological status. Main local anthropogenic activities generating pollution are urbanization, tourism and domestic pollution; nickel extraction and processing activities; agriculture; bush fires. However, this archipelago is also a hot spot of biodiversity of high patrimonial value at the global level. In order to protect its aquatic environments and water resource, a program was carried out by a consortium associating Irstea and two private consultancy offices from 2013 to 2017, under supervision of OEIL (local water observatory) and with other co-financers support. In an initial context of largely unknown local microflora (endemism), it aimed at developing a freshwater diatomic index well suited to local biogeographical condition. Four field sampling campaigns carried out alternately at the dry and wet seasons were proceeded on 74 different stations, allowing to obtain 210 paired data (abiotic descriptors of the environment quality; corresponding diatomic inventories, made of 466 different species).

Various exploratory analyzes allowed us to understand: 1) nature and intensity of local abiotic gradients (natural /vs anthropogenic part); 2) their link with local diatomic communities distribution, helping to identify the most suitable natural framework for evaluation.

In the 2nd phase, an innovative approach mobilized the TITAN program (Threshold Indicator Taxa ANalysis) to detect ecological thresholds in the distribution of taxa, which allowed the selection of alert taxa with respect to 7 elementary metrics of biotic response to anthropization (4 related to nutrient enrichment, and 3 focused on mining impacts).

Following an average calculation by major category of alteration, the IDNC (Diatomic Index of New Caledonia) finally determines the ecological state of the rivers of New Caledonia by retaining, according to the principle of OO-AO (One Out-All Out), the worst state observed between integrated trophic metric and integrated mining metric.

Keywords: New-Calédonian rivers; benthic diatom index; nickel pollution; trophic pollution; ecological status assessment

Discussion on microbiome in ERA

Dr. Marco Pautasso, *Plant Health Risk Assessment team, EFSA, Italy*

Dr. Marco Pautasso is a researcher in the Plant Health Risk Assessment team at the European Food Safety Authority, Parma, Italy. He is interested in network epidemiology, biodiversity conservation, forest health and environmental microbiomes. He supervises the grant work evaluating the impact on/by environmental microbiomes in assessments under EFSA's remit.

He will give a talk on « **Needs and challenges for environmental risk assessment in the context of microbiome** » to introduce the roundtable discussion on microbiome in ERA.

Dr. Frédéric Debode, *Walloon Agricultural Research Center (CRA-W), Gembloux, Belgium*

Dr. Frédéric Debode is active in the development of analytical methods for the authentication of food and feed products. The methods are mainly focused on GMO detection but also on the detection of plant, animal, fungal and bacterial species. Scientific director of the Biological Engineering Unit, he has also launched activities on the study of the environmental microbiome.

Pr. Dr. Ir. Claude Bragard, *UCLouvain, Belgium*

Pr. Dr. Ir. Claude Bragard has been a professor of plant pathology at UCLouvain, Belgium, since 1998. He was the president of the Earth & Life Institute, a research group of ca. 50 principal investigators and 350 researchers from 2015 to 2021. Dr. Bragard's research centers on the interactions between plants and pathogens, comparative genomics, identification of microorganisms in soils and their use as bacterial biocontrol agents, and the detection and identification of new plant or fungal viruses. C. Bragard has been a member of the EFSA Scientific Committee since 2019.

Dr. Véronique Ninane, *Walloon Agricultural Research Center (CRA-W), Gembloux, Belgium*

Dr. Ir. Véronique Ninane is an agricultural engineer and studied the bacterial microbiota with pioneering techniques of high-throughput sequencing in the frame of her PhD (2008). She has been responsible of the CRA-W laboratory for dairy microbiology since 1998. Besides doing microbial research on milk, milk products and dairy starters, she implemented the ISO 17025 standard requirements in the laboratory to properly lead the analyses performed to assess raw milk hygiene for official duty by routine laboratories. The CRA-W laboratory for dairy microbiology took part to the National Reference Laboratory for milk and milk products (MMP) until EURL-MMP ended. These last few years, she has extended the laboratory field of research to soil bacterial communities, which propelled her to join an ongoing regional project (ANTAGONIST) aiming at finding environmental bacteria and conditions that can limit the spread of fungal wheat pathogens in 2018. She has also accumulated knowledge on the soil microbiome in the context of an EFSA project aiming to evaluate the use of environmental microbiomes in risk assessment.

Keynote

Dr. Kathrin Fenner, *Department of Environmental Chemistry, Eawag, Switzerland*

Dr. Kathrin Fenner is Associate Professor of Environmental Chemistry at the Chemistry Department of the University of Zurich (Switzerland) and a senior scientist and group leader at the Department of Environmental Chemistry at the Swiss Federal Institute of Aquatic Science and Technology (Eawag). Her research focuses on experimental and model-based approaches to gain an in-depth understanding of chemical persistence in the environment. The goal of her research is to not only improve current hazard assessment schemes but also provide tools and design principles for better degradable compounds and efficient approaches to bioremediation. In 2015, she received an ERC Consolidator grant to support this line of research. She has published more than 90 scientific research articles in the field of environmental science. Kathrin chairs the section of “Chemistry and the Environment” of the Swiss Chemical Society, has been member of several ECETOC expert groups and has served as Associate Editor with Environmental Sciences: Water Research & Technology up until recently.

Keynote conference - Drivers of contaminant biotransformation in the aquatic environment

Degradation by aquatic microbial communities reduces exposure of water bodies to chemicals. The ability to accurately predict rates and products of microbial biotransformation for a broad variety of chemicals is therefore essential not only for chemical risk management but also in the context of developing new, environmentally safe chemicals. Prediction requires a thorough understanding of the various factors leading to observed biotransformation half-lives and pathways under different environmental conditions.

In my talk, I will discuss experimental and data mining approaches that we employ to improve our understanding of why and how biotransformation efficiency differs between microbial communities and how these differences are modulated by chemical structure and environmental conditions. Specifically, I will report on two large-scale biotransformation studies, one with activated sludge communities and one with river biofilm communities grown with varying proportions of differently treated wastewater. In both cases, I will go from discussing how conditions influence biotransformation of structurally similar chemicals to a more in-depth analysis of trying to link specific enzymes to biotransformation of specific chemical classes.

Dynamics of the total and active bacterial communities in a bioremediation field-trial of As-rich acid mine drainage

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Oxidation of sulfide minerals present in mine wastes leads to the formation of acid mine drainage (AMD). These acid effluents contain elevated concentrations of metals and metalloids and have negative environmental consequences. AMD can be a source of arsenic (As) pollution for aquatic environments.

To treat As-rich AMD, we developed a bioremediation system based on microbially mediated iron oxidation and precipitation. Arsenic is removed from the water by co-precipitation with iron. Biological As(III) oxidation into As(V) enhances its removal by adsorption on Fe(III) precipitates. This study aimed to characterize the dynamics of the bacterial communities developed in two bioreactors fed in-situ with AMD and monitored during one year. Bioreactors were filled with two different biofilm carriers: (i) plastic support (PS) or (ii) a mix of wood chips and pozzolana (WP). Up to 80% of As removal was achieved, with no significant difference between the two bioreactors. Total and active bacterial communities (planktonic and sessile) were analyzed by 16S rRNA metabarcoding. Arsenite-oxidizing bacteria were quantified by qPCR and RT-qPCR targeting *aioA* gene.

At the beginning of the monitoring, distinct bacterial communities colonized the two bioreactors. After three months, the communities evolved towards similar structures. Active communities were dominated by Fe-oxidizing bacteria. The bioreactor filled with WP, characterized by a higher alpha diversity, showed higher stability in its performances towards perturbations compared to the one filled with PS. No clear differences were observed between planktonic and sessile bacteria, suggesting that both communities contributed to the key processes involved in the bioremediation. For the first time, we evidenced the expression of *aioA* gene in bioreactors, corroborating the importance of arsenite oxidation in the treatment process.

The robustness of the treatment system regarding seasonal variations and operational conditions shows great potential for further scale-up and extrapolation of the treatment to other AMD with similar properties.

Keywords: Bioremediation, mine waste water, biofilm carrier, arsenic-oxidizing bacteria

Role of micro-organisms in the leaching of critical metals from tungsten mine wastes: from the microscale to the field scale

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The availability of primary resources will continue to be a growing need to satisfy the increasing global demand for raw materials, with the consequence of the production of waste products from exploration and mining activities. An innovative approach is to consider tailings/wastes from mining in a circular economy concept, as secondary raw materials.
The REVIVING project has been developed in this context, with coupled fundamental and applied approaches, with the objective of obtaining optimized experimental models for efficient recycling of critical metals from mining wastes, based on the manipulation of the indigenous tailing's microbiome.
We tested different leaching processes of metals of interest (Cu, Mn, Mg, Zn, W and Mo) from the mining waste of Panasqueira (Portugal). A first series of batch experiments was carried out with four bacteria isolated from this waste at increasing cell concentrations (107, 108 and 109 cells/ml) and under variable physiological conditions (live, dead, with nutrients...). Another batch reactor is also currently applied to evaluate the acidophilic leaching process via ferro-oxidizing and sulfo-oxidizing micro-organisms enriched from the Panasqueira mine waste. Results from column reactors showed a limitation of the mobility of bacterial cells in the reactor due to the very small waste grain size leading to a strong filtration of the cells. The optimization of the cell transfer process is underway with a system dynamics approach in columns with different filling materials (waste, residues...) and experimental conditions (flow, saturation, geochemistry, microbiology...).
The continuous monitoring of physico-chemical parameters (pH, O2, salinity and concentrations of metals and major ions...) and biological parameters (cell density by cytometry, qPCR and diversity by DNA-metabarcoding) will allow the identification of the dominant processes for a better understanding of the bioleaching phenomenon and therefore to design an efficient system for recycling metals from mining waste on a large scale.

Keywords: bioleaching, bacteria, mining wastes, eco-engineering, critical metals

Microbial role in plastic biodegradation in the marine environment: the case of polyhydroxyalkanoates

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Among actual bioplastics, polyhydroxyalkanoates (PHA) has raised great interest as a substitute to persistent petroleum based plastic materials. Naturally produced by numerous bacteria as a form of carbon storage, PHA show good signs of biodegradability in natural ecosystems.

Here, we evaluate the influence of the chemical structure (eight formulations of short and medium-chain-length PHA) on the biodegradability of PHA in the marine environment. We used an original miniaturized experimental design that allowed transdisciplinary analyses (dissolved inorganic carbon production, oxygen consumption, heterotrophic bacterial activity, microbial biomass and diversity, molecular weight, production of oligomers and monomers). After 30 days of incubation with a natural community, we observed different signs of biodegradability for the eight PHA. In particular, higher microbial activity was monitored in short-chain-length (scl) sample than in medium-chain-length (mcl).

To better understand scl-PHA biodegradation by marine microbes, radio-labeled ^{13}C-PHB was incubated with natural communities for 90 days. Microbial activity and biomass were monitored, and DNA was extracted on days 5, 13, 30 and 90 of incubation. DNA-Stable isotope probing (DNA-SIP) was used in order to describe degrading and opportunist communities. Metabarcoding analyses highlighted taxonomic differences between 13C and 12C fractions, and metagenomic analyses allowed us to decipher the functional difference between the two fractions.

Keywords: plastisphere, biodegradation, polyhydroxyalkanoates, DNA-stable isotope probing

Identifying potential blockbuster trifluoroacetate precursors

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Trifluoroacetate (TFA) is a substance identified by European environmental protection agencies as a major pollutant of concern. As a very persistent and highly mobile substance, TFA easily enters the water cycle, where it accumulates over time. To date, there are no real practical and economical solutions for the subsequent removal of TFA. Thus, reducing the input of TFA precursors into water cycle is seen as the most reasonable measure for a sustainable water management. However, knowledge of where TFA exactly comes from, and which sources lead to elevated concentrations is currently still limited. In addition to being emitted as a base chemical, TFA may be formed from any chemical whose molecular structure includes a C-CF_3 moiety. At present, almost 2000 potential precursor substances are known. Of relevance are chemicals, including pesticides, biocides, and pharmaceuticals, that are released into the environment in significant quantities during their use. Therefore, in this study we investigate the biotransformation of 15 blockbuster chemicals (herbicides and pharmaceuticals with C-CF_3 moieties) as to whether TFA is produced as dead-end metabolite. The selected, potential TFA precursors are provided as carbon sources to a representative panel of aquatic and soil bacteria with or without additional non-fluorinated carbon sources to investigate their metabolic and co-metabolic biotransformation. Ion and liquid chromatography/mass spectrometry are used to quantify parent compounds removal, as well as the formation of fluoride, TFA and other transformation products. Furthermore, gene expression analysis of selected strains will be conducted to identify key enzymes which are expressed during the biotransformation of these chemicals into TFA.

Keywords: Trifluoroacetate precursors, herbicides, pharmaceuticals, biotransformation

How can multi-contamination and redox variation affect metformin biodegradation in continental waters at the sediment-water interface?

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Environmental and human exposure to micropollutants in continental surface waters is a source of concern. Micropollutants reach surface waters due to inefficient wastewater treatment and leaching, and are biologically active at low environmental concentrations. Micropollutants include biocides (*e.g.* metolachlor, terbutryn) and active pharmaceutical ingredients (*e.g.* metformin). Micropollutants dissipate through biotic and abiotic processes at the sediment-water interface, although their behavior and microbial actors involved in degradation remain largely unknown.
Here, we examined the dissipation of metformin, metolachlor, and terbutryn, either alone or in mixtures at the sediment-water interface, in biotic or autoclaved microcosm experiments, and associated bacterial acclimation. Preliminary results suggest different patterns of metformin dissipation in biotic and autoclaved experiments, under oxic or anoxic conditions or undergoing different changes in redox conditions (*i.e.* shifts from oxic to anoxic, and from anoxic to oxic conditions). Dissipation kinetics showed no significant difference under mono- or multi-contamination regimes. Metformin biodegradation was evident, with only 4% metformin remaining after 41 days in biotic experiments, and biodegradation rate increasing upon renewed exposure to metformin, with <10% metformin remaining after 28 days. In contrast, 60% of the metformin remained in the autoclaved experiments after 41 days under both oxic and anoxic conditions, with metformin accumulating upon renewed exposure.
The composition and dynamics of microbial communities in these experiments are currently under investigation. Obtained data may contribute to a better understanding of the role of environmental conditions and associated microbial communities in micropollutant dissipation at the sediment-water interface of continental waters.

Keywords: micropollutants; sediment-water interface; bacterial acclimation; multi-contamination; integrative approach

Metagenomics, metatranscriptomics and statistical learning to enhance the remediation of oil sand processed water in Northern environments

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The mining of oil sands significantly contributes to the Canadian economy. However, the extraction process generates large volumes of oil sands process-affected water (OSPW) that contain a broad class of organic compounds including naphthenic acids (NAs). One potential approach to treat OSPW is by using constructed wetland treatment systems (CWTS). However, at the center of CWTS is a complex interaction between aquatic plants and sediment-, water- and plant-associated microorganisms. Our goals are to 1) understand and describe these interactions, 2) use supervised learning to create microbial-based models, and 3) inoculate specialized microbial communities to optimize NA removal in CWTS. We therefore applied genomic-based methods in a mesocosm-scale CWTS. The plant species *Carex aquatilis* was planted into quadruplicates mesocosms where OSPW was pumped at a constant flow. Metagenomic and metatranscriptomic data from the substrate, the rhizosphere and the endosphere of the plants were analyzed at different time points during the remediation process. Results from these analyses were compared with those obtained from unplanted mesocosms in order to understand the metabolic pathways involved in the degradation of NAs that are specific to the various compartments of the plant environment. The exploration of genomic features with supervised learning algorithms will let us regress the degradation of NAs on the microbial community composition, harnessing the predictive power of microbes. Overall, the novel combination of metatranscriptomics, metagenomics and statistical learning will allow us to identify novel key genes involved in the degradation of NAs and to increase our understanding and control over CWTS bioremediation technologies.

Keywords: phytoremediation, bioremediation, metagenomics, statistical learning, OSPW.

Growth condition dependant glyphosate degradation in *Ochrobactrum pituitosum* strains

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Glyphosate is an increasingly used herbicide, whose non-specific targeting of a crucial amino acid pathway in plants has led to widespread usage in conjunction with genetically modified, glyphosate tolerant crops. There are, however, increasing studies demonstrating the potential negative impacts of glyphosate (GLP) on both macro- and microecology. Current knowledge gaps include the specific effects of GLP on the soil microbiome's structure and function, and the role that GLP biodegraders play in altering its impacts. Finally, the conditions required for soil microorganisms to degrade GLP and the pathways involved in GLP biodegradation remain largely unknown. Here we show that glyphosate degradation in two strains of *Ochrobactrum* is conditional on the presence of a specific carbon source, glutamate, and the lack of phosphate in the medium. These *Ochrobactrum* strains, enriched and isolated from agricultural soil in Southwestern Germany, are closely related to *Ochrobactrum pituitosum* and are capable of degrading GLP *in vitro* at a maximum rate of 94 mg/L*d with a k_m of 4 mg/L when provided with glutamate. Other carbon sources, such as glucose or succinate, failed to stimulate growth or GLP degradation. When supplied with both GLP and organophosphate, GLP degradation is restricted to sub 0.25 mg/L phosphate concentrations. Transcript-to-gene ratios demonstrated that these strains degrade GLP through the sarcosine pathway where the *phnJ* gene attacks the C-P GLP bond to produce sarcosine and phosphate. They cannot, however, utilize GLP as their sole carbon source, thus, the sarcosine pathway is incomplete. While further research is required to link these results to GLP degradation mechanisms in soil microcosms and the environment, they demonstrate that GLP degradation relies on specific environmental conditions. Thus, a careful characterization of GLP-degrading bacteria, coupled to the environmental relevance of their growth conditions, is essential to apply *in vitro* GLP-degraders as putative bioremediators.

Keywords: Glyphosate, genomics, degradation, *Ochrobactrum*

Increased temperature and light availability accelerate the decomposition of glyphosate by stream biofilms

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Climate change projections suggest an increase of temperature by 1 to 3 °C in 2100 while changes in land use will tend to reduce vegetation cover in riparian zones. Variations in these two environmental factors (temperature and light) has been shown to strongly influence biofilm communities, and probably their ability to mitigate pesticides load in flowing waters. Our study aims to determine how variations in water temperature and light availability affect the capacity of the biofilm to degrade glyphosate. With this purpose, we conducted a microcosm study in which two levels of water temperature (Ambient = 19-22 °C and Warm = 21-24 °C) and three levels of light (Dark = 0, Intermediate = 600, High = 1400 µmol photons m-2 s-1) were tested for 21 days on biofilms. Results show that the highest rates of glyphosate dissipation (DT50 = 6.4 ± 0.69 days) and AMPA production (43.57 ± 6.43 µg L^{-1}) were measured in biofilms subjected to warm temperature and high light (WARM_HL). Indeed, biofilms from the WARM_HL treatment showed the highest beta-glucosidase : leucine-aminopeptidase and beta-glucosidase : alkaline phosphatase extracellular enzyme activity ratios and the lowest biomass C:N ratios evidencing that high light availability and warmer temperatures exacerbate the decomposition of organic C compounds in detriment of N or P compounds in microbial communities. This enhanced C-compounds decomposition could be linked to the enhanced transformation of glyphosate into AMPA and glyoxylate, and the subsequent use of glyoxylate as C source for microorganisms. Co-occurrence network analysis revealed that the highest modules complexity was observed in the WARM_HL treatment, the strongest interactions being observed between bacterial taxa belonging to Burkholderiaceae, Moraxellaceae and Mycobacteriaceae families. This study combines, for the first time, ecoenzymatic stoichiometry and xenobiotics biodegradation approaches to understand the functioning of biofilmcommunities in polluted streams.

Keywords: herbicide, biodegradation, global change, aquatic bacteria, network analysis

Keynote

Dr. Robert S. Marks, *University Ben Gurion, Israel*

Prof. Robert S. Marks is a Full Professor at the Ben-Gurion University of the Negev, Israel, at the Department of Biotechnology Engineering, where he created the interdisciplinary Biosensors Laboratory. He has affiliations at The National Institute for Biotechnology in the Negev and the Ilse Kats Centre for Nanotechnology. He is presently the Chair of the Department. He was previously Visiting Adjunct Professor in the NTU-MSE, and a program co-founder and coordinator of the multidisciplinary Singapore NRF CREATE program “Nanomaterials for Water and Energy Management”. Prof. Robert Marks has co-founded, and is the originator of the technologies, for 7 deeptech start ups in Israel, USA, Singapore. Prof. Marks has extensive experience in developing new immuno-biosensors. His work also includes environmental toxicology, such as monitoring water pollution via fiber-optic probes glowing in the presence of toxicants through their associated luminescent bacteria (water on-line monitoring and air toxicity monitoring).

His group participated in developing biochips, enzyme nanolithography, nanofluidics, light-sensitive tissue sutures for surgery, and anti-biofilm materials. Most of his developed biosensors were published and validated with real life samples. He is the Editor-in-Chief of a 2007 2-volume Wiley Handbook in Biosensors and Biochips, edited 5 other books, and founded the Pan Stanford book series ‘the high-tech of biotech’. His is the author of 185+ papers. He has 8 issued patents, as well as, a dozen filed. He has organized 21 international conferences. He was a visiting Professor in lengthy stays in France (Grenoble, Troyes, Paris), Italy (Udine), Finland (Turku), Singapore, USA (Baltimore) and China (Chengdu and Nanjing). He has created an extensive network of collaborations worldwide in most continents, most reflected in publications.

Keynote conference - Cell biosensors: from microorganisms to the systems applied to environmental issues.

Microbial bioreporter organisms are versatile sensing entities that enable toxicity measurements as living organisms in environmental samples. These may either be natural or engineered organisms. These measure bioavailable toxicants, and are, however, restricted to laboratory use. If one uses a panel of differently engineered bioreporters to which the signalling system is integrated to promoters of varying sensibilities (genotoxicity, cytotoxicity, membrane toxicity,...), then one can can fingerprint detection of unknown samples in the sample matrix. In order to make measurements to the field these may be intimately integrated together with a transducer system via bio polymer hydrogels or other chemical entrapment. The advantage of using biosensor systems is that one can produce a fully self-contained system, which is field-operable, and can provide on site measurements. Of particular interest, is the fact that one system to which these bioreporters could be integrated, were fibre-optic transducers. Such as device with at its far-end tip exposing a bacterial bioreporter-coated gel enables the measurement of bioavailable toxicants, without any prior sample treatment, of simply suspended sediment samples. Other systems exist where they have been integrated to biochips, to on-line continuous monitoring and more. An overview of the field will clearly show its potential and future possibilities.

Sensing the environment by distributed microbial sensors: detection of buried landmines as an example

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Whole-cell sensors may be constructed by the fusion of two molecular components: a sensing element (usually a gene promoter induced by the target compound(s)) and a reporting element, gene(s) the activity of which can be monitored quantitatively. These are integrated together into a live cell, generating dose-dependent physical (luminescent, fluorescent, colorimetric, electrochemical) responses to pre-determined conditions. For over two decades, we and others have employed this principle to design and construct microbial bioreporter strains for the sensitive detection of (a) groups of compounds sharing chemical characteristics (e.g. heavy metals, halogenated organics etc.), (b) global biological effects on living systems (e.g. toxicity or genotoxicity), or (c) specific chemicals of environmental concern (e.g. trinitrotoluene). In this presentation I will describe the application of the latter approach for the remote detection of buried landmines.

The major technical problem in alleviating the enormous humanitarian problem created by buried munitions worldwide is not their actual removal but rather their location. Current landmine detection methodologies are not much different in principle from those employed 80 years ago in WWII, in that they require actual presence in the minefield, with obvious risks to personnel and equipment. Other limitations include an extremely large ratio of false positives, as well as a very limited ability to detect non-metallic mines. We have previously described (Belkin et al, Nat Biotech. 2017, 35:308-310) the remote detection of buried landmines using alginate-encapsulated fluorescent microbial (Escherichia coli) bioreporters spread over the tested minefield. Since then we have modified the system to one based on bioluminescent (rather than fluorescent) bacteria, and have employed several synthetic biology approaches to significantly enhance their major performance parameters: higher signal intensity, faster response time, and lower detection threshold of the target explosives.

Keywords: Whole-cell sensors, bioluminescence, landmines

Development of biosensors for the assessment of seawater toxicity: Choice of the bioreporter

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The preservation of the oceans is a major issue of the 21st century. In 2000, the Water Framework Directive harmonized European regulations on water management to protect and restore the quality of aquatic environments, including the marine environment and the coast [1]. If currently, few methods are available for the marine water management [2], there is a real need for global diagnostic tools for the evaluation of cellular toxicity. The aim of our study is to develop a microbial biosensor, using not just a single cell (like currently developed approaches) but a set of representative microorganisms from the autochthonous microbial population that receives the pollution. The principle of our approach is to determine the metabolic functions lost by the microbial community after an exposure to different families of toxics and to identify the bacterial families that carry these lost functions. The first step of this project aimed to expose the microbial community sampled from Roscoff (Finistère, France) to different pollutants characteristic of the marine ecosystem [3][4][5][6]. For this, we have set up an exposure in microcosms. They were composed of sampled seawater exposed to different concentrations of anthracene, benzene, copper chloride, chlorpyrifos and PFOA. After 1 month of exposure, each condition was subject to Phenotype Microarrays for Microbial Cells technology from Biolog®. Thus, it was possible to determine the lost of metabolic functions regarding the cycle of carbon, nitrogen, sulfur and phosphorus following exposure. In parallel, the phenotypic profile of 40 bacterial strains from the same environment was determined with the same tool and for the same biogeochemical cycles. Specific phenotypic profiles were obtained for each of these strains. The selection of bioindicators was carried out thanks to the specific functions that they represent and which are lost following exposure in microcosms. The next step will be the integration of such microbial set into a bioassay and after a biosensor.

Acknowledgements We would particularly like to thank the Roscoff Marine Station for their collaboration. Thanks to the National Research Agency and to the Martera program.

Keywords: seawater, global toxicity, microbial bioindicator, microbial functions

Communication abstracts
Posters

Biogeography for identifying microbial bioindicators reporting ecological status of coastal wetlands in the SUDOE area

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Coastal wetlands represent environments with important ecological issues, composed of a vast diversity of habitats and species and supporting many human activities. However, these environments are in sharp decline, and their deterioration threatens the benefits they provide. Indeed, climate change and human activities tend to modify the functional balance of these environments challenging the management choices. At present, ecosystem-based decision support tools are urgently needed to define management levers for preserving these environments and their biodiversity. It is in this context that the European BIOMIC project (Interreg SUDOE) aims to address this need by developing a toolbox of microbial and trophic bioindicators to assess the ecological status of coastal areas. BIOMIC proposes a biogeography analysis covering 53 coastal wetlands in the SUDOE area (Mediterranean and Atlantic coasts: France, Spain and Portugal) for the development of benthic microbial bioindicators based on environmental DNA approaches. A broad variety of environments have been selected, considering the representativeness and variety of coastal environments of the SUDOE area, and covering coastal management problems, coastal area rehabilitation and biodiversity protection. Microbial communities (bacteria, archaea, fungi) were characterized through 16S and 18S rRNA genes sequencing, and physicochemical parameters and pollutants (metals, pesticides, hydrocarbons) content were determined. Relationships between microbial communities and environmental parameters were evaluated via multivariate analyses including linear discriminant analyses effect size (LEfSe), indicator values (IndVal), threshold indicator taxa analysis (TITAN), MicrogAMBI and network analyses. Potential microbial bioindicators for monitoring the ecological quality of ecosystems in the SUDOE area were identified. Ultimately, BIOMIC aims to implement the use of these bioindicators in existing environmental quality assessment regulations, in order to provide an integrative toolbox combining different food web communities in water and sediment compartments. The identified bioindicators will then be validated by seasonal monitoring and mesocosm experiments.

Keywords: bioindicators; SUDOE coastal area; toolbox; ecosystem health; environmental management.

DIMITRA: an upcoming database on effects of pesticides on the soil microbiome

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Pesticides are still a cornerstone of modern agriculture. Although improvements in their development in the last couple of decades have tried to reduce associated negative effects, studies still indicate that their use might entail a risk for non-target organisms, including soil microorganisms. Soil microorganisms play important roles in maintaining soil health through regulation of nutrient cycling, structure, and diversity of soil flora and fauna. The potential effects of pesticides on soil microorganisms have attracted lots of attention in recent years, as reflected in the increasing number of publications on this topic. However, this wealth of knowledge remains fragmented and should be assessed in a systematic way to allow the development of tools that would be used for defining new target organisms in the soil microbiome. We aim to develop, a novel database which will gather and consolidate all studies to date looking at the effects of pesticides on different soil microbial endpoints. This database is expected to provide (i) pesticide specific studies detailing the effect on soil functional microbial endpoints and (ii) access to curated amplicon sequencing datasets for bacteria and fungi from relevant studies, that would be used for multi-purpose meta-analysis using standard and higher tier ecotoxicology approaches to define relevant microbial taxa that are highly receptive to pesticide applications. In the longer term, this amplicon sequencing database would be expanded to functional microbial groups, such as ammonia-oxidizing microbes, arbuscular mycorrhizal fungi and even protists.

Keywords : pesticides; microbiome; database; function; diversity

Use of metaproteomics for the taxonomical and functional analysis of poplar roots and endophytes

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Phylopeptidomics (1) is an innovative metaproteomics approach that allows quick taxonomical identification of microorganisms even in complex mixtures and their relative quantification, and can give rich insights into the functioning of the biological system. For this purpose, the proteins present in a given sample are proteolyzed and the resulting peptides are analyzed by high-resolution tandem mass spectrometry like with bottom-up proteomics. The detected peptides are further assigned to peptide sequences and then to taxonomical data. This without a priori strategy has been shown successful to identify prokaryotes and eukaryotes from numerous and complex samples (2-5). This powerful technology successfully enables protein and microorganism identification.

Endophytes are the microorganisms that reside within different tissues of a host plant in a commensal or beneficial manner. Plant endophytic community is shaped by many factors, one main being the soil compartment. Indeed most of the endophytes derive from the soil environment. They can confer stress tolerance to the host plant and their role is essential.

The present study focuses on the use of metaproteomics to analyze roots of poplar grown in diverse soils. We report the endophyte microbial community and global proteome obtained by tandem mass spectrometry analysis from roots of *Populus canadensis* grown in potting soil and in a soil from a contaminated industrial site. The analyses were performed in quadruplicates for powerful statistics. At the taxonomical level, major changes were observed in two phyla: Proteobacteria and Actinobacteria. At the proteome level, 289 proteins showed significant abundance changes between the two conditions. The results of this analysis integrated at a more integrative level, i.e. the functional level, will be presented. These results demonstrate the interest of the methodology and the strategy deployed here for the understanding of endophyte mediated molecular processes.

Keywords: Tandem mass spectrometry; metaproteomics; shotgun proteomics; endophytes; poplar

Revealing new taxa and their microbial functions by NGS technologies: example of the cocoa fermentation microbiome

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The emergence of next-generation sequencing (NGS) opened perspectives to the in-depth characterization of microbiological diversity with the application in metataxonomics. This work reveals a complex microbial community in cocoa-producing regions explored by NGS as a powerful tool for microbial identification, enlightening their function and environmental risk assessment. The identified microorganisms were separated into three groups: yeasts, filamentous fungi and bacteria that were further subdivided into four subgroups: acetic acid bacteria (AAB), lactic acid bacteria (LAB), spore-forming bacteria and enterobacteria. Recently, metataxonomic analyses performed on fermented cocoa beans from Brazil, Ivory Coast, Cameroon, Ghana, Nicaragua, and Colombia unveiled the presence of over 330 genera and species. From these, 99 species (30 %) were reported for the first time by NGS in fermented cocoa beans (e.g. *Brevibacillus* sp., *Methylobacterium* sp., *Novosphingobium* sp., *Acinetobacter radioresistens*). Yeast, LAB and AAB are mainly associated with cocoa bean fermentation and their role is well defined. Nevertheless, the new cocoa-related microbial taxa enable better understanding of the process and more efficient control against undesired species. *Gluconobacter* species is undesirable, indicating a fermentation of poor quality through the production of gluconic acid and off-flavors. The appearance of enterobacteria species underlines the importance of ensuring the absence of contaminants. The genera *Erwinia*, *Tatumella*, and *Pantoea* are derived from soil and some of these species are considered as phytopathogens. NGS detected 11 species of spore-forming bacteria *Bacillus* and *Paenibacillus*. *B. subtilis* may contribute to the undesirable acidity and off-flavors of fermented beans. The role of spore-forming bacteria and filamentous fungi remains unclear. Additionally, the isolation and selection of 26 non-*Saccharomyces* yeasts producing the ß-glycosidase enzyme was a useful strategy to obtain species with potential for flavor production. Furthermore, combination with other omics approaches (proteomics, transcriptomics, and metabolomics) is essential to gain a profound understanding of community dynamics and function.

Keywords: Pyrosequencing; Illumina platforms; microbial diversity

Natural fluctuation of metabolome and photosynthetic yield sensitivity of a periphytic biofilm exposed to a model herbicide

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In the context of increasing aquatic chemical pollution, the study of microbial communities such as periphytic biofilms improves the ecological dimension of biomonitoring. Despite a growing knowledge of the effect of contaminants on biofilms, there is a paucity of information about the fluctuation of sensitivity of these communities to chemical stress according environmental conditions. Moreover, usual endpoints often lack of sensitivity and focus only on one component of the biofilm (e.g. autotroph). To tackle this issue, untargeted metabolomics can provide a comprehensive picture of the sensitive molecular response prior physiological/functional responses. In this context, the present study aims to characterize the changes of sensitivity of freshwater periphyton over months through the combined measurement of the photosynthetic yield (ΦPSII) and the metabolomics response based on high-resolution mass spectrometry (HRMS). To do so, periphytic biofilms (4 weeks old) were sampled on a pilot site and exposed during 4h in controlled conditions to a range of six concentrations from 0.3 to 30 µg/L of Terbuthylazine (TBA). The sensitivity of periphyton to chemical was assessed through the determination of Benchmark Doses with a standard deviation of 1% compared to the control (BMD1sd) and their cumulative distribution for metabolomics data. The results indicate a change in the sensitivity over the months for both endpoints. Indeed, BMD1sd of ΦPSII vary from 5.5 to 13.8 µg/L. In the same way, multivariate analyses on metabolomics data showed a response at 0.3 µg/L of TBA. This higher sensitivity of the metabolome was confirmed since 50% of the signals reacted before the ΦPSII BMD1sd. Overall, this study shows that sensitivity of periphyton to chemical stress fluctuates along the year, highlighting the need to consider it for interpretation of field studies. In addition, this work confirms the higher sensitivity of metabolomics against photosynthetic response. The continuation of these investigations along the year will provide additional insight on the influence of environmental parameters on the sensitivity of periphyton to chemical stress. In addition, this should contribute to the identification of which metabolites and pathways are sensitive to environmental conditions against those specifically impaired by the chemical stress.

Keywords: Metabolomics; Freshwater periphyton; chemical stress; Sensitivity shift; Photosynthesis

Impacts of long-term waste water irrigation on the soil microbiome and resistome in Tunisian agricultural soils

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Agricultural wastewater reuse is a common practice in arid regions of northern Africa. We aimed to identify the impact of long-term (20-25 years) and short-term (4-5 years) treated waste water irrigation on agricultural soil properties in a Tunesian case study (*Cebela Borj Touil*). Soils were sampled from replicated irrigated plots (n=4) and from non-irrigated control plots (n=4). Soil pH, salinity, and heavy metal concentrations increased significantly with increasing duration of waste water irrigation practice with total concentrations of Cu, Zn and Pb in long-term irrigated soils reaching mean levels of 55, 120 and 63 mg/kg, respectively. These results were mirrored by significantly increased bioavailability of Cu and Zn as measured by whole-cell bacterial bioreporters. Pollution-induced community tolerance (PICT) is currently being investigated using the [3H]Leucine incorporation technique for metals (Cu, Zn and Pb) and selected antibiotics. We further plan to carry out 16S rRNA gene amplicon sequencing and high-throughput qPCR of antibiotic resistance genes and these data will be presented at the conference.

Keywords: TWW irrigation; agricultural soil; Bacterial communities; Heavy Metals; Antibiotic

Impact and fate of the fungicide tebuconazole in a biofilm/water system: Chemical transformation, fatty acids and taxonomic-related changes

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Fluvial biofilms harbor remarkable adaptability and catabolic potential. Tebuconazole (TBZ) is a triazole fungicide that inhibits cell growth by blocking the ergosterol biosynthesis pathway and is commonly found in rivers as a micropollutant. This study aims to assess the impact of TBZ on community structure, the biofilm fatty acids, and the role of pre-exposure history on microbial dissipation of TBZ. Stream biofilm was pre-exposed for 24 days to 10 and 100 ng/mL of TBZ in a semi-static microcosm test system. Impacts on community structure were assessed as changes in algal, bacterial, and fungal biomass. Impacts on fatty acid profiles were studied using GC-MS analysis. The dissipation capacity was studied using 96h TBZ kinetics tests. LC-MS analysis of TBZ and its metabolite hydroxy-tebuconazole (TBZ-OH) in the water column revealed that microbial biotransformation of TBZ was limited in both non-pre-exposed biofilms and pre-exposed biofilms to TBZ. Interestingly, our results show that the formation of TBZ-OH has a dose and time-dependent pattern. The total algal biomass was slightly impacted by TBZ. Minor changes in the algal community composition were observed in response to TBZ treatment, being cyanobacteria the most sensitive group to TBZ exposure. The quantification of ergosterol confirmed the mode of action of the fungicide, by showing a decrease in ergosterol concentration in exposed biofilms and therefore allowing to link decrease in fungal biomass to TBZ or TBZ-OH toxicity. Phospholipid-derivated fatty acids will be used as markers for bacterial biomass and full fatty-acid profiles will putatively reveal metabolic responses to the TBZ exposure. Planned TBZ-OH toxicity tests will allow to assess the contribution of this product to the overall toxicity observed. Taken together, these findings indicate that negative impacts from TBZ and TBZ-OH might be common in river ecosystems.

Keywords: pesticides; microbial communities; transformation; ergosterol

Microbiome and enzymatic activity as bioindicators of soil pollution by pesticides

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In a world where our population is growing exponentially, modern agriculture faces challenges regarding its productivity and environmental impacts. Pesticides, including fungicides, herbicides and insecticides, are chemicals that are amply used to increase both productivity and quality of agricultural products. However, this use compromises soil health, including its biological, chemical and physical traits. Microbial communities are an important part of soil health, playing a big role in determining several of their physical and chemical properties, as well as what organisms can grow on it. Studies have shown that pesticide application often decreases the number of microorganisms and their diversity, altering the soil enzymatic activity. However, it is still unknown if there is a common and predictable effect of different pesticides on microorganisms (and concomitantly soil enzymatic activity) across different types of soils and habitats. To assess this, we conducted a meta-analysis focusing on studies that quantified the effect of pesticides on soil microorganisms' abundance and enzymatic activity. Preliminary results suggest that variables such as habitat, type of soil and time after pesticide application can shape the effect of pesticides in soil microbiome and enzymatic activity, reducing or increasing its effect. In the long-run, results from this study will allow to draw predictions on how soil microbial communities respond to pesticide application and use the soil microbiome as a bioindicator to identify pesticide pollution in the soil. This knowledge will be crucial to develop better agricultural practices and contribute to a sustainable development of agriculture and our soils.

Keywords: soil microbiome; pesticide pollution; enzymatic activity; bioindicator

Selection of environmentally toxic aquatic microbial communities by bentazone herbicide pressure

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We isolated microbial consortia from pond waters and rainwaters contaminated with bentazone herbicide at levels ranging from 0.48 g/L to 48 g/L, i.e. ten folds more or less than the dose applied in the field. We further tested four consortia for their potential toxicity against the freshwater green algae *Raphidocelis subcapitata* that play a very important role as primary producers in aquatic systems. For that, we incubated a basal culture broth, composed of tryptone (10 g/L) and salt (5 g/L), with the consortia, in the dark and for 11 days. After microbial incubation, we sterilized the culture broth by filtration through a filter of 0.2 µm pore size and added minerals and trace elements that act as growth factors by mixing the filtered broth with the Algaltoxkit F (Microbiotests, Gent, Belgium). Surprisingly, the culture broth incubated with the consortia drastically inhibited algal growth; in these culture broths, the algae grew from two to seven times less than the algae cultivated in the control broth, i.e. the basal culture broth maintained in the same conditions but without any microbial consortia. Three of the tested microbial consortia produced a more toxic broth for the algae than the control broth added with 4.8 g/L bentazone. The microbial composition of the consortia, determined by high-throughput sequencing of the 16S rDNA and ITS regions for bacteria and fungi respectively, revealed common environmental taxa: *Pseudomonas*, *Clostridium*, *Providencia*, *Myroides* and *Comamonas* for the more abundant bacterial genera present in all the consortia; *Debaryomyces* for the only fungal genus present in the four consortia. We showed that herbicide pressure on common aquatic microorganisms might lead to the production of a more toxic component for the aquatic plants than the herbicide itself. That was observed in a laboratory assay; what happens in nature?

Keywords : Water ; Herbicide contamination ; Indirect side effect ; Microbial algaecide

Assessment of the impact of pesticides on microbial communities with different trophic complexity and predator-prey interactions

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The use of pesticides in agricultural soils is increasing worldwide in order to sustain global food demand. Toxicity of these compounds to natural ecosystems has been a constant concern, however, ecotoxicological assessments have mostly focused on aquatic organisms and terrestrial macro-organisms. Little is known about the impact of pesticides on soil microbial networks including microbial predators (protists); protists might be directly impacted by pesticides, with potential cascading consequences for predator-prey interactions and soil functioning. This is in particular important, since interactions between microbial predators and prey influence bacterial diversity, productivity, plant growth and the flux of nutrients to several trophic levels.

The aim of this study is to evaluate the toxic effects of the fungicide etridiazole and the insecticide acetamiprid in a microbial food web context including bacteria and different micro-predators (protists). We performed a microcosm experiment with gamma-sterilized soil in which we assembled microbial communities of different levels of trophic complexity. Three levels of trophic complexity were established: (i) bacteria only (i.e., bacterial community extracted from soil and re-inoculated to the microcosms), (ii) bacteria plus bacterivorous protists, and (iii) bacteria plus protists with different prey preferences. Microcosms were established in quintuplicates for each experimental variation and sampled at 0, 3, 7, 14, and 21 d after the exposure to each pesticide (1 mg kg^{-1} final concentration). We assessed the effect of pesticides on the eukaryotic and prokaryotic soil communities using 18S and 16S rRNA gene amplicon sequencing, respectively, and on microbial abundances using qPCR. Additional measurements on functional parameters are planned. We envisage, that our findings will shed light on the role of microbial trophic complexity for the response to pesticides.

Keywords: food-web, protist, pesticides, soil, microbial ecology

Effect of glyphosate on the microbiota of rainbow trout, *Oncorhynchus mykiss*

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The herbicide glyphosate has been widely used in the past 40 years, under the assumption that side effects were minimal. In recent years, its impact on microbial compositions and potential indirect effects on plant, animal and human health have been strongly suspected. Glyphosate and co-formulates have been detected in various water sources, but our understanding of their potential effects on aquatic animals is still in its infancy compared with mammals. We investigated the effect of chronic exposure to an environmentally relevant concentration of glyphosate (pure active substance and two commercial formulated products) on bacterial communities of rainbow trout (*Oncorhynchus mykiss*). Gills, gut contents and gut epithelia were then analyzed by metabarcoding targeting the 16S rRNA gene. Our results revealed that rainbow trout has its own bacterial communities that differ from their surrounding habitats and possesses microbiomes specific to these three compartments. The glyphosate-based herbicide treatment significantly affected the gill microbiome, with a decrease in diversity. Glyphosate treatments disrupted microbial taxonomic composition and some bacteria seem to be sensitive to this environmental pollutant. Lastly, co-occurrence networks showed that microbial interactions in gills tended to decrease with chemical exposure. These results demonstrate that glyphosate could affect microbiota associated with aquaculture fish.

Keywords: gill microbiome, glyphosate-based herbicides, dysbiosis, chronical exposition.

Gut microbiota impairment is associated to physiological alterations in *Xenopus laevis* tadpoles exposed to graphene oxide

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Graphene-based nanomaterials (GBMs) such as graphene oxide (GO) possess unique properties triggering high expectations for the development of technological applications in many areas. Thus, GO is likely to be released in aquatic ecosystems acting as a pollutant receptacle. It is essential to carefully evaluate its ecotoxicological potential to ensure a safe use of these nanomaterials. In amphibians, previous studies highlighted tadpole growth inhibitions following GBMs exposure together with metabolic disturbances. As it is known that GO possess bactericidal properties and that the gut microbiota constitutes a compartment of crucial importance in the host homeostasis regulation, this study investigates the link between gut microbial communities and host physiological alterations. For this purpose, *X. laevis* tadpoles were exposed during 12 days to GO (from 0 to 10 $mg.L^{-1}$). Growth rate was monitored every 2 days and genotoxicity was assessed through enumeration of micronucleated erythrocytes. Genomic DNA was extracted from the whole intestine to quantify gut bacteria and to analyze the community composition. GO exposure led to a dose dependent growth inhibition and genotoxic effects were detected following exposure to low doses. A transient decrease of the total bacteria was noticed with a persistent shift in the gut microbiota structure in exposed animals. Genotoxic effects were related to a strong gut microbiota remodeling characterized by an increase of the relative abundance of the strain *Bacteroides fragilis*. The growth inhibitory effects were associated to a shift in the Firmicutes/Bacteroidetes ratio while metagenome inference suggested an alteration of multiple metabolic pathways and upregulation of detoxification processes. This work indicates that the gut microbiota compartment should be considered as an integrative marker for ecotoxicological studies as structural or functional impairments could lead to host physiological alterations.

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Keywords: Nanomaterials, amphibian, Intestinal microbiota, aquatic ecotoxicology

Parabens impact interactions among key members of the early-life gut microbiome

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Parabens constitute one of the most commonly found preservatives worldwide and are known to impose adverse impacts on human health by exerting endocrine-disrupting activities. They have been associated with dysbiosis in the gut microbiome, however, their direct role in inter-microbial interactions is unknown. Here, we aim to identify the effects of parabens on five mixed gut microbial communities and dissect the mechanisms that are involved in microbe-microbe interactions as a response to paraben toxicity. Mixed microbial culture compositions were exposed to propyl-, methyl-, and butyl-parabens (200 mM) and characterized using 16S rRNA gene sequencing. The growth of paraben-sensitive and -tolerant microorganisms was tracked by optical density and qPCR assays. Paraben-sensitive species were either cultivated in spent supernatants of paraben-tolerant species and/or grown in co-cultures with the paraben-tolerant species to test interspecies interactions against paraben toxicity (2000 mM). The concentration of parabens was monitored with high-performance liquid chromatography. In mixed communities, all tested parabens decreased the relative abundance of phylotypes from genera *Escherichia*, *Blautia,* and *Bacteroides,* while increasing the relative abundance of *Enterococcus*, *Faecalibacterium,* and *Coprococcus*. Mono-culture assessment of strains to paraben toxicity showed that *Lactobacillus reuteri* and *Enterococcus faecalis* growth were not impacted by parabens whereas *Escherichia coli* and *Bacteroides caccae* were severely inhibited by the propyl-paraben. *L. reuteri* and *E. faecalis* reduced the concentration of propyl-paraben by 20% after incubation for 24 hours. Moreover, cultivation of *E. coli* and *B. caccae* in spent supernatants of *E. faecalis* but not *L. reuteri* improved the growth of both strains in the presence of propyl-paraben. In summary, our results showed that parabens influence gut microbiome dynamics and paraben-tolerant members of the gut microbiome may protect sensitive species.

Keywords : Microbiota ; benzoic acid ; toxicity ; biotransformation

Chronic exposure to antiseizure drugs impacts the gut microbiota community and function.

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Diet and medications are major factors affecting the microbiota composition and exposure to xenobiotics may lead to gut microbial dysbiosis. While the impact of some xenobiotics, such as antibiotics, on the gut microbiome is widely recognized, the influence of chronically administered host-targeted drugs on the gut microbiome is less known. Our goal was to explore *in vitro* effects of chronic exposure to three antiseizure drugs (carbamazepine, valproate, and levetiracetam) on the gut microbial composition and metabolic functions.

Microbial microcosms were established by cultivating four fecal inoculums from young children (11 months to 4 yo) over 24 hours. Using a semi-batch process, these fecal-derived microcosms were cultivated for 11 cycles (24 hours each) with the three antiseizure drugs or drug-free controls; the first 7 cycles with daily transfer to fresh media containing 2mM of the drugs, followed by 4 cycles of potential recovery period without any drugs. The impact of this exposure on the microbial community dynamics (diversity and composition) was determined by 16S rRNA gene sequencing of cycle 1 and 7 cultures. Metabolism of the microbial community after cycle 1 and 7 of exposure to carbamazepine was evaluated by a non-targeted metabolomics approach (LC-MS).

Our results show that, out of 3 antiseizure drugs, carbamazepine had the strongest impact on the gut microbiota structure and metabolism. More specifically, carbamazepine exposure influenced the relative abundance of six genus-level phylotypes (*Lachnospiraceae incertae sedis*, *Roseburia*, *Butyrivibrio*, *Clostridium XIVa*, *Escherichia/Shigella*, *Bacteroides*). We also observed that microbiome communities did not recover from chronic exposure after 4 drug-free cycles. After 7 cycles of exposition to carbamazepine, metabolites from dicarboxylic acid and dipeptides were decreased.

Understanding how the antiseizure drugs change the gut microbiota can help develop strategies, to modulate the response of the microbiota after treatment to mitigate the consequences for the host.

Keywords : antiepileptic ; microbiota ; metabolism ; biodiversity

Interactions of anthelmintic veterinary drugs with the soil microbiota: Toxicity or growth-linked enhanced biodegradation?

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Anthelmintic compounds are used to control gastrointestinal nematodes in productive animals. Upon their administration, they are only partially metabolized in animal tissues with 40-90% of the administered dose found in animal excreta. These are used as manures leading to their dispersal in agricultural soils. Once in soil, anthelmintics interact with soil microorganisms, with the outcome of this interaction being either detrimental (inhibition of soil microorganisms), or beneficial (growth-linked microbial degradation). We aimed to disentangle these complex interactions between anthelminthics veterinary drugs and soil microorganisms. Two soils which identified previously as « fast » or « slow » degrading soils for the compounds albendazole, ivermectin and eprinomectin, were subjected to three repeated applications at two dose rates (1 and 10 mg kg-1). We hypothesized that this application scheme will lead to enhanced biodegradation in « fast » soils and a gradual accumulation and toxicity in « slow » soils. Repeated application of albendazole resulted in different transformation pathways in the two soils and a clear acceleration of its degradation in the fast soil only. This was not the case for ivermectin and eprinomectin whose application resulted in accumulation of their residues. We noted a strong and long-lasting inhibition of nitrification and a significant reduction in the abundance of ammonia-oxidizing bacteria and archaea. In case of commamox bacteria the abundance reduction was not so clear. We also studied the effects of these compounds on the abundance of total bacteria, total fungi and total crenarchaea. Notable reduction in the abundance of total fungi and total crenarchaea was observed, mainly in albendazole-treated soils. Amplicon sequencing analysis, targeting the above microbial groups, is ongoing and will be presented at the conference. Our findings provide first evidence for the toxicity of these compounds on key soil microbial groups that might have to be considered in their regulatory framework.

Keywords : anthelminthics ; AOMs ; degradation ; ecotoxicity ; soil microbiome

In situ translocation experiment to assess the adaptation and resilience of periphytic communities to pharmaceutical substances using a PICT approach

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Numerous studies have demonstrated the worldwide occurrence of pharmaceutical contamination in aquatic environments, in which they can affect microbial periphytic communities. Recent monitoring in a small river located in Lake Bourget watershed (Tillet River, Savoie, France) showed the accumulation of pharmaceuticals in its downtream urban section. In this context, we sought to (i) assess if this chronic exposure to contamination can cause an increase in the tolerance of microbial communities to a set of pharmaceutical substances (‘pollution induced community tolerance' concept, PICT) and (ii) study the tolerance dynamics (tolerance acquisition, or loss) according to exposure level changes.

To reach these objectives, we carried out an *in situ* translocation study in the Tillet River, considering an upstream (reference) and a downstream (contaminated) site. After a 4-week growth phase in each site, periphytic biofilms were transferred between the sites to simulate, during 6 weeks, a restoration (down- to upstream) or deterioration (up- to downstream) of the chemical water quality. Water contamination was monitored using polar organic chemical integrative samplers (POCIS), and biofilm community tolerance was assessed using a PICT approach. Acute toxicity tests were performed on biofilms sampled every 2 weeks, using 7 pharmaceuticals tested individually (ofloxacin, atenolol, diclofenac, paracetamol, erythromycin, sulfamethoxazole, sulfamethazine) and measuring the following biological activities: β-glucosidase, photosynthesis, or growth.

Our results confirmed the expected increase in pharmaceutical concentrations in surface water from up- to downstream. In parallel, an increased biofilm tolerance mainly towards diclofenac and atenolol was observed at the downstream station, from the toxicity tests on photosynthesis. The translocation approach also revealed an increase of tolerance levels in communities transferred downstream, and a decrease of tolerance levels in communities transferred upstream, suggesting resilience processes. This work demonstrates the relevance of the PICT approach for *in situ* diagnosis of the impact of pharmaceuticals on natural microbial communities.

Keywords : Biofilms ; microbial ecotoxicology ; PICT (pollution induced community tolerance) ; POCIS (polar organic chemical integrative sampler) ; rivers

Bacterial and fungal microbiome changes along a gradient of organic micropollutants in a fjord at the Swedish West coast

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Scientific evidence widely supports adverse effects on vertebrate and invertebrate organisms living in aquatic systems impacted by organic micropollutants. Nevertheless, different responses have been measured when the effect is assessed on microbial organisms. Diversity can be promoted due to favorable and rich environmental conditions but also impeded due to the toxic pressure of contaminants leading to microbial community shifts.

In this study, we investigated the structure and biodiversity of the bacterial and fungal community under the effect of physicochemical parameters and organic micropollutants using environmental DNA (eDNA). Six water samples were collected along Hakefjorden around the city of Stenungsund – the centre of Sweden's petrochemical industry. Additionally, three freshwater tributaries were sampled: a wastewater treatment plant (WWTP) effluent and two streams debouching from agricultural and urban areas. GC- and LC-MS analysis were performed to quantify a wide-array of organic micropollutants and eDNA was extracted for further amplicon sequencing (16S rRNA and ITS regions).

Our results showed significantly lower bacterial and fungal α-diversity in marine microbiomes compared to freshwater ones with about half as many observed Amplicon Sequence Variants (ASVs). Interestingly, the marine site closest to the WWTP effluent displayed considerably higher bacterial α-diversity measures than all other marine sites. Our results suggest that proximity to the effluent affect β-diversity for both kingdoms. These dissimilarities in the marine environment could not be explained by physicochemical parameters only. In addition, the site close to the effluent was characterized by a high percentage of unique ASVs and shared more than twice as many ASVs with the effluent itself than other marine sites. Since the observed community shifts were limited to one site and not observed further downstream, our results suggest a spatially restricted effect of sewage effluent only.

Keywords : marine microbiome ; WWTP ; bacteria ; fungi ; organic micropollutants

Can plastics become vectors of harmful microorganisms? An example from greenhouse plastics

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The increase of plastic waste in the environment is a serious environmental concern. After plastics are discarded and abandoned, they do not remain stationary. Instead, they can be indefinitely transferred between different habitats (such as soil, river, ocean etc.). Plastics have several ecotoxicological impacts; one of the most unexpected is that they can become a habitat for different microorganisms. This new ecosystem, known as "plastisphere", can represent an ecological risk because plastics moving between environments may introduce alien species, pathogenic or antibiotic resistance bacteria in new habitats. This study aims to study the potential of greenhouse plastics in an intense agricultural area in Cyprus to be a vector for bacteria from the time of use until they are discarded, abandoned, and through mismanagement, end up in the sea. Five locations were selected to represent the environmental fate of greenhouse plastics from use to their abandonment in soil and subsequent transport to the river and the sea, taking samples of plastics and the surrounding environments (soil and water). Scanning Electron Microscope (SEM) analysis showed a gradual evolution of the biofilm developed on the surface of the plastic in each location sampled. The bacterial community of each sample was studied through 16S rRNA metabarcoding. The β-diversity analyses confirmed significant differences between the plastic and the surrounding environment. In addition, the attached community hardly changed in terrestrial environments. The presence of six genera, *Flavobacterium* (potential pathogen), *Altererythrobacter*, *Acinetobacter* (human infections), *Pleurocapsa* (antibiotic resistance), *Georgfuchsia* and *Rhodococcus* on plastic, regardless of their sampling location, confirmed the potential of plastics to serve as a vector of microorganisms between ecosystems. Future studies should employ full sequencing or qPCR identification to confirm their risk to ecosystems and human health.

Keywords : Bacterial communities ; Greenhouse plastic ; Plastic cycle ; Plastisphere

Presence of polyethylene micro-particles in an agricultural soil affects microbial activities and plants

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The large quantities and diverse sources of plastics released into agricultural soils is a serious source of pollution that became a major concern regarding soil functioning and fertility due to plastic persistence in soils. The present study takes place in a context of recent awareness and still scarce knowledge about the consequences of micro-sized plastic (MP) residue persistence. It aims to assess the effects of polyethylene (PE), a plastic currently widely used, on soil microorganisms especially those involved in nitrogen cycle due to their role in soil fertility and on plant functional traits. In order to assess the possible role of the exposure concentration and of the plant type on microbial response, we compared the exposition of a pristine soil to two MP concentrations (19.3 and 75 mg/g) in soil microcosms planted with a fodder plant (*Dactylis glomerata*) and a field plant (*Zea mays*). The effects of MP were assessed after 15, 30 and 60 days by measuring microbial activities (respiration, nitrification and denitrification) and plant traits. For each treatment, the chemical signature of PE has been investigated using pyrolysis/ gas chromatography/ mass spectrometry on manually collected PE particles.
No effect was observed on nitrification whatever the treatment. Contrasting effects were observed on respiration and denitrification depending on doses and plant presence and plant type. After 60 days, denitrification decreased in the soil exposed to the highest dose with *D. glomerata* and increased for the lowest dose with *Z. mays*.
Preliminary results on the main pyrolysis products derived from PE did not allow to identify an effect of the treatments on the chemical signatures of the particles. Further investigations are currently conducted on organic matter pyrolysis products, such as N-containing molecules to test the effect of the experimental treatments on the development of microbial community on the particle surface.

Keywords: Microplastic, microbial activities, plant traits, plastic characterization

Prokaryotic, microeukaryotic and fungal composition in a long-term polychlorinated biphenyl-contaminated brownfield

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Polychlorinated biphenyls (PCBs) were recognized as persistent organic pollutants, and accumulated in organisms, soils, waters, and sediments, causing major health and ecological perturbations. Litterature reported PCB bio-transformation by fungi and bacteria *in vitro*, but data about the *in situ* impact of those compounds on microbial communities remains scarce. The present work investigated for the first time the PCB impact on microbial diversity from the three-domains-of-life in a long-term contaminated brownfield. Soil samples were ranked according to their PCB concentrations. Extracted DNA from each sample was used to illumina sequencing and qPCR process. Significant increase in microbial activity and abundance was shown according to increased concentrations. Microbial communities structure showed a segregation from the least to the most polluted samples. Moreover, *Bacteria* belonging to *Gammaproteobacteria* class, as well as *Fungi* affiliated to *Saccharomycetes* class, including some species known to transform PCBs, were abundantly retrieved in the highly polluted soil samples. Co-occurrence network revealed potential interactions between species in presence of PCBs that might be adapted and possibly able to transform PCBs. This *in situ* analysis highlighted a trend of PCB to select bacterial and fungal species, possibly involved in PCB transformation and these compounds might also lead to specifics interactions involving species from different domains of life.

Keywords : polychlorinated biphenyls ; metabarcoding ; microbial communities ; soil

Deciphering the response of a microbe-poplar holobiont to a PAH contamination gradient: exploration and integration of multi-omics data

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants mainly found in anthropized soils where they can cause environmental and health issues on a global scale. Phytoremediation could be a cost-effective and reliable method for soil PAH decontamination. Studies on the dynamic of PAH dissipation in soil-plant systems have mostly focused on rhizospheric microorganisms. However, it has recently been shown that some of the PAHs in contaminated soils could be taken up and transported into plant tissues. Endophytes could have a key role in the fate of internalized PAHs and mitigate the plant response to PAH toxicity. An integrative approach combining multi-omics data and direct biochemical activity measurements is a promising way to study complex biological processes in a holistic way. Exploration of the variations at multiple levels such as transcriptome, proteome, and metabolome could help determine the physiological response of the plant holobiont in response to a contamination gradient and unravel key mechanisms taking place.

As part of the ANR-EndOMiX project, this study aims to explore the response of microbial endophytic communities and their host (*Populus canadensis*) in a gradient of PAH contamination. A multi-contaminated soil, from a former coking plant (Homécourt, 54, France; GISFI) was chosen. It was spiked with increasing concentrations of phenanthrene from 0 up to 2000 mg.kg-1. Samples from roots and leaves were collected after 4 weeks. Transcriptomic, metabolomic, proteomic and biochemical analyses have been realized on these samples. A total of 27,561 transcripts, 2908 metabolites, and, 5310 proteins were identified in both plant tissue complemented by 6 biochemical activities. Each data set was studied on its own and then, the three ‘omics' and the biochemical data sets were combined through the mixOmics package. The integration of the data revealed the importance of certain transcripts, proteins, and metabolites. Ultimately, these data will allow us to propose scenarios concerning the molecular mechanisms employed by the microbes-plant systems in response to PAH contamination.

Keywords : Soil ; PAHs ; integrative approach ; mixOmics package ; poplar

Impact of synthetic musks and substrata material on single and multi-species biofilms formed by bacteria isolated from drinking water

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The presence of emerging contaminants in drinking water (DW) has been reported worldwide. Musk fragrances are among the contaminants that have been detected in DW at trace concentrations. However, information about their impact on the microbial communities and particularly on DW microbial quality is scarce. This work provides a pioneer evaluation of the effects of two synthetic muss contaminants, tonalide (AHTN) and galaxolide (HHCB), in microbial biofilms formed by bacteria isolated from DW (*Acinetobacter calcoaceticus, Burkholderia cepacia* and *Stenotrophomonas maltophilia*). Single and multi-species biofilms were formed on two different surfaces, polyvinyl chloride (PVC) and stainless steel AISI 316 (SS316), in the presence of the selected musk contaminants. The impact of musks was assessed directly on the pre-established biofilms and on the bacteria motility, ability to form biofilm and biofilm susceptibility to chlorination. An increase in the cellular density and viability, and in the extracellular polysaccharides content was observed for multi-species biofilms formed on SS316 after exposure to AHTN. On the other hand, the exposure of biofilms formed on PVC to HHCB resulted in alterations in the ability of bacteria to form new biofilms affecting also their susceptibility to free chlorine.

The results allowed to conclude that the presence of musks at residual concentrations influenced DW bacterial dynamics, with the potential to impact the DW quality and safety. Most of the alterations caused by the direct exposure of biofilms to musks were observed when SS316 was used. On the other hand, most of the alterations observed in the ability of bacteria to form new biofilms and their susceptibility to chlorine treatment were mainly observed when the exposure to musks took place on PVC surfaces.

Keywords : biofilm ; galaxolide ; polyvinyl chloride ; stainless steel ; tonalide

Heavy metal contamination of sediments along Nigerian Coastal waters and its influence on the bacterial diversity

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Bacteria communities in sediments are important players in regulating essential biogeochemical processes and play roles in degrading a range of anthropogenic pollutants. In this study, culture-independent molecular analyses (Illumina Miseq) targeting the V4 region of the bacterial 16S rRNA gene was used to describe the diversity of bacterial communities associated with heavy metals-contaminated coastal river sediments. Metal concentrations were determined from 45 sediment samples from five Nigerian rivers using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). Metals concen v.ninane@cra.wallonie.be trations at all sites often exceeded the World Health Organization (WHO) sediment guidelines. Bacterial diversity was reduced with higher metal concentration levels. The bacteria group that predominated in the sediments were Proteobacteria, Planctomycetes, Epsilonbacteraota, Euryarchaeota, Chloroflexi, and Cyanobacteria and included mainly polychlorinated biphenyls and poly aromatic hydrocarbons degraders. The dissimilarity between the rivers (Beta diversity measures) showed several abundances (Alpha diversity measures) of bacterial communities that were shared. This study highlights that bacterial diversity in Nigerian metals-contaminated coastal and riverine sediments varied significantly, and contain numerous taxa that may be useful bioindicators for monitoring heavy metal contamination.

Keywords: Bacterial diversity, river sediments, metal contamination, 16S rRNA, ICP-MS

Drying effects on resistance and resilience of hyporheic microbial communities exposed to copper contamination

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The hyporheic zone can be defined as the water saturated sediments below and beside a riverbed. Microbial communities colonizing the hyporheic sediment play a key role in the biogeochemical and ecological river processes such as organic matter decomposition or pollutant degradation leading to natural surface water purification. However, anthropogenic activities can disturb the hyporheic zone functioning. In an agricultural watershed context, copper used as a fungicide in conventional and organic agriculture can be leached and found in river sediment. Negative effects on microbial structure and functions (e.g. denitrification, respiration) have been observed in river sediments with a copper concentration of 56 mg Cu.kg-1. In the context of global change, chemical contamination is not the only stressor affecting freshwater ecosystems, drying is an increasing phenomenon affecting both pristine and contaminated rivers. The copper contamination and river drying effects have been explored independently on hyporheic microbial communities, but their combined effects are still unexplored. In this study, we evaluate the drying effects on resilience and resistance of microbial communities exposed to copper contamination. We hypothesized additive negative effects of drying and copper contamination on microbial functions. To test this hypothesis, we set up an experiment in microcosms consisting of columns filled with sediment contaminated or not by copper at a nominal concentration of 70 mg.kg-1. Microbial communities were exposed to a 4-week drying period followed by a rewetting period. Sediments were collected at 3 column depths at 4 times: before, during the drying period, and after 2 days and 3 weeks of rewetting. For each sediment samples, copper distribution and microbial functions (respiration, denitrification, exo-enzymatic activities, leaf litter degradation) were measured. This study will provide a better understanding of combined stressors effects in the hyporheic zone, which remains an understudied river compartment.

Keywords : combined stressors ; microcosm ; PICT ; microbial functions ; sediment

The seasonal dynamic of benthic microbial communities in coastal marshes (Charente Maritime, France) reveals microbial indicators considering metal content

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Coastal marshes shelter a vast species diversity, thanks to a high diversity of environments and habitats. However, coastal areas are highly impacted by human activities, receive contaminants from both the ocean and the continent and undergo important physicochemical modification due to climate change. These disturbances can greatly affect the functional balance and benefits these environments deliver. It is therefore necessary to further study these ecosystems in order to better perceive how these major changes will affect them.

Most of these ecosystems' functions are provided by benthic microbial community, which functional diversity provide them a leading part in trophic webs and biogeochemical cycles. However, this community's regulation is complex, depending on both abiotic and biotic parameters. Among them, a major biotic parameter is trophic interaction with the rest of the benthic community such as meiofauna and heterotrophic protists. Hence, it is of paramount importance to consider communities that interact with microorganisms in order to properly apprehend changes in microbial communities. Abiotic parameters are also decisive in benthic community regulation. Factors such as pollution, salinity, temperature or light affect organisms in many ways and should be considered while studying coastal ecosystem communities. Metal pollution, for example, leads to a community composition shift, promoting metal-resistant-species in benthic communities.

This study's aim is to assess the structural dynamic of benthic microbial community of the Charente Maritime wetlands in a 4 seasons monitoring comparing 8 marshes of various salinities. Benthic community composition was studied through environmental DNA sequencing (archaeal/bacterial 16S and protist/meiofaunal 18S rRNA genes). Biological information gathered were combined with metallic pollutant concentrations using multivariate analyses revealing key microbial taxa associated with metal pollution. Thus, this study provides new insights for understanding the effect of pollutants on microbial community composition, useful information to define potential bioindicators for evaluating the ecological status.

Keywords : trophic web ; environmental DNA ; seasonal monitoring ; bioindicator ; metal pollution

Impact of ZnO nanoparticles on the Actinomycetes isolated from soil

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Actinomycetes perform a number of biological functions in the soil and are considered as indicators of soil health and fertility actinomycetes are considered as a permanent member and geosmin producer of higher bacterial communities found in soil. These microorganisms perform a very important function of degrading organic polymers like cellulose and chitin.

Zinc (Zn) is an important nutrient for plant growth and other metabolic activities in plants. Due to the increasing Zn deficiency in the soil, the application of ZnO nanoparticles is gaining momentum in global agriculture. It is observed that ZnO nanoparticles have antimicrobial properties. Scientists come up with a new concept of biological nanoparticles in which less toxic nanoparticles are synthesized from biological sources like plant leaves, stems etc. The authors investigated the impact of ZnO nanoparticles on isolating the actinomycetes from different geographical locations in India. Results indicated that both biological and chemical synthesized nanoparticles are showing negative impact on the isolated species of actinomycetes (*Streptomyces* species). It was observed that significant cell disruption was observed when treated with both biological and commercial nanoparticles. An average size of the biologically synthesized and commercial nanoparticles were around 90-120nm and 40-60nm respectively. The Minimum Inhibitory Concentration (MIC) ranges from 0.11 to 0.98 mg/ml for biological and 0.04-0.39 mg/ml for isolated actinobacterial species. So, it can be considered as the biological nanoparticles are safe in comparison to the chemical but on the other hand these biological nanoparticles also have toxicity.

Keywords : Actinomycetes ; ZnO nanoparticles ; organic polymers ; Toxicity ; Streptomyces

Interactive effects of temperature and bismuth exposure on fatty acid composition, antioxidant enzymes and lipid peroxidation in snails fed on bismuth-contaminated algal biofilms

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Bismuth (Bi) was long used as an alternative to lead to reduce environmental contamination from various industrial applications. In this study, we investigated the effects of Bi on primary producers (biofilm) and its subsequent effects on primary consumers (freshwater snails) through environmental and dietary pathways. In addition, we investigated the interactive effects of temperature stress (in the context of climate change) on the response of primary consumer physiology to Bi. Biofilms were cultured with and without Bi contaminated water (2 µM) for 21 days. Bi content and fatty acid (FA) composition were determined, and biofilms were fed to snails. Snails were maintained in 24 microcosms divided in two groups: 12 microcosms at ambient temperature (19°C), and 12 microcosms at 25°C. The 12 microcosms were then evenly divided into one of four Bi exposure groups (1) control, (2) Bi-contaminated water (2 µM), (3) Bi-contaminated diet, or (4) Bi-contaminated water and diet. At the end of exposure period (28 days), we determined snail Bi content, FA composition, the activities of antioxidant enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase and Glutathione S transferase) and lipid peroxidation by measuring the concentration of malondialdehyde (MDA).

In Bi-exposed biofilms, we observed an increase in polyunsaturated FA which could reflect a protective strategy to preserve cell structure and integrity. In snails, fatty acid composition reflected that of their diet. Under the different conditions, antioxidant enzymes responded differently suggesting an induction of oxidative stress under Bi exposure and thermal stress. In Bi-exposed snails, an increase in MDA was observed, suggesting a deficiency of antioxidant enzymes to prevent lipid peroxidation.

In this study, we highlighted the importance of food as an exposure pathway for primary consumers. The assessment of toxicological effects of metals based only on aqueous exposure seems not to be sufficient suggesting that dietborne contamination should be considered.

Keywords : Bismuth ; temperature ; biofilm ; snails ; fatty acid

Impact of Copper oxide nanoparticles on *Bacillus megaterium*

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Copper substances are utilised in various agrochemicals from the last century as a potential fungicides and algaecides. With the advancement of nanotechnology in the agro-sector, copper oxide (CuO) nanoparticles were replaced with the traditional copper. Different studies conducted on this aspect concluded that increased concentration of nanoparticles in soil was directly proportional to increased application. This indicated high residual potential of CuO nanoparticles.

Bacillus species is one of the most important members of the rhizosphere. In our study we isolated *Bacillus megaterium* from rhizosphere soil and treated it with different concentrations of nanoparticles. In the preliminary studies it was observed that there was significant negative impact on *Bacillus megaterium* when treated with different concentration of the CuO nanoparticles in aqueous medium. The disc diffusion and well diffusion studies indicated no significant impact but ROS was observed for the same concentration. Further studies with the help of FACS confirms the internalisation of Copper oxide nanoparticles into the microbial cells. It was also observed that CuO nanoparticles affect important functions like IAA production of *Bacillus megaterium.* To the best of our knowledge it is the first study on *Bacillus megaterium* indicating the impact of copper oxide nanoparticles in growth and plant growth promotion activities.

Keywords : CuO Nanoparticles ; rhizosphere ; ROS ; IAA ; *Bacillus megaterium*

Challenges, benefits and limitations of different soil amendments

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Nowadays, agriculture address multiple challenges to meet the criteria for higher crop production. The main purpose of fertilizer application is to improve soil physicochemical properties, water retention, nutrient utilization, alteration of pH and increase soil organic carbon. All of these changes influence the soil microbial communities which is crucial for overall soil health. The impact of organic and inorganic fertilizers after 20 years of regular amendments was assessed. In addition to the effect on bacterial and fungal community structures and soil properties, attention has also been paid to the presence of antibiotic resistance genes and occurrence of pathogenic microorganisms. Furthermore, apart from organic (manure and sludge) and inorganic fertilizers, biochar belongs to the most extensively studied soil amendments, which can improve the soil properties. However, the impact of biochar on soil strongly depends on type of material, temperature of pyrolysis and application rate. Therefore, we compared the impact of biochar prepared from two different feedstock (plant biomass and waste from poultry slaughterhouse) under two different temperatures (350°C and 500°C) which were applied at two ratios, 2% and 5% (w/w), in two different soils, Cambisol and Luvisol. Their impact on soil (in terms of soil enzyme activities, diversity and microbial community structures and physicochemical properties of treated soils) were evaluated at different time intervals within 12 months. Furthermore, the impact on growth and root endophytes of *T. aestivum* L. was evaluated.

Acknowledgement: The project was funded by the Czech Science Foundation projects no. 19-02836S.

Keywords : biochar ; manure ; sludge ; soil and endophytic communities

Predicting pesticide biodegradation potential from microbial community composition: new tools for bioremediation

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Bioaugmentation is receiving increasing attention as a green technology to treat contaminated areas by inoculating specific biodegrading microorganisms. However, our understanding of the role of microbial community composition and structure in the expression of contaminant degradation potential is yet to improve. It could help making wise choice for microorganisms – community or specific strain – to be inoculated in contaminated soils with consideration to their indigeneous microbiota.
Here we tried to predict the microbial degradation of two herbicides, glyphosate and isoproturon by means of penalized regression and machine learning methods routinely used in genomic selection. To this end, we conducted experimental modifications of these two herbicides degrading communities by applying biocide treatments coupled with serial dilutions. We then applied three selected genomic selection methods (*i.e.* Ridge Regression, LASSO and Random Forest) on these community variants to link their OTUs composition to their herbicide degradation capacities.
Resulting predictions power is compelling with more than 80% correlation between predicted and actual herbicide degradation capacities. Moreover, OTUs detected as having an impact on herbicide degradation were confronted to literature and validated. To go further and test the robustness of our methods, an experimental validation of the theoretical prediction was set up. Mixed resulting prediction quality calls for promising advances in the field of soil bioremediation with the need of further improvement. Finally, futures applications in a bioremediation perspective are considered.

Keywords: glyphosate, isoproturon, microbial degradation, microbial community composition, genomic selection

Isolation characterization and identification of pesticide-degrading strains of soil microorganisms: the case of an innovative cropping system in Belgium

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Among the processes that determine the fate of pesticides in soils, microbial degradation is recognized as one of the most important. Biodegradation of pesticides seems to be achieved through the contribution of a few key species that exhibit degradation capabilities. However, in field conditions the real effect of these key species on the fate of pesticides is still poorly understood. We attempted to isolate and identify some of them capable of degrading metamitron, metazachlor, bentazon, s-metolachlor and chlortoluron after selective enrichment cultures in minimal medium. Their degradation capacities were evaluated in a minimal liquid medium with pesticide as the only source of carbon and nitrogen. The feasibility of assessing the degradation behavior of degrading strains on undisturbed soil columns was performed. A special focus was made on the effect of autoclaving on soil properties that might control the fate of pesticides. In total, 85 pesticide-resistant cultures were isolated. 27 of them showed significant oxidative enzyme capacity with ABTS as substrate which may indicate their ability to degrade phenolic compounds. Three isolated strains (SM 12, SM 2 and SM 14) were suggested to be able to degrade metolachlor. Three other strains (MET 8, MET 17 and MET 12) were proposed to be able to degrade metamitron. One strain (MZ 3) was potentially able to degrade metazachlor. The soil column study revealed for the first time that autoclaving may influence the saturated hydraulic conductivity by affecting the soil structure. This can lead to inaccurate assessments of pesticide fate in experiments conducted under abiotic soil columns and inoculated with degrading strains.

Keywords: pesticide, herbicide, degradation, agroecology

Toxicokinetic of a pesticides cocktail pulse on stream biofilms with different hydrological histories

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The potential adverse effects of pesticides on stream ecosystems subjected to extreme hydrological events has been poorly explored. Therefore, there is an urgent need to increase our knowledge to propose suitable pesticide mitigation strategies in the context of global change scenarios affecting stream ecosystems. In this study, a microcosm experiment was performed to assess the dissipation kinetics of a pulse of pesticide mixture (glyphosate, aminomethylphosphonic acid (AMPA), tebuconazole, terbuthylazine and imidacloprid) by stream biofilms with a different hydrological history. The effects of two types of drought events caused by hydropeaking (short and more frequent droughts) and agricultural practices (long and less frequent droughts) on aquatic biofilm structure-function were evaluated and compared with an immersed control, before and after the pesticide cocktail pulse. Different biomass and functional parameters were analyzed in biofilms to determine the combined impact of droughts and pesticides, whereas the dissipation kinetics of each pesticide molecule of the cocktail was evaluated in the water as well as in the biofilm. Algal biomass measured as chlorophyll-*a* concentration was significantly lower under drought conditions compared to the control and these differences were more remarkable after the application of the pesticides cocktail. Microbial respiration per unit of microbial (algal and bacterial) carbon was higher in biofilms subjected to droughts, which tended to accumulate lower extracellular polymeric substances (EPS) comparing to the immersed control. Droughts also modified the organic matter decomposition fingerprint of stream biofilms, independently from pesticides cocktail exposure. The diffusion of glyphosate and AMPA into biofilms was lower than that of tebuconazole, terbuthylazine and imidacloprid and this was explained by the differences in molecules' hydrophobicity. However, long droughts tended to further decrease the diffusion of pesticides per unit of biofilm EPS in comparison the short droughts and control treatments.

Keywords: pesticides pulse, biofilms, droughts, toxicokinetic, toxicodynamic

Preventive bioremediation for agricultural soils to reduce pesticide contamination

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Application of pesticides is a still an unavoidable practice on field cereal crops and it results in quasi-systematic contamination of all environmental ecosystems. To limit their persistence and leaching, a preventive bioremediation method was devised by simultaneously applying a pesticide with its degrading microorganism[1]. The herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) and the bacterial strain Cupriavidus necator JMP134 were first investigated in standard soil planted microcosms with an agricultural context (commercial formulation of 2,4-D, agricultural dose) but under controlled laboratory conditions and with a fresh bacterial culture. This approach led to a 3-fold reduction of the 2,4-D persistence in soil without reducing the herbicide efficiency or affecting the bacterial diversity[2]

The aims of our research work were: first, to validate this preventive bioremediation approach by testing a galenic formulation of the microorganism usable by farmers and studying the effect of various pedoclimatic conditions on the laboratory scale (different soils, humidity, etc.) and outside, under natural conditions; secondly to extend the concept to other herbicides.

Our approach was applied on several agricultural soils using a galenic formulation of the strain in laboratory conditions. This still showed a reduction of 2,4-D persistence (3-fold factor) and conservation of herbicide efficiency. Reduction of 2,4-D transfer from soils treated with C. necator towards groundwaters was also shown, confirming that the environmental contamination was limited.

The new targeted herbicide was the sulfonylurea nicosulfuron, widely used on maize crops and regularly detected in surface and groundwaters, as well as its two main metabolites: ASDM (N,N-dimethyl-2-sulfamoylpyridine-3-carboxamide) and ADMP (4,6-dimethoxypyrimidine-2-amine). Until now, several nicosulfuron-degrading strains were isolated from agricultural soils but all produced ASDM and ADMP. Other strains are currently studied for their metabolite degradation capacity. The most promising candidates will be tested in consortia to assess the impact of our preventive bioremediation approach for a more sustainable herbicide use.

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Keywords: 2,4-dichlorophenoxyacetic, microorganisms, Bioremediation

Optimizing chlordecone reduction in soils of the French West Indies using biodegradation coupled to in situ chemical reduction

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Soil, surface water and groundwater in Martinique and Guadeloupe (French West Indies) are contaminated with chlordecone (CLD; C10Cl10O), a very persistent organochlorine pesticide. In soils, its persistence is estimated as several decades in nitisols, centuries in ferralsols and half a millennium in andosols. CLD accumulates in food chains. Long-term chronic exposure to CLD through food and drinking water can have consequences for human health.

In recent years, several remediation methods have been tested in the laboratory and sometimes *in situ*. In particular, an approach using zero valent iron (ZVI) for ISCR (*in situ* chemical reduction) has been shown to reduce CLD in soils in favor of less chlorinated hydrochlordecones which are less toxic although more mobile. One of the main drawbacks of this method is the amount of ZVI required to reduce CLD. Thus, the present work was carried out with the aim of finding an intermediate method of enhanced biodegradation-ISCR by favoring microbial iron reduction, which in turn, could favor CLD reduction.

In microcosms, tests with different amounts of ZVI, equivalent or lower than the quantities used for the ISCR, were applied under water saturated conditions and monitored over time for CLD and transformation products (TPs), bacterial biomass and diversity. Sugar cane residues were added as a carbon source issue from local distilleries. This experiment showed that with ¼ of the amount of FZV used for ISCR, a decrease of CLD of around 50% was achieved, with formation of hydrochlordecones and other TPs. Analysis of microbial communities showed an impact of ZVI treatments on bacterial biodiversity, in particular by increasing and diversifying genera and families known for iron-reducing activities, which was one of the objectives and is therefore encouraging for future work.

Finally, preliminary results from in situ trials set up using ¼ of the amount of FZV used for ISCR will also be presented.

Keywords : chlordecone ; soils ; remediation ; bacterial diversity ; iron reduction

Mycelial nutrient transfer facilitates bacterial co-metabolic organochlorine pesticide degradation in nutrient deprived environments

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Biotransformation of soil contaminants such as organochlorine pesticides (OCP) often is impeded by a lack of nutrients relevant for bacterial growth and co-metabolic biotransformation. By providing space-filling mycelia, fungi may promote contaminant biodegradation by facilitating bacterial dispersal and the mobilization and release of nutrients. We here tested whether mycelial nutrient transfer from nutrient-rich to nutrient-deprived areas facilitates bacterial OCP degradation in a nutrient deficient habitat. The legacy pesticide hexachlorocyclohexane (HCH), a non HCH-degrading fungal species (*Fusarium equiseti* K3) and a co-metabolically HCH-degrading bacterial isolate (*Sphingomonas* sp. strain S8) from HCH-contaminated soil were used in spatially structured model ecosystems. Using 13C-labelled fungal biomass and protein-based stable isotope probing (protein-SIP), we traced the incorporation of 13C fungal metabolites into bacterial proteins while simultaneously determining the biotransformation of the HCH isomers. The computed relative isotope abundance (RIA), labeling ratio (LR), and the shape of isotopic mass distribution profiles of bacterial peptides indicated the transfer of 13C-labeled fungal metabolites into bacterial proteins. Distinct 13C incorporation into the haloalkane dehalogenase (linB) and 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase (LinC), as key enzymes in metabolic HCH degradation, underpin the role of mycelial nutrient transport and fungal-bacterial interactions for co-metabolic bacterial HCH degradation in heterogeneous habitats. Nutrient uptake from mycelia increased HCH removal by twofold as compared to bacterial monoculture. Fungal-bacterial interactions hence may play an important role in co-metabolic biotransformation of OCP or recalcitrant micropollutants.

Keywords : Biotransformation ; fungal ; bacterial interactions ; nutrient transport ; mycelia ; pesticides ; protein ; SIP

Bioaugmentation for the treatment of pesticides polluted soils: selection of carrier materials for microbial biofilm formation and inoculation in soil

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The use of pesticides in agriculture can lead to soil and groundwater contamination due to the migration of pesticides and their metabolites through soil. The addition of pesticide-degrading microorganisms to top soil, via a bioaugmentation process, is a green way to reduce pesticides contamination without soil excavation or chemicals addition. The efficiency of bioaugmentation approaches is closely linked to the viability and survival of the microorganisms once in soil. Inoculants viability into soil can be increased when inoculant are introduced as microbial biofilms grown on carrier materials. In the frame of BIOPEPS and EPURSOL projects, various carrier materials for biofilm development were tested for their ability to favor the growth and activity of pesticide (MCPA)-degrading bacterial consortia. Various carrier materials (zeolite, pozzolana, oyster shell) as natural or modified (modification of surface properties) materials were put in contact with a selected microbial planktonic consortium capable of MCPA biodegradation. The biodiversity and MCPA biodegradation activity of the various biocomposites (biofilm + carrier material) were evaluated. T-RFLP and High-throughput sequencing on the 16S rRNA genes suggested that modifying the surface of carrier material influenced bacterial diversity as well as MCPA degrading activity. Growth as biofilm allowed higher activity than growth as planktonic state. This suggests that taking into account the nature of the carrier material should improve the efficiency of bioaugmentation process for bioremediation purposes.

Keywords: Bioaugmentation, pesticides, carrier materials

Microorganisms: actors governing the degradation/transformation of diuron in sewage sludge during biological treatment

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The omnipresence of micropollutants in effluents from urban wastewater treatment plants (WWTP) pose a serious risk for the environment and human health. Among them, diuron is considered as a "Priority Hazardous Substance" because its high toxicity. In spite of biodegradation pathway of diuron into 3,4-dichloroaniline which exhibit a higher toxicity than diuron is well known in soil, it remains unclear in sewage sludge during the biological treatment. Moreover, in urban WWTP, the higher concentration of diuron were found in the effluents than those in the influents. These observations raise questions about the metabolic potential of diuron degradation and transformation in WWTPs. To date, few data are available in the literature on this point. This work aims to test the hypothesis that an additional quantity of diuron is produced during wastewater biological treatments from the transformation of other compounds including 3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)-urea and 1,2-dichloro-4-nitrobenzene. The second hypothesis concerns the presence of the microorganisms in sewage sludge, which are capable of diuron degradation. Hence, the experiments on bacterial cultures were set up with the settled water and the microbial inoculum extracted from sewage sludge of two WWTP with the presence of diuron and its precursors. The first results showed that the microorganisms have well developed in these medium during the incubation period. The chemical analysis were then carried out in order to determinate the fate of these micropolluants. The result obtained are (i) a significant decrease of 1,2-dichloro-4-nitrobenzene concentration, and (ii) no change for diuron concentration. However, we observe that there is an appearance of two new peaks around the diuron elution zone. We assume that the potential formation of diuron by nitrobenzene is compensated for the part of diuron degraded or transformed. Microbial activity and diversity were performed to determinate which microbial groups were appliqued in this phenomena.

Keywords: microorganisms; diuron; sewage sludge; biodegradation; biological treatment

Combining gamma irradiation and bioaugmentation enhances Treated Waste Water's quality for its reuse in agricultural irrigation

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Treated Waste Waters (TWW) reuse in agriculture can be beneficial for the environment as it reduces their discharge into ecosystems and because they are rich in nutrients helping plant growth and soil fertility. However, TWWs are also considered as 'hotspots' of dissemination of bacteria, antibiotics and heavy metal resistance genes, as well as pathogens. This study aims to develop an innovative strategy for TWW depollution consisting in the combination of gamma irradiation and bioremediation. Gamma irradiation will enable pathogen elimination and complex organic matter degradation while bioaugmentation will use consortium of microorganisms specialized in metal sorption. Bacterial strains were isolated from soils irrigated with TWW. Those strains were characterized and selected for their tolerance to 0.2 g/L, 1 g/L and 1.5 g/L of Cd, Pb and Cu respectively. A consortium composed of *Bacillus selenatarsinatis*, *Bacillus simplex* and *Streptomyces brasiliensis* was then selected and its metal biosorption capacity was evaluated after 24h of contact with TWW and 4 KGy gamma irradiated TWW. Depolluted TWW was then applied on Pea (*Pisum sativum*) germination for 9 days, in dark at 25°C. Results showed that the consortium was able to bioaccumulate 0.1801, 8.085 and 0.1249 mg/g of dry weight of Cd, Pb and Cu, respectively in depolluted TWW. After irrigation of Pea seeds with depolluted TWW, we noticed a positive significative impact of the germination as we obtained 0.63 *versus* 0.24 g and 0.25 g *versus* 0.13 g for embryonic biomass and 20.8 cm *versus* 9.25 cm and 12.8 cm *versus* 5.58 cm for elongation, in shoots and roots with depolluted *versus* non depolluted TWW respectively. Finally, the new developed strategy seems to be very promising for safety TWW reuse and enhancing crop's productivity by alleviating contaminants' stress on agricultural plants.

Keywords: Treated Waste Water; heavy metals biosorption; gamma irradiation water depollution; Pea abiotic stress alleviation.

Impact of a nitrate- and oxygen-dependent remediation strategy (Schäfersee-Verfahren®) on a contaminated urban lake

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Lake Schäfersee, located in Berlin, showed declining water quality over several decades due to the discharge of untreated rainwater from a highly urbanized area of 260 hectares. As a consequence, the lake was regularly depleted of oxygen, developed fish-toxic hydrogen sulfide formation, fish dye-offs occurred, and organic and inorganic persistent pollutants accumulated in the sediments. To alleviate the lake, a lake remediation company installed a system to add calcium nitrate (Ca(NO3)2) and O2-enriched water simultaneously to the hypolimnion (Schäfersee-Verfahren®). Ca(NO3)2 has been previously used successfully in lake remediation. Nitrate shall serve as an alternative electron acceptor for microbial respiration, reducing phosphate release and hydrogen sulfide formation. The supplementation of O2-enriched water shall stimulate microbial oxygenase-mediated degradation of organic pollutants. However, the precise effects on microbial processes and pollutant removal in lakes with different contamination profiles are still not well understood.
In this study, we investigate the impact of Ca(NO3)2 and O2-enriched water on (semi)metal mobilization, pollutant transformation, and microbial sediment communities in lake Schäfersee. Preliminary results show that redox conditions changed to more positive values during Ca(NO3)2 addition. Sulfate increased, indicating reduced hydrogen sulfide formation. Data suggest phosphate is demobilized. Despite the occurrence of Pb in the sediments, data show no increase in its concentrations in the bottom water, contrary to assumptions of previous lab-scale studies. Zn increased concomitant with Ca(NO3)2 addition but did not exceed permissible drinking water thresholds. For PAHs and long and short-chain hydrocarbons, no clear trend toward a decrease in their concentration in the sediments was observed contrary to previous studies using nitrate for bioremediation. More in-depth analyzes of the role of the microbial communities in this process are in progress. Together, this study contributes to a better understanding of the advantages and risks of the addition of Ca(NO3)2 and O2-enriched water to stratified contaminated lakes.

Keywords: Pollutant transformation Metals Polycyclic aromatic hydrocarbons (PAHs)

Biodegradation of PBAT/PLA mulch film particles in defined bacterial cultures

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The application of mulch films is an increasing agricultural technique with a covered area of over 4,000 km^2 per year in Europe alone. Approximately 93% of mulch films are conventional films based on polyethylene (PE). With a loss rate of 3,2%, conventional films contribute to the incorporation of microplastic in terrestrial systems. Hence, biodegradable mulch films are a promising alternative. However, the biodegradation is highly dependent on location factors like the soil type and the composition of the microbial community. To avoid deliberate littering of microplastic in agricultural soil systems, the complete depolymerization and metabolization of the monomers of film particles must be guaranteed and researched. With a deeper insight into this topic, potential risks can be assessed and future applications for an accelerated bioremediation can be found.
In this study, the biodegradation of biodegradable mulch films is tested using particles composed of a blend of poly-(butylene adipate-*co*-terephthalate) (PBAT) and polylactic acid (PLA). The particle degradation is conducted with two different microbial cultures that were found to be capable of utilizing all four monomers of the PBAT/PLA polymer: a pure culture of *Rhodococcus opacus* DSM 43250 and a mixed culture of *Cupriavidus necator* and *Pseudarthrobacter polychromogenes*, which were isolated from agricultural soils and identified via MALDI-TOF. The growth of the cultures on PBAT/PLA-particles was determined via CO_2-measurment using gas chromatography and the release of PBAT monomers was evaluated via high performance liquid chromatography. Possible adsorption of pesticides onto the plastic is discussed in the literature. Here, potential growth inhibition due to the presence of the pesticides Tebuconazol and Thiacloprid were investigated. The results are discussed in the context of sustainable use of mulch films in the agricultural sector.
The research is part of the iMulch project funded by the European Fonds for Regional Development (EFRE).

Keywords: biodegradation, micro plastic, pesticide, bioremediation

Bioplastics in the environment: Depolymerization and monomer metabolization of biodegradable mulch film

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Plastic mulch films are increasingly applied in agriculture to enhance crop production. To avoid soil plastic pollution, either removal of the mulch films post-harvest or full biodegradability of the materials are crucial. Focus of our work is the investigation into the biodegradation and full metabolization of biodegradable mulch film as sustainable alternative to conventional plastics. Next to polyethylene-film (PE), the biodegradable alternative made of poly-(butylene adipate-*co*-terephthalate)-polylactic acid (PBAT/PLA) was used. We investigated the impact of different dosages of PE and PBAT/PLA microplastic particles on the microbial communities of field soils via 16S rRNA amplicon sequencing. In addition, potential PBAT/PLA metabolizing microbes were isolated from field soil samples and identified via MALDI-TOF. Of the microbes best performing in monomer metabolization studies, *Rhodococcus opacus* stood out, as this strain could utilize all four monomers which was investigated via high performance liquid chromatography of the supernatant. Next, the metabolization of mulch film enzymatic hydrolysate was measured, followed by microbial growth monitoring on mulch film particles by CO_2-development via gas chromatography (GC). To finally explore the possibility of enhanced microbial bioremediation at the end of crop season, pieces of PBAT/PLA mulch film were incubated in soil and sprayed with *R. opacus* cultures. Possible improvement of film metabolization was measured using scanning electron microscopy and CO_2 evolution by GC. In an alternative end-of-life utilization, mulch film removal from the field combined with microbial upcycling of the PBAT/PLA to neutral lipids triacylglyerol (TAG) has been demonstrated with *R. opacus* for the monomer mixture and the enzymatic polymer hydrolysate. The results will be discussed in the context of purposefully bringing plastic in the environment and options for minimizing the impact of mulch film use, including emergency biodegradation and upcycling options.

This research is part of the iMulch project funded by the European Fond for Regional Development (EFRE).

Keywords: Biodegradation, Soil, Plastics

Characterization of metal(loid)s -tolerant bacterial isolates from Touissit-Boubker district (Morocco) and their interaction with plants for soil remediation

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Soils polluted by metal(loid)s are present all over the world they pose a great threat to global ecosystems as well as to the human health. They are often the results of mining activities that generally produce sandy waste with low organic matter content. These wastes have the particularity to be easily moved to nearby sites by the wind and to the groundwater by runoff and infiltration. This soils need to be recovered and one of the most proposed process is phytomanagement; which consist in using plants associated with microorganisms, both of them tolerant to pollutants, in order to develop a new ecosystem, that stabilizes the soil and holds metal(loid)s back of dispersal.
In order to obtain the best results, both metal(loid)s-tolerant plants and microorganism should be selected. In this association, microorganisms may play multiple roles; first transforming metal(loid)s to less hazardous states and/or accumulating metal(loid)s and second by improving plant performance in this environment to ensure high soil metal(loid)s stabilization rates. Hitherto, our goal is to develop bacterial inoculants able to survive and develop in such polluted soils and to use them in combination to indigenous plants for recovering the mining technosols from Touissit-Boubker district (Morocco).
Several bacterial strains were isolated from *Vicia faba*'s nodules growing on the polluted soil from Touissit amended or not with biochar (7 isolates in all); 6 bacterial isolates were obtained directly from the mine soil and one isolate from a non-polluted soil from the region. These isolates showed resistance to both Zn and Pb up to 200 mg L^{-1}. Further characterization of the isolates is in progress, as well as their interaction with plants and other microorganisms, in order to find the best candidates to create bacterial inoculants to remediate polluted soil from the Touissit region.

Keywords: phytomanagement, metal(loid)s-resistant bacteria, technosol, plant-bacteria interaction, soil remediation

A roadmap for the integration of environmental microbiomes as a new tool for risk assessment

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The microbiome refers to communities of microorganisms and their genome in a defined environment.

Microbiome research has accelerated in the last decade. Presently, there is however no universal guidance or proper methodology to account for the structure and dynamics of environmental microbiomes. Even more so, the way environmental microbiomes (plants, wildlife, soil) can be included in risk assessment remains to be determined.

The Walloon Agricultural Research Center (CRA-W) and the Catholic University of Louvain (UCLouvain) have joined their expertise to explore existing data and tools. The purpose of the project, co-financed by EFSA (GP/EFSA/ENCO/2020/02), is to define a roadmap for the potential integration of microbiome considerations under risk assessments, within EFSA's remit.

The first step consists of an extensive review of the existing literature on the subject, which encompasses various ecosystems and wildlife organisms. The information is scrutinized in order to define what a healthy baseline is, if it exists, how it can be described, and what are the beneficial or detrimental impacts on a given microbiome.

Another aspect relates to the different omics techniques and tools associated with microbiome studies. Data quality, harmonization and correct interpretation are essential for the comparison of results between laboratories and the identification of potential risks. At this level, genomic methods based on amplicon high-throughput sequencing are the most advanced for a proposition of guidelines ensuring the quality of results provided by each laboratory. It also includes recommendations such as testing the suitability of the targeted regions, their evaluation on mock communities, determining the sensitivity of the methods and the use of appropriate databases and bioinformatics pipelines. The integration of longer amplicon sequencing technologies, allowing for more precise taxonomic identification, is also considered.

Keywords: environmental microbiome ; omics techniques ; standardization ; networking

Microbial ecotoxicology for marine management: towards microbial inclusion in water quality guidelines

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Marine ecosystems are facing increasing pressures at local and global scales. Strategies to minimise the impacts of pollutants in these ecosystems are typically informed by water quality guideline values, which comprise toxicity thresholds for relevant indicator species. While microorganisms are critical members of marine environments, underpinning food chains and provisioning ecosystem services, they remain absent from water quality guidelines. Quantifying effects of stress on microbial communities is challenging due to their diversity, complex interactions and functional redundancy, which can lead to unpredictable outcomes for community structure and functions. Here we describe the development of a novel ecotoxicological framework to derive microbial stress threshold values relevant to their community ecology and functions. Seawater microbial communities were exposed to a concentration gradient of the reference toxicant, copper. Shotgun metagenomic sequencing was coupled with cell enumeration (ddPCR and flow cytometry) to generate quantitative gene- and genome-centric microbial community profiles for each copper treatment. Concentration-response relationships for taxonomic groups and functions of interest were developed by quantifying changes in gene or taxon abundance relative to the concentration of the stressor. From these relationships, threshold-effect concentrations can be estimated for multiple taxa and functions, then combined in a cumulative sensitivity model to describe the proportion of taxonomic groups/functions affected as the stressor concentration increases. This integrative method allows insight into the relationships between seawater microbial community composition, interactions, and functional potential with respect to copper stress and represents a significant step towards the inclusion of microbial data in derivation of water quality guideline values.

Keywords: marine ; bacteria ; function ; genomics ; quantitative

The soil microbiome as a potential toolbox for risk assessment in the food chain

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The soil microbiome plays an important role in the safety of primary food production for human health and in plant protection. The soil microflora naturally carries human and plant pathogens, as well as antibiotic resistance genes (ARGs) potentially dangerous for human health. Well managed however, the soil microflora provides a suitable environment for healthy plant growth; farming practices indeed determine the level of foodborne pathogens, the risk of ARGs spreading in soil and from soil to food, and the level of plant resistance to pathogen attacks. Human activities often induce a structural modification of soil microbial communities, allowing for the proliferation of the best-adapted microorganisms. Another response may be an increase of ARGs and of mobile genetic elements in soil bacterial communities. As they adapt quickly, microorganisms are interesting indicators of soil health; numerous microbial indicators have therefore been developed to assess it. The aim of the project presented here is to evaluate the feasibility of using them for risk assessment in the food chain. We pointed out three potential uses to assess. First, since the microbiome is a potential source of risk, e.g. by introducing pathogens or ARGs through feed crop irrigation with the waste water of vegetable-rinsing operation or by spreading manure for grass and forage crop production, we suggest using microbial indicators to monitor the prevalence of zoonotic micro-organisms in animal populations. A second use would be as an indicator of soil health disorder in pesticide risk assessment, microorganisms being considered as non-targeted organisms. The third suggested use of the soil microbiome is as a tool to evaluate the probability of plant pests spreading in an area. In this option, the microbiome is considered as an indicator of soil suppressiveness against plant pathogens. All experts welcome.

Keywords: Pesticides ; Biological hazards ; Plant health

Microsoil - Investigation of alternative test methods to correctly assess the impact of plant protection products, biocides and pharmaceuticals on soil microorganisms

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For the risk assessment of plant protection products (PPP) potential effects on soil microorganisms are addressed by investigating if N-transformation (OECD 216), an important soil function, may be affected. However, by focussing on only one central function of the microbial community, other effects can be overseen.

To determine potential other effects of PPP on functional microbial activity, one aim of the project ‘Microsoil' is to investigate the sensitivity of three alternative test methods, i.e. substrate induced respiration (MicroRespTM), measurement of enzymatic activities (ISO 20130) and effects on ammonium oxidizing bacteria (AOB, ISO 15685). To gather information about the sensitivity of these methods, six model substances were investigated (fungicides: tebuconazole, pyraclostrobin, propamocarb; herbicide: ethufomesate; antibiotic: tiamulin hydrogen fumarate; biocide: dodecyl dimethyl ammonium chloride (DDAC)). For sensitivity assessment, results are compared to OECD 216 data.

Up to now, tests were performed in different soils, e.g. LUFA 2.1. Three to four nominal test concentrations, e.g. 1x, 5x and 10x of the intended application rate, were tested. Measurements for each test method were performed after 14 and 28 days. If effects above 25% occurred after 28 days, the test duration was extended up to 84 days.

Thus far results are available for the test substances applied on LUFA 2.1. In this soil most of the chosen PPP did not have an effect. For tiamulin and DDAC inhibitions of AOB were found above 25% after 28 days exposure. Additionally, e.g. ethofumesate, pyraclostrobin and tiamulin also affected specific enzymes. Upcoming experiments with other soils may help to identify potential influence of soil properties to ecotoxicological results. A synopsis of these results can lead to a comprehensive method review for the modification of the test strategy within current regulations.

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Keywords: functional biodiversity ; risk assessment ; soil microbial community

In vitro screening of soil representative nitrifying strains as potential microbial indicators for regulatory use

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Pesticides are environmental pollutants regulated by a stringent EU framework. This is based on toxicity tests that are well-established for aquatic organisms and terrestrial macro-organisms, but not for soil microorganisms. Recent benchmarking research has pointed to ammonia-oxidizing microbes (AOM) as ideal microbial indicators of the effects of abiotic stressors on the soil microbial community due to their key functional role, their sensitivity to external perturbations, and the availability of established tools to measure their activity and abundance. Our main objective is to develop and standardize pioneering *in vitro* tests, as a first conservative step to assess the toxicity of pesticides on soil AOM. Here, to identify the most sensitive strain per group of nitrifiers, we assayed the toxicity of selected pesticides (herbicides, insecticides, fungicides) with a range of phenotypically and ecologically distinct strains of AOB (*Nitrosomonas europaea*, *Nitrosomonas communis*, *Nitrosospira multiformis*), AOA (*Ca.* Nitrosocosmicus franklandianus, *Nitrososphaera viennensis*, *Ca.* Nitrosotalea sinensis) and NOB (*Nitrobacter sp.* NHB1, *Nitrobacter hamburgensis*, *Nitrobacter winogradsky)* representing globally distributed lineages found in soil. Toxicity was determined at the functional level via monitoring nitrite production or consumption in liquid cultures of AOB/AOA and NOB respectively, amended with a broad range of pesticides concentrations, and relevant toxicity endpoints (EC_{50s}) were calculated. All pesticides affected at least one non-target nitrifier with the different strains exhibiting various levels of sensitivity. The acidophilic *Ca.* N. sinensis was the most sensitive strain among AOA, while the sensitivity of AOBs was pesticide dependent. Categorizing by pesticide target revealed contrasting toxicity patterns, with fungicides like pyraclostrobin and etridiazole being highly toxic to all nitrifying strains, while insecticides (e.g., chlorpyrifos) and herbicides (e.g., metsulfuron-methyl) imposing greater effects on AOA and AOB strains, respectively. Overall, our work is expected to provide novel ecotoxicity tools for the systematic estimation of the impact of pesticides on non-target soil microbes.

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Keywords: ammonia-oxidizing microorganisms, microbial indicators, pesticides toxicity, risk assessment scheme

Evaluation of the estrogenic activity in natural water using the YES test

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The estrogenic activity of freshwater samples from different sampling points characterized from various anthropogenic disturbance (e.g. agricultural, industrial practices) were evaluated by the YES test, using a recombinant yeast strain *Saccharomyces cerevisiae* BJ1991 (ISO 19040-1:2018). This microorganism contains a human estrogen receptor (hER) gene stably integrated into the genome of yeast cells BJ1991. When an estrogen receptor agonist binds to hER, the receptor-ligand complex capable of binding to the ERE (estrogen response elements in expression plasmids) and the transcription of the reporter gene Lac-Z is initiated, β-galactosidase is synthesized. In presence of β-galactosidase, the chromogenic substrate chlorophenol red β-d-galactopyranoside (CPRG) in the medium undergoes a colour change from yellow to red. The change of absorbance can be measured at 570 nm. The overall results show the sensitivity of the YES test to the mixture of chemical residues occurring in river water. The recombinant yeast strain *Saccharomyces cerevisiae* is as an effective microbiological tool for the determination of the overall level of water pollution without identifying specific chemical components. This tool can be particularly useful in the case of chemical mixture pollution, which include legacy (e.g. pesticides) and emerging (e.g. pharmaceuticals and metabolites of pesticides) contaminants.

Keywords: yeast, freshwater, chemical mixture, emerging contaminants, water quality

Bacterial ecotoxicology of azole antifungal agents

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Azole antifungal agents are widely used active ingredients in pharmaceuticals, personal care products, and pesticides. This promotes the uncontrolled release into the natural environment. Azole fungicides are classified as emerging environmental contaminants. Prolonged exposure to these compounds may cause significant negative effects on the biotic components of natural ecosystems. A group of organisms that specifically might be affected by exposure to such pollutants are environmental bacteria.

The goal of this study was (a) to understand response of environmental bacterial strains to exposure to four typical azole fungicides, with different structures and directions for use (fluconazole, clotrimazole, climbazole, epoxiconazole); (b) to compare biological and chemical degradation processes of azole fungicides; (c) to detect transformation products generated during fungicides degradation and investigate their toxicity towards beneficial bacterial strains.

In this study, new bacterial strains were isolated from the natural environment using selective culture method. Changes in the metabolic activity, membrane permeability and enzymatic activity induced by exposure to individual azole fungicides were determined using spectrophotometric methods. Biological and photo(cata)lytic degradation were determined by LC-MS/MS.

The results showed that each compound affects the bacterial cell surface differently, however, the short-term contact of bacteria with fungicides promotes a decrease in the cell metabolic activity. Among all fungicides tested, only climbazole was partially susceptible to biological removal while other fungicides remained resistant to biodegradation. Under optimized photolysis conditions, the removal efficiency of clotrimazole, climbazole and epoxiconazole was significantly higher than that of biodegradation. The Fenton process supported by UV irradiation allowed to complete degradation of these fungicides. This study increases the understanding of the environmental impact of azole derivatives, their degradation, and toxicity.

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Keywords: azole fungicides, environmental bacteria, degradation, toxicity, membrane permeability

Development of a collaborative web platform documenting the diversity and extent of diatom deformities

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The Biological Diatom Index (BDI) is a regulatory indicator used for the assessment of the biological quality of French water bodies within the Water Framework Directive. In particular, this index considers the proportion of deformed diatoms, whose presence probability profile is an indicator of stress. Although this is a sensitive criterion, it is rarely informed as there is no consensual objective approach for attributing a teratological character to a diatom when the deformation is subtle. In this context, our project aims at documenting the most common teratologies by developing an iconographic collection of deformed diatoms on the French territory. This tool, which will be designed and fed by surveillance operators, will provide them assistance in determining the teratological status. For this purpose, a collaborative web interface will be implemented in order to acquire a large database of teratology images, illustrating the specific diversity, or on the contrary the stability, of the types of deformations encountered for French mainland diatom species. On the long term, sharing knowledge on the deformations observed on the territory and associated environmental conditions will be key to guarantee the quality of the existing BDI, by optimizing the use of diatom deformation for the assessment of toxic stress. This poster will illustrate the first steps of this crowdsourcing approach.

Keywords: diatoms; teratologies; crowdsourcing

Arising difficulties in the implementation of DNA-Metabarcoding Vs Morphological analysis of diatom samples

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Diatoms are unicellular eukaryotic organisms that have been exploited for effective freshwater bioassessment. As excellent bio-indicators, they are routinely used in national environmental monitoring programs in Europe within the Water Framework Directive (WFD) 2000/60 /EC (Foster et al., 2000) and CEN standards (CEN, 2018).

New technologies, such as the application of DNA metabarcoding for characterising benthic diatom communities, aim at faster and maximised freshwater bioassessment. Through this technique, individual species found in one environmental sample are established using genetic variability and are characterised by short DNA fragments called a barcode (Valentin et al., 2019).

The Wat-Dimon project aims at the creation of a novel genomic test for identifying European benthic diatoms. The project intends to optimise the DNA-based test methodology for complementing and/or replacing the traditional ecological assessments based on the morpho-taxonomy methodology approach that requires taxonomic expertise and is subjected to scientific bias. With the development of a complementary bioinformatics tool, the biotechnological interpretation of results can be facilitated. This will allow the prompt response to the environmental needs, the early assessment of environmental quality and early treatment response. This work compared the two methodologies used for diatom ecological biomonitoring, DNA-metabarcoding and morphological characterisation. DNA barcodes were amplified using universal primers for diatoms and sequenced using Illumina MiSeq. Bioinformatic analysis was performed using the DADA2 pipeline to quality-filter the high number of sequences from the samples and identify them by comparison with reference databases (Diat.Barcode). The project focuses on the *rbc*L gene. Results show a positive correlation between morphological and molecular IPS scores. Three sampling sites have been identified as problematic with limitations of both techniques influence points deviating from the norm. Finally, comparing the two methodologies was crucial for the gap analysis, identifying potential diatom candidates for barcoding studies and enriching already existing diatom barcode databases.

Keywords: diatoms ; DNA metabarcoding ; morphology

Deciphering new exposure biomarkers in ciliates and bacteria exposed to pyrethroids by transcriptomic and volatolomic analysis

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The assessment of pesticides impact is mainly driven by chemical measurements in different environmental compartments. These kind of data provide an overview of the environmental contamination but makes it difficult to predict the impact on biological community (biocenosis). Determining the biological mechanisms that enable to respond to a disturbance is the key to studying an organism's response to a given stress. “Omics” approaches represent a good way to achieve this and allow to consider a biomarker approach for pesticides exposure.

Using basic doses/effects tests on aquatic ciliates (*Paramecium tetraurelia*) and/or an edaphic bacterium *Pseudomonas fluorescens* (with cell cycle duration as descriptor) we found that the observed effect could greatly differ depending on pyrethroid and organism used. Using a pool of metabolic and gene signatures obtained under controlled conditions, two analytical approaches were developed and are described below as non-targeted and targeted studies. Results are obtained following a deltamethrin exposure.

For targeted analysis, we focused on the level of *tspo* gene transcription as marker of cell stress. This marker has been previously noted in *P. tetraurelia* exposed to pyrethroid insecticides. An abnormal *tspo* transcriptional level has been observed in *P. tetraurelia* and *P. fluorescens* cultures and on data from human intestinal microbiota exposed to low doses of insecticide.

For non-targeted approaches, analysis based on volatolome (i.e. study of Volatiles Organic Compounds, VOCs) were performed on *P. fluorescens* and *P. tetraurelia*. We demonstrated that there is a clear deviation of the volatolome when cells are exposed to a low dose of insecticide and that specific VOCs signature could be considered as new exposure biomarkers.

In close future, it will be interesting to determine the potential of these different functional markers sensitive to pyrethroids insecticides in order to build a pan-specific picture of their (eco)toxicity.

Keywords: (Eco)toxicogenomics ; Pesticides ; Biomarkers ; Volatiles ; Gene expression

Functional diversity of the Île-de-France's peri-urban lakes' microbial diversity: Impact of seasonality and degree of eutrophication

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Peri-urban lakes are ecological buffer of highly anthropized areas such as the Île-de-France region and are of major significance to urban ecology. Heavily impacted by autochthonous and allochthonous inputs, these ecosystems are hotspots of microbial biodiversity and elements cycling. Due to their quick and intense response and adaptation capacity, peri-urban lakes are environmental sentinels of global change.

Besides the phytoplanktonic autotrophs compartment, which is well described in lake ecosystems, the functional importance of the heterotrophic microbiome (bacteria, archaea) remains unclear and rarely considered in the lakes' ecosystems' response to anthropic pressure. A 16S rDNA amplicon and metagenomics spatio-temporal series analysis of the impact of a eutrophication gradient on peri-urban lakes of the Île-de-France region will unravel the structural (taxonomical and functional diversity, resilience and redundancy) and metabolic (N, C and P cycles) responses capacity of these microbiomes.

Keywords: Eutrophication, Freshwater, Microbiome, Functional diversity, Space-Time series

The need for standardization in the use of metabarcoding approaches for the use of microorganisms in environmental risk assessment

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There is increasing interest in the use of the microorganisms within a regulatory context for environmental risk assessment. In this regard, there has been considerable discussion amongst academic communities and regulatory bodies about the use of metabarcoding approaches for this purpose. However, there has been little consideration as to how to translate the complexities of metabarcoding DNA sequencing technologies and the data generated into the highly standardized arena of regulatory science and environmental risk assessment. Microbial community data generated through metabarcoding approaches can be highly variable in both the quality of data and the quantity of reads. Such factors can lead to decisions regarding data processing and downstream analysis to be taken on a per dataset level, which could lead to considerable variation between outcomes of potential ecotoxicological studies. This does not align well with existing frameworks for ecotoxicological studies to be used for environmental risk assessment, which require highly standardized, repeatable studies. This poster discusses the challenges associated with the use of metabarcoding technologies in generating standardized, repeatable and robust data suitable for use in a risk assessment context. We will discuss how data processing decisions made regarding: bioinformatics pipeline selection, variable DNA quality, clustering algorithms, erroneous or contaminant sequences, the inclusion of highly rare taxa, endpoint calculation, or the rarefaction (or not) of data to an even sampling depth. Many of these decisions are taken on a case-by-case basis by individual researchers. However, standardised methodologies and data processing guidelines are required to enable such technologies to be used within an environmental risk assessment. Our goal is to present a challenge to the microbial ecotoxicology community to consider the how their research, testing methods and analysis pipelines can be translated into a regulatory science context.

Keywords: Microbiome ; Sequencing ; Standardisation ; Regulatory ; Bioinformatics

Harmonizing bioinformatics procedures in microbiome amplicon high-throughput sequencing

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The last decade has witnessed a fast development of high-throughput sequencing (HTS) technologies and associated procedures. They allowed, among others, to study the microbiome composition of complex samples from very contrasting environments using amplicon HTS. Bioinformatics workflows have been developed to deal with the huge amount of generated data, and meta-analysis tools like Qiita have been created to analyze microbiome data in the context of tens of thousands of other samples from other studies. However, to allow such an approach, sequencing data must be generated and processed in the same way so that the results from different studies can be appropriately compared. Hence, the major steps of a traditional bioinformatics workflow dedicated to microbiome amplicon HTS are presented here, together with the main available options, pros/cons and recommendations for each of them. Slightly upstream of the bioinformatics data processing, the choice of the amplified target(s) must be cautiously made as the different loci do not provide the same taxonomic coverage and they may be differently affected by amplification biases. Robust results are provided by evaluating the suitability of markers using mock samples and by amplifying several loci. Combining short- and long-read sequencing technologies is also promising. Regarding the bioinformatics workflow itself, it can be divided into four main steps/components: the read merging, where an appropriate trimming strategy should be applied to avoid unexpected sequence losses during contig generation. The read clustering into representative sequences is another key point with a consensus in favour of ASVs to replace OTUs. Finally, reference databases and classifiers should be carefully selected/parametrized according to the study specifications, some combinations being irrelevant. All together, these harmonizing efforts will help increasing the range of microbiome studies by allowing the comparison of sequencing data from different works in a relevant way, based on results produced with identical procedures.

Keywords: Microbiome ; high ; throughput sequencing ; bioinformatics ; harmonization

Combined sewer overflows continually disseminate cocktails of biochemical pollutants into surface waters

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At the beginning of typically sudden and intense Mediterranean rainfalls, soil surfaces are washed away, river sediments remobilized, and sewer pipes drained. These waters are qualified as “first flushes” and the materials they carry enter directly into surface waters by runoff or are collected by the sewage network to end at wastewater treatment plants. There is large evidence that “first flushes” are major contributors of contaminants to surface waters. Through a study of coupled physical-biogeochemical-pollution parameters at a Mediterranean extreme storm event, we were able to impute without ambiguity the highest level of pollution mixtures including wastewater-borne microorganisms ever recorded in the coastal Têt river to the drainage of combined sewer overflows (CSOs). The goal of this communication was to deepen our knowledge on the spatial and temporal dynamics of pollutant mixtures through a 5-year sampling at rural and urban stations along the same river. We quantified micropollutants, fecal bacteria markers, pathogens and integron-integrases and antibiotic resistant genes (ARGs) and could establish significant links on their evolution along time and space. Average biochemical pollutant levels at urban sites were significantly higher than those at rural sites. At the several CSOs studied, pollutants exceeded by several orders of magnitude average levels at urban sites. Recorded levels were correlated with the intensity of first flushes rather than with the river flow. In conclusion, combined sewer overflows chronically disseminate a massive cocktail of micropollutants, ARGs, and fecal and pathogenic microorganisms into surface waters because they occur whenever there is an intense rainfall. Biochemical indicators that increased systematically during CSOs could be incorporated into tools to detect on-site the sewers that contribute the most to pollution cocktails, which in turn would help decide which combined sewers should be prioritized in their conversion into sewers that conduct rain and waste waters separately. These tools will help to mitigate CSOs threat to freshwater and coastal ecosystems and to human health.

Keywords: first flushes; ARGs; fecal biomarkers; micropollutants; pathogens

Microbial indicators, antibiotic resistance, metabolic changes, and energy metabolisms driven by long-term exposition to multiple pollutants in a highly impacted tropical coastal Bay

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The microbial community inhabiting impacted coastal sediments reflects on their structure and functional patterns the effects of chronic exposition to contaminants. Since sediments accumulate pollutants released from anthropogenic activities, monitoring its quality through appropriate and accurate tools is urgently requested. The Sepetiba Bay (SB, Rio de Janeiro – Brazil) presents long-term contamination by metals (mainly Zn and Cd) since the 1960s. The current contamination scenario is mostly influenced by the urban expansion that contributes to high organic and pollutant loads to the bay from the release of untreated sewage to the main tributary rivers, mainly impacting the SB internal sector stations (eastern portion). While external sector stations (western portion) are less polluted and mostly influenced by tidal currents. Using bacterial community composition data (16S rRNA gene amplicon sequencing - V4-V5 region) and functional prediction analysis (Tax4Fun2), the present study aims to decipher the impact of *in situ* pollution by pointing out the microbial indicators of metal pollution, the main pathways/energy metabolisms/genes favored by the metallic contamination, and the co-selection of antibiotic-resistant genes (ARG) driven by metals. Our study shows that anaerobic metabolism microbial indicators dominated the most polluted stations while aerobic metabolism indicators dominated the less polluted stations. Such observation brings new insight into microbial community organization highlighting that the most polluted sites own methanogenic metabolism – in which predicted genes were more abundant in the internal sector - contributing also to greenhouse gas emissions. In addition, correlating with metal contamination data, the predicted function of microbial communities showed that different functional patterns predominate in each SB sector. Our study also revealed a strong correlation between metal resistance genes (MRG) and antibiotic resistance genes (ARG), indicating that MRG and ARG are co-selected by the metallic contamination prevailing in SB.

Keywords: Microbial ecology ; functional ecology ; ecotoxicology ; risk assessment

Evolution of the environmental dimension of antibacterial resistance research: a scientometric analysis (1990 – 2021)

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Over the past few decades, the number of scientific publications on antimicrobial resistance has steadily increased. Similarly, and although this dynamic started a little later, the number of works specifically addressing the environmental dimension of this problem − that is, release, fate, and effects of antimicrobials, antimicrobial residues, and resistant microorganisms in the environment − has also continued to grow.

Part of an ongoing research in history and sociology of science, this scientometric analysis traces how the international research field on the environmental dimension of antibacterial resistance has been structured and transformed over the past 30 years. It is based on the scientific literature published in English since 1990 and available via the Web of Science.

Distinct from more "traditional" bibliometric methods that are limited to statistical − but static − descriptions of a research field over a given period, this presentation proposes an analysis of the dynamics according to which this field has evolved throughout the period considered (1990-2021). Various quantitative analyses and in particular network analyses are performed with CorText Manager (a collaborative web application for analysis and mapping of heterogeneous data, sustained by the french Institute For Research and Innovation in Society (IFRIS) and France's National Research Institute for Agriculture, Food and Environment (INRAE) : https://www.cortext.net).

These analyses focus on different entities − research objects (environmental compartments, types of antibacterial drugs, bacterial species...), methods, topics and research communities − and retrace the evolution of both (i) the importance of these different entities within the research field and (ii) the strength of the links that connect them. This presentation thus shows how this research field is structured today and offers a view on the epistemic, technical, and temporal dynamics that led to the current structure by describing how it has evolved over the last three decades.

Keywords: scientometric analysis ; antibacterial resistance ; environment ; network analyses ; temporal dynamics

Evaluation of the ecotoxicological effects of sulfamethoxazole, ciprofloxacin, and tetracycline alone or in chemical mixtures on the bacterium *Aliivibrio fischeri*

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The ubiquitous presence of antibiotic residues as environmental emerging contaminants of ecosystems has become a serious concern. Antibiotic (AB) consumption in huge amounts in human and veterinary medicine cause their environmental spread, through wastewater plants, reclaimed water and agricultural practices. Antibiotics potential effects on non-target microorganisms are not well known. An AB can be found in natural environments as a single compound or in a mixture with other antibiotics or contaminants. Consequently, different additive and synergic effects can occur on a microbial population, influencing for example its abundance and functioning.
The use of an ecotoxicological standard test makes it possible to evaluate direct acute effects of a substance in terms of effective doses (e.g. EC_{50}) and to compare different chemicals for their potential toxicity. Owing to its intrinsic biocide effect, an antibiotic is expected to be harmful for bacteria. The *Aliivibrio fischeri* bacterium has be largely employed to evaluate the acute toxicity of many chemicals (metals, IPAs, PAHs) commonly found in environment. In this context, this study provides an ecotoxicological evaluation of some antibiotics (sulfamethoxazole, ciprofloxacin, and tetracycline) alone or in mixture for evaluating the bioluminescence inhibition (%), in accordance with the UNI EN ISO 11348-3:2019. Moreover, the same ABs were tested with a heavy metal, such as copper. Finally, a soil co-contaminated by the three antibiotics and copper was tested in order to evaluate the ecotoxicological effect on the test bacterium of a real matrix. The main results will be discussed.

Keywords: Antibiotics, non-target microorganisms, ecotoxicological test, soil mixture effects

Transcriptomic and proteomic responses of *Microbacterium* sp. C448 exposed to sulfamethazine antibiotic

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The *Microbacterium* sp. C448 was isolated from a soil regularly exposed to sulfamethazine (SMZ), for its ability to partly mineralise this antibiotic and other related sulfonamides.

The aim of our study was to explore its metabolic adaptation towards exposure to SMZ environmental (10 mg/L) and medicinal (250 mg/L) concentrations. Its transcriptomic and proteomic responses were analysed by focusing on the degradation regulon (*sad* genes) and resistance genes (*folP* and *sul1*).

The transcriptomic and proteomic results were essentially congruent whatever the concentrations tested. In culture conditions, exposure to the highest concentration of SMZ led to the highest *sad* expression and Sad production, confirming these proteins' role in sulfamethazine degradation *in cellulo*. Moreover, when sulfamethazine was completely degraded, Sad production tends to return to the basal level observed under control conditions. Congruence of transcriptomic and proteomic results was also observed for resistance genes. Although Dihydropteroate synthase (DHPS)-Sul1 protein was 100-fold more produced than that of DHPS-FolP, both were expressed at a basal level and were not affected by SMZ exposure. Moreover, non-targeted analyses showed overexpression and overproduction of a putative sulphate exporter W0Z8D9 in *Microbacterium* sp. C448 exposed to the highest SMZ concentration. In addition to *sul1* resistance genes, this efflux pump could be involved in the sulfamethazine detoxification process by exporting the SO_2 formed during its degradation.

Acknowledgements : The authors thank the National Research Agency for its financial support (ANTIBIOTOX project ; Grant ANR-17-CE34-0003).

Keywords: Sulfonamide antibiotic, *Microbacterium*, Biodegradation regulon, Resistance genes, Omic approaches

The effects of selected pharmaceutical contaminants on Acinetobacter calcoaceticus isolated from drinking water

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The occurrence of emerging contaminants (ECs) in drinking water (DW) is unquestionable, as well as the unavoidable biofilm formation in DW distribution systems (DWDS). However, the exposure of DW biofilms to ECs is a reality often disregarded, and the effects from this exposure may affect the water quality. It is crucial to recognize the ECs impact on DW biofilms for further ECs prioritization. This work studied the effects of the exposure of *Acinetobacter calcoaceticus* biofilms to four pharmaceutical compounds already found in DW at residual concentrations (µg/L or ng/L): caffeine – CAF, carbamazepine – CBZ, ciprofloxacin – CIP and ibuprofen – IBP. The main goal of the work was to understand the role of ECs exposure (for 7 days) in bacterial behaviour, particularly the ability to form new biofilms, bacteria susceptibility to antibiotics (CIP, levofloxacin - LEV, tetracycline - TET and trimethoprim-sulfamethoxazole - TMP-SMX), tolerance to free chlorine, motility and growth rate. It was found that IBP exposure decreased biofilm formation ability by 11%, while an increase of 16% was observed after the exposure to the mixture of all selected ECs (MIX). Moreover, CAF-exposed bacteria had lower susceptibility to TPM-SMX, while CBZ- and CIP- exposed bacteria were more susceptible to TMP-SMX. Furthermore, exposure to CIP resulted in a decrease of *A. calacoaceticus* susceptibility to CIP and LEV. Also, IBP exposure decreased *A. calcoaceticus* susceptibility to LEV and TET. Higher *A. calcoaceticus* growth rates were obtained after CIP exposure. These results demonstrate that the selected ECs affected the behaviour and growth dynamics of bacteria under conditions typically found in DWDS. Among the selected ECs, CIP and IBP were those causing the most concerning effects.

Keywords: antibiotic ; antimicrobial susceptibility ; biofilm ; drinking water ; pharmaceutical contaminants

Genotypic and phenotypic analysis of antimicrobial resistance in the denitrifying isolate *Pseudomonas veronii*

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We investigated the antibiotic resistance in a non-pathogenic, denitrifying bacteria *Pseudomonas veronii*. This isolate, from a river sediment, is capable of complete denitrification from NO3- to N2. *P. veronii* was tested towards a range of antibiotics via the classical disc method as well as a cultivation method allowing to determine dose-effect curves. In parallel antibiotic resistance genes in the genome of the denitrifier were identified via annotation through MaGe from the MicroScope platform.

The genotypic antibiotic resistance of the denitrifier P. veronii showed the presence of several genes encoding efflux pumps (e.g. AcrAB-TolC). *P. veronii* was resistant towards macrolides (erythromycin and tylosin), tetracycline the quinolone flumequine, chloramphenicol, the B lactam amoxicillin and the aminocoumarin novobiocin. On the other hand, P. veronii was sensitive towards the sulfonamide sulfamethoxazole and the quinolones ciprofloxacin and ofloxacin. A tolerance, i.e. a delayed impact, was observed for the beta-lactam ampicillin and fosfomycin. Resistance towards the large lipophilic antibiotics (novobiocin, erythromycin and tylosin) is most likely related to the exclusionary properties of the cell envelope. The presence of efflux pump genes most likely explains resistance towards the other antibiotics tested, but needs to be confirmed. Our results show that the non-pathogenic denitrifier *P. veronii* exhibits resistance towards several antibiotics, however sensitivity and tolerance towards others. The latter most likely affects the functioning of this species in the environment and its denitrifying activity which will be investigated in the future.

Keywords: denitrification ; antibiotics ; environment

Following the route of veterinary antibiotics tiamulin and tilmicosin from livestock farms to agricultural soils

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Veterinary antibiotics (VAs) are hardly metabolized in the animal body after administration and are mostly excreted in feces. Their use for soil fertilzation or energy production may facilitate VA dispersal impacting environmental quality and human health. We studied the persistence of two VAs, tiamulin (TIA) and tilmicosin (TLM) after their administration to pigs towards receiving environments. We asked the questions: (a) how different administration modes affect their excretion; (b) how anaerobic digestion and ambient storage affect VAs and *vice versa*; (c) how persistent are VAs in agricultural soils. TLM was detected in feces at levels folds higher (4.27-749.6 μg g^{-1}) compared to TIA (0.55-5.99 μg g^{-1}), with both VAs peaking during the administration period followed by a gradual dissipation during the withdrawal period. Administration through water resulted in delayed or lower levels for TIA and TLM, compared with feed administration. TIA and TLM (fortification levels 0.5, 5 and 50 μg g^{-1}) dissipated gradually during manure stockpiling (DT_{50} 5.85-35.9 and 23.5-49.8 days respectively). Both VAs persisted longer during anaerobic digestion (DT_{90} > 365 days) and negatively affected biomethanation at levels > 5 μg g^{-1}. In direct soil application, TLM was more persistent than TIA with fumigation extending their persistence, suggesting a major role of soil microbiota in their degradation. Soil application of VAs through feces increased their persistence, probably due to sorption to organic fecal matter. Our results suggest that manure incorporation in agricultural soils of TIA/TLM treated pigs will likely disperse VAs in soil with yet unexplored consequences for the environment and human health.

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Keywords: veterinary antibiotics, tiamulin, tilmicosin, dissipation, anaerobic digestion.

Use of reclaimed water for soil irrigation: role of biotic pressure on the antimicrobial resistance dissemination

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The increasing demand for water has made the planned use of water an issue of major concern. In this context, the use of treated wastewater (TWW) for irrigation appears as an alternative for solving water availability problems. Through irrigation, the soil endogenous microbiome is exposed to exogenous organisms (including pathogens, antibiotic resistant bacteria) and chemicals (like antibiotics) that may interfere with their growth and life but could also play a role in the mitigation of the dissemination of antimicrobial resistance (AMR). Indeed, interactions between these endogenous and exogenous microbiomes may rule out the AMR dissemination. In order to explore this point, lab-scale microcosms were set up using 2 soils with a four-year history of irrigation with (i) tap water and (ii) TWW, implying thus a different AMR pressure. Inside glass flasks, 25 g of each soil was weighed and an equivalent of 100 L/m2 irrigation water was added. Four type of water were tested with various microbiome load: tap water, TWW, treated and filtered 0.2 µm wastewater, treated and filtered 1 kDa wastewater. TWW was also added on the soils that were sterilized before irrigation in order to reduce the diversity of the endogenous microbiome. These flasks were incubated and sequential samplings were performed at 0, 7, 14, 30, 45, 60, 75, 90 days after irrigation. Sequencing and quantitative PCR analysis on soil and water samples will serve to understand the respective role of soil and water microbiome on AMR dissemination. Other agronomic data were also collected to verify their influence in biotic interactions. These data can serve for future decisions, mainly if we discover that the reclaimed water has an important role in biotic pressure and resistance spread. In this case, considering the possibility of filtering the reclaimed water before use it for irrigation will be a feasible alternative.

Keywords: resistant genes ; antibiotics ; water reuse ; microbiomes

Is environmental epidemiology suitable for monitoring emerging antibiotic resistance in human ? a pilot study in wastewater of hospital buildings

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Antibiotic resistance is a major global threat, especially to human health. Bacteria display more and more resistance genes and Multi-Dug Resistant (MDR) bacteria are now endemic worldwide, for instance enterobacteria producing extended spectrum betalactamase (EBLSE) such as CTX-M betalactamase. Extremely resistant bacteria (XDR) are emerging and their epidemiology should be monitored thoroughly. Among XDR bacteria, carbapenemase-producing enterobacteria (CPE) are of particular concern [1,2] because they produce specific enzymes that hydrolyze carbapenems (last ressort antibiotics in severe infections [2,3]) [4] but also most molecules in the β-lactam antibiotic class. In France, the most widespread carbapenemases are firstly oxacillinases of type OXA-48, then NDM metallo-β-lactamases [5]. A second type of emerging XDR bacteria are vancomycin-resistant enterococci (VRE) carrying *vanA* resistance gene. Surveillance of antibiotic resistance is a key to prevention mainly for emerging XDR [6]. Monitoring of pathogens in wastewater has been effective during the SARS-CoV-2 pandemic. In this study, we developed a strategy to monitor XDR genes $bla_{\text{OXA-48}}$, bla_{NDM} et *vanA* in wastewater from three hospital buildings occupied by patients with various risk for XDR carriage. We also compared the rate of emerging XDR genes to the rate of blaCTX-M chosen as marker of endemic MDR.

Freshwater microbial communities as a potential nature-based solution for wastewaters tertiary treatment

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In this presentation the results of two experiments, one carried out at bench scale, under controlled conditions, and the other performed in a pilot reactor located in a wastewater treatment plant (WWTP), will be presented. Both experiments aimed to investigate the efficiency of freshwater microbial communities to improve the overall quality of urban wastewater after conventional treatments. A wide set of water quality parameters were monitored at the influent and effluent of the bioreactors including nutrients concentration (total and inorganic forms), fecal bacteria (*E.coli*) and contaminants of emerging concern to calculate the removal rates achieved. A set of biological parameters of the benthic and planktonic communities (organic matter content, algal biomass and composition, and its photosynthetic efficiency) were also measured simultaneously to be related with bio-reactor removal performance. Preliminary results show an overall improvement of treated wastewater quality, both at bench scale and in the pilot plant. In particular, under controlled conditions, the bioreactor based on the benthic microbial communities' activity showed the highest percentages of nutrients and fecal bacteria removal. More specifically, these biofilm-based reactors were able to reduce continuously more than 50% of the total phosphorus and nitrogen still present in treated wastewater, during the 9 weeks of the bench scale experiment duration, and these removal capacities were significantly correlated with biofilm algal biomass. Moreover, the removal efficiencies of fecal bacteria gradually increased during the experiment and finally resulted around 65% the last three weeks. Similar results are expected for the pilot reactor of the WWTP in which preliminary assays evidenced that an overall reduction of nitrogen and phosphorus emissions of about ~5000 kg year-1 (TN) and ~1000 kg year-1 (TP) to receiving freshwater ecosystems could be achieved by implementing a biofilms-based reactor, only considering 6 of the 538 WWTPs currently operating in Catalonia (NE Spain).

Keywords: microbial communities ; wastewater treatment ; nature ; based solution ; freshwaters

Performic acid: disinfection effectiveness and ecotoxicology on receiving environment

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To address the growing demand for water and threats to water safety from both emerging biotic and abiotic contaminants through treated wastewater discharges, many advanced emerging technologies for wastewater treatment have been developed. Recently, performic acid (PFA) has received increasing interest due to its high oxidizing properties. Most of the research work has focused on the disinfection of target indicator microorganisms. However, its ability to disinfect of microorganisms in a real wastewater matrix and its effect on microbial ecotoxicology in receiving water remains unknown. This study has two objectives. The first one is to evaluate the PFA-disinfection efficiency on microorganisms in the effluent. For this purpose, experiments with effluent from the Colombes WWTP treated with PFA at 0.8, 2 and 4 mg/L were set up in the laboratory. The second is to evaluate the PFA-effect on the microbial ecotoxicology of the Seine water. Hence, mixing experiments of the effluent treated by PFA (2 mg/L x 10 minutes) with Seine water (10/90, v/v) were performed in the laboratory. Subsamples were collected at different time points. Abundance, activity and diversity of microbial communities were characterized using flow cytometry, enzymatic assay and next generation sequencing. Microbial abundance results showed that the PFA-efficiency in wastewater treatment (expressed as the number of damaged cells per liter) increased significantly and linearly with increasing PFA concentration and contact time. Moreover, microbial abundance (expressed as the number of intact cells per liter) and activity (expressed as the amount of fluorescein produced per hour per liter) in the Seine water mixed with the PFA-treated effluent decreased gently with increasing contact time. Microbial diversity was performed to determine which microbial groups were most sensitive to PFA treatment. The results of this work will assist authorities in making decisions regarding the application of PFA to treat wastewater on a large scale.

Keywords : performic acid ; disinfection ; wastewater treatment ; ecotoxicology ; receiving water

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