

EcotoxicoMic 2020

2nd International Conference on Microbial Ecotoxicology

Virtual Edition (6th-9th October, 2020)



https://ecotoxicomic.org/

Programme & Book of Abstracts



Conference website : https://ecotoxicomic20.sciencesconf.org/

Contact : ecotoxicomic20@sciencesconf.org



https://fcsrovaltain.org/

Welcome to EcotoxicoMic 2020!



Following the success of the First International Conference on Microbial Ecotoxicology that was held in Lyon in 2017, it is our great pleasure to welcome you to this second edition.

Given the overall situation with the COVID-19 pandemic, it has been very challenging to organize such an event. That's why we are proud to offer an original virtual conference gathering the international scientific community dealing with microbial ecotoxicology in a wide range of ecosystems.

This was possible thanks to a motivated organisation team and the support of our many partners. We hope the quality of the presentations and the presence of world-renowned speakers will make EcotoxicoMic 2020 a scientific success.

We are already looking forward to seeing you all again in Montpellier for the EcotoxicoMic2022 conference that will be held from November 15th to 18th, 2022.

We wish you a nice and fruitful virtual conference!



Stéphane Pesce, INRAE Co-Founder of the EcotoxicoMic Network

Lese



Philippe Garrigues, CNRS President of the Rovaltain Foundation

Programme at a glance

(Time slots correspond to French Time Zone)

0:00.200	Tuesday October 6	h Wednesday October 7th	Thursday October 8 th	Friday October 9 th
12:00am	DRomics tool training course (specific registration is required)	SESSION 2 (9:00-12:40 am) Microorganisms in complex systems: biological interactions, pollution & climate change Keynote Conference: H. Guasch	SESSION 1 (9:00-12:20 am) Impact of contaminants on microbial functions Keynote Conference: S. Hallin	Virtual POSTER SESSION (9:00-11:00 am) Scientific Committee meeting (deliberations for awards)
2:00pm		SESSION 2 (2:00-3:20 pm)	SESSION 1 (1:40-2:20 pm) End of the session	SESSION 5 (2:00-2:40 pm)
OPENING SESSION (2:30-3:30 pm) Welcome & Practical information Opening Conference: L. Wick Virtual POSTER SESSION (3:30-5:30 pm)		SESSION 4&5 (3:40-5:20 pm) Microorganisms for ERA -&- Microbial ecotoxicology: from research to end-users	SESSION 3 (2:20-5:40 pm) Microorganisms role in contaminant (eco)dynamics & application to bioremediation	Keynote Conference: I. Lavoie AWARDS CEREMONY CLOSING SPEECH (→3:30 pm)
6:00pm		Keynote Conference: C. Griebler	Keynote Conference: J. Lloyd	

Organizers

Coordinator

Operational team



Christophe Leboulanger IRD



Delphine Delaunay Rovaltain Foundation (CEO)



Stéphane Pesce INRAE

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- Maialen Barret, ENSAT, Toulouse, OC, SC
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Partners

Platinum Partner



Frontiers is a community-rooted, open-access academic publisher. Their grand vision is to build an Open Science platform where everybody has equal opportunity to seek, share and generate knowledge, and that empowers researchers in their daily work.

Frontiers in Microbiology (IF=4.235) is a leading journal in its field, publishing rigorously peer-reviewed research across the entire spectrum of microbiology.

Exclusively for attendees of EcotoxicoMic 2020, Frontiers in Microbiology will offer a **50% discount on the full publishing price (limited to the 40 first submissions)**.

Gold Partners

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Environmental Science and Pollution Research (ESPR)





To answer global challenges, the **French National Institute for Agriculture, Food, and Environment (INRAE)** is deploying targeted research, combining fundamental and applied research, providing long-term solutions and short- and medium-term innovations. The institute is committed to an open, participatory and interdisciplinary science approach to build transitions with society.

Occitania is the most attractive region in France, with nearly 47,000 new inhabitants each year. Thanks to this dynamism, the Region supports economic development that is a source of social progress, ensures equal opportunities and access to employment, guarantees the sustainable development of territories, strengthens solidarity.

The **French National Research Institute for Sustainable Development (IRD)** is a crucial player in providing scientific advice on the major challenges of development. It takes an original approach to research, expertise, training and knowledge-sharing for the benefit of countries and regions, making science and innovation key drivers in their development.

A special funding from IRD has allowed to offer **free registration grants to a dozen of scientists** from eligible countries such as Syria, Tunisia or Mexico.

The Environmental Science and Pollution Research (ESPR) Journal form Springer serves the international community in all areas of Environmental Science and related subjects with emphasis on chemical compounds.

Thanks to a special grant from ESPR, the **best oral and poster presentations by young researchers will be awarded**, with a **total prize of 4,500€**.

The **French Office for Biodiversity (OFB)** is a French Government agency that conducts research and enforces government policies on wildlife and the environment. It provides support for government environmental policies and assists managers of national areas. CFB also strives to educate the public on biodiversity issues and gain their support for conservation.

With 31 municipalities and more than 470,000 inhabitants, **Montpellier Méditerranée Métropole** participates in the vast movement of municipal, urban and peri-urban regrouping, committed throughout France. The metropolis, with its 31 municipalities, is the heir to a long history. Marked by migrations, the richness of its economic and cultural activities, this territory is ideally located, at the crossroads of influences that stir up the entire Mediterranean.

Silver Partners













HydroSciences Montpellier









The **French geological survey (BRGM)**, is France's reference public institution for Earth Science applications in the management of surface and subsurface resources and risks. Around geosciences, BRGM is developing expertise to contribute to a harmonised management and controlled use of the soil and subsoil of cities and territories.

Set up in 2009, the **French ECOTOX network of ecotoxicologists**, has broadened to include the French community active in this field and currently involves around 150 members from some twenty INRAE research units and from external partners (INERIS, IRSN, CNRS, CEA, MNHN, universities and specialised schools, etc.).

The Mediterranean Centre for Environment and Biodiversity Laboratory of Excellence (CeMEB LabEx) is a federation of ten research units in the Montpellier area. Research focuses on the dynamics and functioning of biodiversity and ecosystems in a context of marked environmental change, notably due to human activities. An important objective is to use scenarios to anticipate both the biological consequences of global change, and anticipate the evolution of ecosystem services and human societies.

The University of Montpellier (French: Université de Montpellier) is a French public research university in Montpellier in south-east of France. Established in 1220, the University of Montpellier is one of the oldest universities in the world. University of Montpellier was ranked 1st in the world in Ecology in the subject rankings of Academic Ranking of World Universities 2018.

The University of Perpignan (French: Université de Perpignan; Catalan: Universitat de Perpinyà Via Domitia) is a French university, located in Perpignan. The first university of Perpignan was established in 1349 by King Peter IV of Aragon. It is one of the oldest regional universities, following the steps of close centers, such as University of Toulouse and University of Montpellier.

HydroSciences Montpellier (HSM) is a joint research unit depending of CNRS, Université Montpellier and IRD. It gathers some 150 researchers and teaching staff. The main objective of HSM is to quantify and predict how climate change and human activities affect water resources in the Mediterranean and tropical regions.

The **CEFREM** is a research unit of the university specialised in the coastal environment. The researches are focusing on material and energy transfers at the interfaces of the coastal system. The research team is composed of sedimentologists, geochemists, biologists and physicians. The new equipment will be used in courses for the Master in Marine Geosciences and Aquatic Environments.

The engineering schools of Toulouse INP relies on approximately 700 researchers grouped in 17 laboratories associated with the CNRS, INRAE and other universities in Toulouse. The two main fields of research of Toulouse INP are Physical science and Engineering for Energy, Climate, Digitalisation and Materials; and Life Sciences and Engineering for Agronomy, Agribusiness and Environment.

Research at **EcoLab** covers various aspects of the dynamics of biodiversity and ecosystem functions in a context of global change to study the effects of both natural variability and anthropogenic disturbance on a local to global scale, and to analyse physiological, biological, ecological and biogeochemical responses as well as the feedbacks between biodiversity and environmental change over time periods ranging from organismal life cycles to millenia.

The Observatory for Research on the Mediterranean Environment (OREME) is an OSU devoted specifically to assessing unpredictable variations in Mediterranean environments and their vulnerability —through the resources provided by the Station Marine de Sète, with the involvement of the University of Montpellier (UM) and the MEDIterranean platform for Marine Ecosystem Experimental Research (MEDIMEER).

Join the EcotoxicoMic Network



EcotoxicoMic, the international network on Microbial Ecotoxicology (powered by the Rovaltain Foundation), aims at establishing and fostering contacts and scientific partnerships within the community of microbial ecotoxicologists, at improving the visibility of research and innovation in the field of Microbial Ecotoxicology and at promoting the transfer of knowledge between the scientific community, the environmental managers and the society.

Created in 2018, following the First International Conference EcotoxicoMic2017, **this informal international network already gathers more than 150 scientists from 29 countries** (Researchers, Professors or Associate Professors, Post-Docs, PhD Students...) interested by the field of Microbial Ecotoxicology.

Becoming a member of the international EcotoxicoMic Network is free-of-charge. The only thing you need to join the EcotoxicoMic Community is to fill online a membership form, available on the EcotoxicoMic website (<u>https://ecotoxicomic.org/</u>).

Publish your work in the next EcotoxicoMic Research Topic



Microbial Ecotoxicology Advances to Improve Environmental and Human Health Under Global Change

<u>Editors</u>: F. Martin-Laurent, INRAE ; A. Cébron, CNRS ; M. Schmitt-Jansen, UFZ ; D. Karpouzas, University of Thessaly; S. Morin, INRAE; C. Palacios, University of Perpignan https://www.frontiersin.org/research-topics/14404/microbial-ecotoxicology-advances-to-improve-environmental-and-human-health-under-global-change

This **Research Topic in** *Frontiers in Microbiology* (IF=4.235) welcomes original results concerning these challenging questions as well as articles addressing the latest advances in microbial ecotoxicology.

Exclusively, for attendees of EcotoxicoMic 2020, Frontiers in Microbiology offers a 50% discount on the full publishing price (this 50% discount for the EcotoxicoMic2020 attendees will be only available for the 40 first manuscripts submitted). The **articles will be published as soon as they are accepted**. They will be further compiled into a free-of-charge and free-to-access eBook, which we can be distributed electronically.

Consult the first Frontiers e-book on Microbial Ecotoxicology

https://ecotoxicomic.org/2020/07/17/frontiers-e-book-on-microbial-ecotoxicology/

The first Frontiers e-book on Microbial Ecotoxicology is freely available online.

Co-edited by S. Pesce (INRAE), F. Martin-Laurent (INRAE), E. Topp (AAFC) and J.-F. Ghiglione (CNRS) following the EcotoxicoMic2017 conference, this free e-book brings together **42 articles co-authored by 281 investigators**.



Tuesday, October 6th

2:30 – 3:30 pm	Opening Session	(Chair: Christophe Leboulanger)
2:30-2:50	Introduction (Christophe Let	ooulanger, Philippe Garrigues)
2:50-3:30	Keynote Conference (Lukas	Wick)

- 3:30 5:30 pm Virtual Poster Session
- 5:30 pm End of the Day

Wednesday, October 7th

9:00 – 10:40 am Session 2 "Microorganisms in complex systems" – Part I (Chair: Chloé Bonnineau)

- 9:00-9:40 Keynote Conference (Helena Guasch)
 9:40-10:00 Bacterial transfer from plastic to deep-coral microbiomes: harmful effect and holobiont concept (Anne-Leila Meistertzheim)
- 10:00-10:20 A disturbed environment impacts the gut microbiota of the Mediterranean pond turtle Mauremys leprosa (Schweigger, 1812) (Carmen Palacios)
- 10:20-10:40 Fish gut microbiome and metabolome responses after an exposure to toxic cyanobacterial blooms (Alison Gallet)

10:40 – 11:00 pm Coffee Break

11:00 – 12:40 amSession 2 "Microorganisms in complex systems" – Part II
(Chair: Carmen Palacios)

- 11:00-11:20 Resistance and resilience of soil N cycle-related bacterial processes to drought and heat stress in rehabilitated urban soils (Mehdi Fikri)
- 11:20-11:40 Toxic bloom-forming Planktothrix (cyanobacteria) stressed by high light and high temperature (Sandra Kim-Tiam)
- 11:40-12:00 Microbial production of geosmin within freshwater biofilms: driving factors in a global change scenario (Lorenzo Proia)
- 12:00-12:20 Particle-attached riverine bacteriome shifts in a pollutant-resistant and pathogenic community during a Mediterranean extreme storm event (Mégane Noyer)
- 12:20-12:40 Metal resistance genes rich biofilm communities selected by biocide-containing surfaces in temperate and tropical coastal environments (Jean-François Briand)

12:40 am - 1:00 pm Lunch Time

2:00 – 3:20 pm Session 2 "Microorganisms in complex systems" – Part III (Chair: Mechthild Schmitt-Jansen)

- 2:00-2:20 Does pesticide pressure affect the grazing behaviour of chironomids on microalgae? (Soizic Morin)
- 2:20-2:40 Ecotoxicity of graphene-based materials toward a diatom-bacteria biofilm (Lauris Evariste)
- 2:40-3:00 Is plastic a real threat to freshwater detrital ecosystem? A trophic approach. (Daniela Batista)
- 3:00-3:20 Effects of thorium on periphytic biofilm community, taxonomic structures, nutritional quality, and its trophic transfer to the snail Lymnaea sp. (Caroline Doose)
- 3:20 3:40 pm Coffee Break

Wednesday, October 7th

3:40 – 5:20 pm	Sessions 4&5 "Microorganisms for ERA & microbial ecotoxicology from research to end-users" (Chair: Fabrice Martin-Laurent)
3:40-4:20	Keynote Conference (Christian Griebler)
4:20-4:40	Characterizing the effects of a complex contamination by pesticides: Preliminary results on the Cleurie River (France), a pilot site for an interdisciplinary approach based on phototrophic biofilms. (Martin Laviale)
4:40-5:00	Test of biodegradable polymers to replace the European-banned conventional plastic sticks : from research to end-users (Jean-François Ghiglione)
5-00-5:20	Application of toxicophenomics in ecotoxicity tests using marine phototrophs: merging bio-optics and artificial intelligence. (Bernardo Duarte)
5:20 pm	End of the Day

Thursday, October 8th

9:00 – 10:40 am	Session 1 "Impact of contaminants on microbial functions" – Part I (Chair: Jean-François Ghiglione)		
9:00-9:40	Keynote Conference (Sara Hallin)		
9:40-10:00	The power of the dose-response framework to uncover molecular functional responses of micro-organisms to chemicals: Dromics (Floriane Larras)		
10:00-10:20	Side effects of pesticides in groundwater: impact on bacterial denitrification (Caroline Michel)		
10:20-10:40	Which potential of antibiotic resistance of environmental bacteria? The case of denitrifiers (Pseudomonas veronii) (Chen Chen)		
10:40 – 11:00 pm Coffee Break			
11:00 – 12:20 an	n Session 1 "Impact of contaminants on microbial functions" – Part II (Chair: Dimitrios Karpouzas)		
11:00-11:20	BactoTrait : A functional trait database to evaluate how contaminants govern the assembly of bacterial communities (Aurélie Cébron)		

- 11:20-11:40 The impact of urban contamination on antibioresistance in microbial communities from periphyton and sediments (Chloé Bonnineau)
- 11:40-12:00 Wastewater effluent impacts the microbial diversity of periphyton in artificial streams and leads to increased community tolerance to micropollutants (Louis Carles)
- 12:00-12:20 Dynamics and main players of the N cycle in microbial mats are affected by chronic hydrocarbon contamination (Marisol Goñi-Urriza)

12:20 am - 1:40 pm Lunch Time

1:40 – 2:20 pm Session 1 "Impact of contaminants on microbial functions" – Part III (Chair: Dimitrios Karpouzas)

- 1:40-2:00 Metabolic response of Microbacterium sp. C448 exposed to environmental and medicinal concentrations of sulfamethazine antibiotic (Laurianne Paris)
- 2:00-2:20 How to anchor metabolic responses of microbial communities to community functioning? (Stefan Lips)

Thursday, October 8th

2:20 – 4:00 pm Session 3 "Microorganisms role in contaminants (eco)dynamics & application to bioremediation" – Part I

(Chair: Jennifer Hellal)

- 2:20-3:00 Keynote Conference (Jonathan Lloyd)
- 3:00-3:20 Dichloromethane degradation at contaminated sites Insights from biomolecular and dual-element isotope analysis approaches in model aquifers (Stéphane Vuilleumier)
- 3:20-3:40 Nutritional inter-dependencies and a carbazole-dioxygenase are key elements of a Sphingomonas dependent consortium for thiabendazole degradation (Sotirios Vasileiadis)
- 3:40-4:00 Elucidating the micropollutant biotransformation potential of natural stream biofilms (Werner Desiante)
- 4:00 4:20 pm Coffee Break

4:20 – 5:40 pm Session 3 "Microorganisms role in contaminants (eco)dynamics & application to bioremediation" – Part II

(Chair: Jennifer Hellal)

- 4:20-4:40 Adaptability of iron-oxidizing bacteria on printed circuit boards bioleaching: effects of a metal-rich leachate. (Juan Anaya)
- 4:40-5:00 Antibiotics favor the establishment of antibiotrophic bacteria in agricultural soil microbial communities, but are not always sufficient to enhance antibiotic-degradation: manure spreading can help. (Loren Billet)
- 5-00-5:20 Organic micropollutant degradation in a membrane bioreactor inoculated with activated sludge. (Ana Rios Miguel)
- 5:20-5:40 Emerging micropollutants distribution and degradation in marine Capbreton Canyon (North Altantic Ocean) : from sediments to pure strains (Rémy Guyoneaud)

5:40 pm	End of the End of the Day
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Friday, October 9th

9:00 – 11:00 am	Virtual Poster Session
11:00 – 12:00 am	Deliberations for best oral and poster awards (Members of the Scientific Committee only)
12:00 am – 2:00	pm Lunch Time
2:00 – 2:40 pm	Session 5 "Microbial ecotoxicology from research to end-users" – Part II (Chair: Soizic Morin)
2:00-2:40	Keynote Conference (Isabelle Lavoie)
2:40 – 3:30 pm	Closing Session
2:40-3:10	Awards Ceremony (Delphine Delaunay)
3:10-3:30	Closing Speech (Christophe Leboulanger)
3:30 pm	End of the Conference

Posters (1/2) (presented in alphabetical order of first author)

#	First Author (and presenter if ≠)	Poster Title	pdf/video
1	Abdennadher et al.	Alexandrium minutum from the Gulf of Gabès (Southeastern Mediterranean): Morphology, toxicity and molecular characterization towards risk assessment for Paralytic Shellfish Poisoning	pdf
2	Artigas et al.	Interaction between glyphosate and dissolved phosphorus on bacterial and eukaryotic communities from river biofilms	pdf
3	Astaikina et al.	Soil microbial community responses to pesticides	pdf
4	Bagagnan et al.	Spatio-temporal variation of microbial communities of the Seine River surface waters at the scale of the Parisian agglomeration	video
5	Batisson et al.	Agricultural adjuvants: impact on the microbial toxicity and biodegradation of the pesticide active substance?	pdf
6	Batista et al.	Are aquatic fungi able to evaluate microplastic impacts on leaf litter decomposition process?	video
7	Ben Othman et al. (Leboulanger)	Toxicity of PAHs to phytoplankton: an example of legacy contaminants with effects still not adequately addressed	video
8	Bernarde et al.	Evolution of the biodiversity and interspecific relationships in river biofilms from a preserved environment or exposed to pharmaceuticals	pdf
9	Bourhane et al.	Bacterial diversity reports contamination around the Ichkeul Lake (Tunisia)	video
10	Camotti Bastos et al. (Henry)	Study of the recovery of bottom ashes from the combustion of wood-sludge mixtures from wastewater treatment plants in the context of agricultural amendment in France	pdf
11	Carles et al. (Batisson)	Bioremediation of agricultural soils: a natural preventive approach to reduce the environmental contamination by pesticides	video
12	Chonova et al.	Passive samplers to highlight the presence of pharmaceuticals and their potential effect on microbial communities	video
13	Colls et al. (Chonova)	The URBIFUN project – Urbanization effects on the relationship between microbial diversity and ecosystem functioning	video
14	Diaz-Vanegas et al.	Field testing of two filling materials for the bioremediation of arsenic-rich Acid Mine Drainage	video
15	D'Incau et al.	Production of biosurfactants and biodegradation of total hydrocarbons (THC) by an endemic microbial consortia and Burkholderia thailandensis	video & pdf
16	Duperron et al.	Microbiome-aware ecotoxicology of organisms: relevance, pitfalls and challenges	video
17	Evariste et al.	Gut microbiota and host-related responses of Xenopus laevis tadpoles exposed to nanoparticles	video
18	Fadhlaoui et al.	Effects of temperature and contaminants on periphytic biofilms	pdf
19	Fraissinet-Tachet et al. (Maucourt)	Impact of PCBs on the structure of microbial communities	pdf
20	Gréau et al.	Response of Populus trichocarpa and associated endophytic communities to PAH contamination gradient	video & pdf
21	Hanano et al.	Exposure of Aspergillus flavus NRRL 3357 to Dioxin Results in a Hyper Aflatoxicogenic Phenotype: A Possible Role for Caleosin/Peroxygenase (AfPXG)	video
22	Hellal et al.	Bacterial biotransformation of chlordecone and two degradation products from the In Situ Chemical Reduction process (ISCR)	video
23	Holub et al.	Impact of natural radioactivity on microorganism communities in different mineral springs in Auvergne (Massif Central, France)	pdf
24	Jabiol et al.	Array of microbial indicators, a promise for a better monitoring of pesticide effects on stream biological quality	pdf

Posters (2/2) (presented in alphabetical order of first author)

#	First Author (and presenter if ≠)	Poster Title	pdf/video
25	Jouffret et al.	Deep exploration of the microbial community in sediments from the Seine river basin by combining metagenomics & metaproteomics	video
26	Jusselme et al.	Microorganisms: actors governing the degradation/transformation of diuron in sewage sludge during biological treatment	pdf
27	Karas et al.	Exploring the potential of biobeds to treat the pesticide-contaminated effluents from various agro-food industries: insights into the role of microbiome and plasmidome	pdf
28	Lajnef et al.	Variability in microbial diversity during harmful algal blooms in coastal Tunisian waters	pdf
29	Langarica-Fuentes et al.	Effect of glyphosate application on microbial community dynamics of four German agricultural soils	pdf
30	Lavergne et al.	Microbial diversity and pathways mediating mercury transformations in Antarctic aquatic ecosystems	video
31	Mahamoud Ahmed et al. (Pesce)	Chronic-exposure of river sediment microbial communities to environmental concentrations of copper induces diversity changes and tolerance acquisition without increasing sensitivity to arsenic	pdf
32	Malbezin et al.	Response of river biofilms to herbicide contamination: taxonomic and functional changes and their implications for nutritional quality	video
33	Martins et al.	The unexpected role of bioaerosols in the Oxidative Potential of atmospheric Particulate Matter (PM)	pdf
34	Michel et al.	Preservation of microbial consortia: impact of storage at 4°C and -80°C on an As(III)-oxidizing community	pdf
35	Nasri et al.	Nematodes trophic groups changing via reducing of bacterial population density after sediment enrichment to ciprofloxacin antibiotic: Case study of Marine Mediterranean community	pdf
36	Noyer et al.	Archaea and Bacteria evolve differently in response to a Mediterranean extreme storm event	video
37	Palacios et al.	Removal of microbial communities (Archaea and Bacteria) from wastewaters by photo-oxidation on TiO2 under controlled radiation	video
38	Papadopoulou et al.	Comparison of the in vitro activity of novel and established nitrification inhibitors used in agriculture on soil ammonia- and nitrite-oxidizers: working out more effective nitrification inhibition strategies	video
39	Papadopoulou et al. (Bachtsevani)	In soil assessment of the efficacy and off-target microbial toxicity of quinone imine and other established nitrification inhibitors used in agriculture	video
40	Papazlatani et al.	Isolation and characterization of a Mycosphaerella tassiana isolate able to rapidly degrade the recalcitrant fungicide Imazalil	video
41	Pesce et al.	Benthic communities as indicators of large lake surface sediment contamination: A case-study in Lake Geneva.	video
42	Petric et al.	Exploring climate change feedbacks of the extreme weather events on the interaction between rhizosphere microbiota and plants	pdf
43	Polst et al.	To shift or not to shift: Agricultural run-off induced regime shifts in phototrophic communities	video
44	Proia et al.	Natural freshwater biofilms: a potential nature-based solution for sewage waters treatment	video
45	Rimet et al. (Chonova)	Diat.barcode: a DNA tool to decipher diatom communities for the evaluation environmental pressures	video
46	Rolando et al. (Barra Caracciolo)	Bioaugmentation involving a bacterial consortium isolated from a foaming agent conditioned soil from tunneling	pdf
47	Thiour-Mauprivez et al.	Bacterial hppd: a biomarker of exposure of soils to beta-triketone herbicides?	video
48	van Bergen et al.	Biodegradation rates of pharmaceuticals in activated sludge are dependent on concentration	video

Keynote Speakers

Opening conference



Dr. Lukas Y Wick is head of the Bioavailability Group and deputy head of the Department of Environmental Microbiology at the **Helmholtz Centre for Environmental Research – UFZ in Leipzig** (Germany). His research interests relate to the bio-physical and ecological drivers of microbial transformation of organic contaminants for improved environmental (bio)-technology. He received his PhD in synthetic bio-organic chemistry from the University of Basel (Switzerland). After postdoctoral stays in environmental chemistry and microbiology at the Massachusetts Institute of Technology MIT (USA) and the Swiss Federal Institute of Aquatic Science and Technology EAWAG he joined the Soil Science Laboratory of the Swiss Federal Institute of Technology Lausanne EPFL. In 2004 he became head of the Bioavailability Group the Helmholtz Centre for Environmental Research – UFZ. He was appointed adjunct professor at the Faculty of Engineering of the University of Alberta, Canada (2012-2015) and is since 2014 speaker of UFZ' key research area 'Controlling Chemicals Fate'.

Session 1



Dr. Sara Hallin is professor and holds the chair in Soil Microbiology at the **Swedish University of Agricultural Sciences** since 2012. Her research area is microbial ecology of bacteria and archaea, especially those involved in nitrogen cycling. Her research spans several environments and systems, including different terrestrial ecosystem such as agricultural soils, arctic soils, and wetlands, aquatic environments and engineered systems. She has a MSc in agronomy and a PhD in microbiology, focused on denitrification in wastewater treatment systems. After her PhD, she did pioneering work to target denitrifying bacteria in the environment by developing molecular markers targeting genes encoding key enzymes in the denitrification pathway at the time when only taxonomic markers were used. In 2005 she was appointed Associate professor and became professor in microbial ecology in 2011. She is internationally known for her work on the ecology and genomics of denitrifying and nitrous oxide reducing microorganisms. Currently, she is continuing her research on nitrogen cycling ecology with the aim to understand relationships between the ecology of microbial communities, the biogeochemical processes they perform and the corresponding ecosystem functions.

Session 2



Researcher at the CEAB of the CSIC in Blanes, Girona (Spain) since August 2018, Dr. Helena Guasch has been teaching ecology subjects as Adjunct Professor of the Department of Environmental Sciences of the University of Girona (UdG) and doing her research at the Institute of Aquatic Ecology for more than 15 years. Her main expertise is fluvial ecology and ecotoxicology, focused on the ecological effects of chemicals at different levels of biological complexity (from biomarkers to ecosystem processes) and scales of study (in micro- mesocosms and field experiments and watershed-scale investigations). Her background is fluvial ecology (with a doctoral thesis on drivers of primary production). She expanded her scope to investigate the ecology of human impacted aquatic systems. In this context, she has been looking for biological indicators of different types of impact and of exposure to different pollutants: molecular biomarkers of stress, diatom indicators and ecosystem functions, among others. Her research is now considering the principles of sustainable development, as the best way to integrate research questions into a common framework that also takes into account the economic and social challenges. In the most basic aspects of the research, she is very interested in validating several "ecotoxicomic" approaches to be able to understand the reciprocal relationships between human alterations and the role played by microbial communities in the quality of water and the health of ecosystems, understanding the causes and effects of nutrient pollution, an old but prevalent and growing environmental problem and in the challenging study of the interlinks between biofilms and the "plastisphere".

Sessions 3



Professor Jonathan Lloyd currently leads a multidisciplinary geomicrobiology group working at the interface between biology and geology at the **Manchester University (United Kingdom)**.

His research focuses on the mechanisms of microbial metal reduction, with emphasis on the environmental impact and biotechnological applications of metal-reducing bacteria. Current activities are supported by funds from NERC, BBSRC, EPSRC, the EU and industry. He is also a Senior Visiting Fellow at the National Nuclear Laboratory, which helps support the development of his nuclear geomicrobiology programme..

Session 4



Professor Christian Griebler (University of Vienna, Austria) is a groundwater ecologist by heart. He studied zoology and limnology and received his PhD in 1998 from the University of Vienna. After a PostDoc stay (2000-2003) in Tuebingen, Germany, at the Center of Geoscience, he accepted a group leader position at the Helmholtz Center Munich, Germany. In 2014, he became acting head of the Institute of Groundwater Ecology in Munich. In 2019 he returned to Austria accepting a full professorship in limnology at the University of Vienna. C. Griebler's research foci are in groundwater microbial ecology, food web dynamics, carbon cycling, contaminant biodegradation and the assessment and monitoring of ecosystem health. He is advocate for the implementation of ecological measures to groundwater monitoring schemes in national and international water laws.

Session 5



Professor Isabelle Lavoie (INRS, Canada) develop research dealing with the monitoring of aquatic ecosystems health status using biological descriptors. She is particularly interested in responses of river biofilms to anthropogenic disturbances (eg, urban context, agricultural, mining). The Water biological integrity assessment tool DIEC (Diatom Index for Eastern Canada), developed with colleagues in 2005, is now a water quality indicator used by a variety of water management organizations (Quebec and Ontario Ministries of the Environment, Government of Canada, watershed organizations, private companies). Her research objectives are to develop bioindicators and biomarkers of stress, by studying the effects of anthropic disturbances on the nutritional quality of biofilms (eg fatty acids) and the consequences on the food chain.

Programme of keynotes (Time slots correspond to French Time Zone)

Date	Time slot	Session	Keynote
			speaker
Tuesday, October 6 th	2:50-3:30 pm	Opening Session	L. Wick
Wednesday, October 7 th	9:00-9:40 am	Micro-organisms in complex systems: biological	H. Guasch
		interactions, pollution & climate change (#2)	
Wednesday, October 7 th	3:40-4:20 pm	Micro-organisms for environmental risk	C. Griebler
		assessment (#4)	
Thursday, October 8 th	9:00-9:40 am	Impact of contaminant on microbial functions	S. Hallin
		(#1)	
Thursday, October 8 th	2:20-3:00 pm	Micro-organisms role in contaminants	J. Lloyd
		(eco)dynamics & application to bioremediation	
		(#3)	
Friday, October 9 th	2:00-2:30 pm	From research to end-users (#5)	I. Lavoie

Abstracts – Oral communications (1/4)

Session 1: Impact of contaminants on microbial functions

Thursday, October 8th

- 9:40-10:00 am The power of the dose-response framework to uncover molecular functional responses of micro-organisms to chemicals: Dromics <u>Floriane Larras</u>, Elise Billoir, Stefan Lips, Marie Laure Delignette-Muller, Mechthild Schmitt-Jansen
- 10:00-10:20 am Side effects of pesticides in groundwater: impact on bacterial denitrification <u>Caroline Michel</u>, Nicole Baran, Laurent André, Catherine Joulian
- 10:20-10:40 am Which potential of antibiotic resistance of environmental bacteria? The case of denitrifiers (Pseudomonas veronii) <u>Chen Chen</u>, Anniet Laverman, Céline Roose-Amsaleg
- 11:00-11:20 am BactoTrait : A functional trait database to evaluate how contaminants govern the assembly of bacterial communities <u>Aurélie Cebron</u>, Emna Zeghal, Philippe Usseglio-Polatera, Florian Lemmel, Albin Meyer, Corinne Leyval, Pascale Bauda, Florence Maunoury-Danger
- 11:20-11:40 am The impact of urban contamination on antibioresistance in microbial communities from periphyton and sediments <u>Chloe Bonnineau</u>, Agnès Bouchez, Anais Charton, Christophe Dagot, Marion Devers, Jérôme Labanowski, Emilie Lyautey, Fabrice Martin-Laurent, Leslie Mondamert, Stephane Pesce
- 11:40-12:00 am Wastewater effluent impacts the microbial diversity of periphyton in artificial streams and leads to increased community tolerance to micropollutants <u>Louis Carles</u>, Simon Wullschleger, Bettina Wagner, Adriano Joss, Kristin Schirmer, Nele Schuwirth, Christian Stamm, Rik Eggen, Ahmed Tlili
- 12:00-12:20 am Dynamics and main players of the N cycle in microbial mats are affected by chronic hydrocarbon contamination *Eléonore Attard, Johanne Aubé, Pavel Senin, Christophe Klopp, Patricia Bonin, <u>Marisol Goñi-Urriza</u>*
- 1:40-2:00 am Metabolic response of Microbacterium sp. C448 exposed to environmental and medicinal concentrations of sulfamethazine antibiotic <u>Laurianne Paris</u>, Muriel Joly, Marion Devers-Lamrani, Nadine Rouard, Didier Viala, Christophe Chambon, Michel Hébraud, Pascale Besse-Hoggan, Fabrice Martin-Laurent, Isabelle Batisson
- 2:00-2:20 am How to anchor metabolic responses of microbial communities to community functioning? <u>Stefan Lips</u>, Floriane Larras, Mechthild Schmitt-Jansen

The power of the dose-response framework to uncover molecular functional responses of micro-organisms to chemicals

<u>Floriane Larras</u> 1, Elise Billoir 2, Stefan Lips 1, Marie Laure Delignette-Muller 3, Mechthild Schmitt-Jansen 1

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Microbial ecotoxicologists are facing many challenges such as assessing the effect and risk of chemicals on ecological functions, or even linking their repercussion across biological functional levels. The recent rises of functional omics methods (e.g. transcriptomics, proteomics, metabolomics) have strongly supported the investigation of those challenges but most of the time, functional responses assessed at the molecular level consist in the comparison of two or three treatments and are reported as a fold change compared to the control. In comparison to a dose-response framework, traditionally applied in ecotoxicology, this approach is not suitable for trend response exploration, sensitivity values derivation and comparison or Adverse-Outcome-Pathway application and therefore of limited value for risk assessment. However, putting into practice a dose-response framework in multi-omics studies rises several questions like: i) how to deal with molecular functional data of different nature (e.g. microarray, RNA-seq, metabolome), ii) how to interpret the biological functional meaning of those outputs, iii) what is the added-value of omics functional data compared to the apical endpoints classically measured (e.g. growth, photosynthesis), and iv) how to link multi-omics levels? Because we have to handle those questions to tackle the previously stated challenges, we have built a turnkey tool called DRomics (https://lbbe.univ-lyon1.fr/-DRomics-.html) which: i) detects which molecular items (e.g. contigs, metabolites) significantly respond to a stressor along a contamination gradient, ii) defines the best model fitting their response, and iii) derives a benchmark dose for each item. To illustrate the workflow, DRomics was applied to a functional response exploration of the microalgae Scenedesmus vacuolatus to the biocide triclosan. The transcriptome, metabolome, growth and the photosynthesis responses were assessed after 14h of exposure to a gradient of triclosan concentrations. Numerous metabolic pathways have been identified as potentially impacted by the biocide but the highest sensitivity values of the metabolites involved in lipid metabolism pathway as well as their main decreasing response trend along the concentration gradient pointed out a possible direct interaction between this biocide and this microalga. The results illustrate the applicability of DRomics to assess microbial functional responses. Further examples will be outlined exploring the effect of any stressors (as long as a pressure gradient was studied) on any function-related endpoint (from the molecular to the apical level) and biological settings (single-species as well as communities).

Side effects of pesticides in groundwater: impact on bacterial denitrification

Caroline Michel 1, Nicole Baran 1, Laurent André 1, 2, Catherine Joulian 1

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The increasing pesticide use in agriculture involves the presence of both parent pesticides and their transformation products (metabolites) in various environmental matrices and notably groundwater. One drawback is that pesticides and metabolites (even referred to as emerging groundwater contaminants) are now known to have side effects on non-target surface and subsurface living organisms. The environmental consequence of side effects of pesticides and their metabolites on microbial ecosystems is that they can threaten the ecosystem services based on microbial activities in soil (litter degradation, plant growth, nutrient cycling, degradation of pollutants...) and groundwater (production of drinking water, nutrient cycling, degradation of contaminant...).

Most studies on side effects of pesticides and their metabolites have been conducted in soils, showing main impacts on microbial abundance, presence or absence of microbial species, increase or decrease in gene expression (mainly linked to the N cycle), and increase or decrease of the functional diversity (activities linked to P, N, S, C cycles). Moreover, pesticides presence was shown to usually lead to the selection of microorganisms having the ability to degrade them. Pesticides and their metabolites impacts on soil microbiology are rather well documented, but knowledge on their side effects on groundwater microbial ecosystems is scarce. The few studies conducted in groundwater have underlined potential side effects on groundwater communities as pesticides presence could increase microbial biodiversity. The role of pesticides contamination history on pesticides impact on groundwater communities has also been underlined.

In this context, the impact of two pesticides, S-metolachlor and Propiconazole, and their metabolites, ESA-metolachlor and 1,2,4-triazole, was studied on microbial denitrification, a key function for nitrate removal in groundwater. Laboratory experiments were performed with or without pesticides or metabolites at 2 and 10 µg/L. Kinetics of nitrate reduction along with nitrite and N2O production all suggest that S-metolachlor has no or only little impact, whereas its metabolite ESA-metolachlor inhibits denitrification by 65 % at 10 µg/L. Propiconazole and 1,2,4-triazole also inhibit denitrification (by 29-38 %) at both concentrations, but to a lesser extent than ESA-metolachlor. When inhibition occurs, substances affect the reduction of nitrate into nitrite. At the end of batch experiments, no significant change in narG and napA genes abundance was detected, suggesting an impact of pesticides at the protein level rather than on bacteria abundance. Community diversity fingerprints and Illumina sequencing indicate no major impact of pesticides on bacterial diversity except for ESA-metolachlor at 10 µg/L that induces an increase of biodiversity indices. General growth parameters such as bacterial biomass and acetate consumption suggest no impact of pesticides, except for propiconazole at 10 µg/L that partially inhibits microbial metabolism (as acetate uptake). In conclusion, pesticides and their metabolites can have side effects on microbial denitrification in groundwater at realistic environmental concentrations, and may thus affect ecosystem services based on microbial activities.

Which potential of antibiotic resistance of environmental bacteria? The case of denitrifiers

Chen Chen 1, Anniet Laverman 1, Céline Roose-Amsaleg 1

1 : Ecosystèmes, biodiversité, évolution

Universite de Rennes 1, INEE, Observatoire des Sciences de l'Univers de Rennes, Centre National de la Recherche Scientifique : UMR6553

Microbial facilitated denitrification is a major process mainly including reduction of nitrate and generation of molecular nitrogen through a series of intermediate gaseous nitrogen oxide products. Denitrifiers are essential to biogeochemical nitrogen cycling in a wide range of environments. Denitrifiers in the environment are exposed to pollutants such as antibiotics (ATBs), for example aquatic environments receiving waste water from hospital, farms and industry. The overuse of antibiotics in our society and the great adaptability and horizontal gene transfer of microorganisms has resulted in the appearance of antibiotic resistant bacteria both in medical as well as in the natural environment.

The goal of this study was to investigate ATB resistance in non-pathogenic, denitrifying bacteria. We aimed to study ATB resistance level, on both apparent and genomic level, of a denitrifying species. Through this study, we want to assess the status of ATB contamination and antibiotic resistance gene (ARG) distribution for natural denitrifiers in sediments. We studied an environmental denitrifying bacterium, *Pseudomonas veronii 11V444*, isolated from river sediment of the Charmoise a tributary of the Seine River (France). The genome sequence of the strain 11V444 was determined using PacBio RS II sequencing.

The ATB resistance level on *Pseudomonas veronii* was evaluated against 11 ATBs, which were selected based on the amount of usage and the types, via an ATB-bacterial co-cultivation method allowing to determine dose-effect curves. The tested ATBs were macrolides (erythromycin and tylosin), sulfonamides (sulfamethoxazole), aminocoumarin (novobiocin), β -lactam antibiotics (amoxicillin and ampicillin), tetracycline (tetracycline), quinolones (ciprofloxacin, ofloxacin and flumequine) and the small antibiotic molecule fosfomycin. In parallel we checked antibiotic resistance genes via annotation through MaGe from the MicroScope platform.

Our experiments showed that *Pseudomonas veronii* 11V444 was resistant towards macrolides, sulfonamides, and aminocoumarin, but sensitive towards tetracycline and quinolones. For other types of antibiotics (β -lactam antibiotics and small molecule fosfomycin), we observed a certain inhibition of the growth curve within the first 6 hours, but then slowly recovered. The sensitive strains exhibited EC50 of 1.9 mg/L for tetracycline, 0.25 mg/L for ciprofloxacin, 1.25 mg/L for ofloxacin and 6.1 mg/L for flumequine.

So far, 5 efflux pump genes were found in the genome of *Pseudomonas veronii*. Since these multidrug efflux pump genes are commonly distributed among *Pseudomonas*, activation in *Pseudomonas veronii* 11V444 needs to be confirmed. Further analysis will be done to compare ATB resistance with genomic results. By combining experimental data analysis with genomic prediction using a bioinformatics platform, we are improving our understanding on ATB resistance of the denitrifier *Pseudomonas veronii*.

BactoTrait – A functional trait database to evaluate how contaminants govern the assembly of bacterial communities

<u>Aurélie Cebron</u> 1, Emna Zeghal 1, Philippe Usseglio-Polatera 2, Florian Lemmel 1, Albin Meyer 2, Corinne Leyval 1, Pascale Bauda 2, Florence Maunoury-Danger 2

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In the environment, and particularly in soils, the microbial diversity observed locally is conditioned by a certain number of ecological filters: abiotic (climatic conditions, pedology, physico-chemical parameters), biotic (interactions between microorganisms, vegetation and fauna), but also anthropogenic (disturbances, stress, pollution). A key question in microbial ecotoxicology is to understand the impact of anthropogenic filters on microbial diversity and ecosystem functioning. Studies based on the taxonomic composition and on the measurement of certain microbial functions do not always make it possible to highlight the impact of the presence of contaminants on the microbial community structure. This observation has led us to develop a new approach based on microbial functional traits. This approach allows a simplified representation, by proxys of functions and not by species, and leads to a generalizable approach over a larger range of ecosystems having different bacterial diversity. Functional traits of bacteria include all the characteristics (morphological, physiological, genomic, etc.) describing a given strain and having a range of values individually measured.

We have created the first database of bacterial functional traits, thanks to the properties of more than 19,493 bacterial strains. Among the 19 traits listed in this database, there are for example: oxygen preference, size and shape of bacteria, motility, optimum of pH and temperature, genome GC percent, trophic type. After a "fuzzy coding" step, this trait database was used to transform taxonomic diversity information obtained by high-throughput sequencing of 16S rDNA from 30 soil samples of 10 sites with different levels of metal and polycyclic aromatic hydrocarbon (PAH) contamination. This "traits" approach makes it possible to better discriminate soils according to their pollution levels and type. Certain bacterial traits are even good indicators of specific contaminants (metals and polycyclic aromatic hydrocarbins, PAH).

The impact of urban contamination on antibioresistance in microbial communities from periphyton and sediments

<u>Chloe Bonnineau</u> 1, Agnès Bouchez 2, Anais Charton 1, Christophe Dagot 3, Marion Devers 4, Jérôme Labanowski 5, Emilie Lyautey 2, Fabrice Martin-Laurent 4, Leslie Mondamert 5, Stephane Pesce 1

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Since the early 20's antibiotics have been massively produced and consumed for the benefit of both human and animal health. Nevertheless, antibiotics have also reached the aquatic environment through diffuse sources (*e.g.* veterinary treatment, contaminated manure application...) and through wastewater. Consequently, antibiotics concentrations between the ng/L and μ g/L range are regularly detected in surface water and those molecules have also been found in sediments and aquatic biota. The ubiquitous presence of antibiotics exerts a selective pressure on microbial communities leading to the acquisition and dissemination of antibioresistance in the environment. While both antibiotics and antibioresistance have been found in different aquatic compartments, more investigation is required to better understand their distribution and to identify hot spots of accumulation.

In this context, we investigated the repartition of antibiotics and antibioresistance in different aquatic compartments on 4 stations belonging to regional observatories and presenting contrasting levels of pharmaceuticals: 2 on the Arve river belonging to Sipibel observatory and 2 on Lake Geneva belonging to the Observatory on Lakes. On the Arve river, the 2 stations were located up- and down- stream the discharge place of a wastewater treatment plant (WWTP) collecting both urban and hospital wastewaters. On Lake Geneva, one station was located in a relatively pristine area while the second was close to the discharge of an urban WWTP. To better identify a potential temporal dynamic of antibiotics and antibioresistances over seasons, 6 samplings were conducted during 1.5 year. On each sampling campaign, the following parameters were determined: (i) antibiotics levels in water, sediments and periphyton; (ii) antibioresistance in periphyton and sediments using various techniques: detection of resistance genes, integrons quantification, detection of tolerance acquisition *via* a PICT (Pollution Induced Community Tolerance) approach; (iii) antibiotics biodegradation potential of microbial community from sediments (by radiorespirometric measurement); (iv) diversity of bacteria and diatoms in periphyton and sediments; (v) physico-chemical parameters and (vi) metallic contamination in sediments.

While microbial resistance to antibiotics is commonly assessed by quantifying resistance genes or isolating antibiotic resistant bacteria, antibiotic resistance can also be estimated by measuring the acquisition of antibiotics tolerance at community level, following a PICT approach. In our study, periphytic microbial communities from the Arve river were found to have a higher tolerance to the tested antibiotics (ciprofloxacin, ofloxacin, sulfamethazine and erythromycin) than communities from Lake Geneva, in agreement with the expected levels of contamination. In addition, in some cases, a higher tolerance was also found at stations close to WWTP effluents than in upstream/protected stations. For example, periphytic microbial communities collected downstream the WWTP on the Arve river were generally found to have a higher tolerance to ciprofloxacin than the upstream communities. Comparing whole community tolerance to other classical indicators of antibioresistance and to antibiotics levels in the aquatic ecosystems allows us to better understand the interconnection between pharmaceutical exposure, *in situ* tolerance and genetic potential for antibioresistance.

Wastewater effluent impacts the microbial diversity of periphyton in artificial streams and leads to increased community tolerance to micropollutants

Louis Carles 1, Simon Wullschleger 1, Bettina Wagner 1, Adriano Joss 1, Kristin Schirmer 1, Nele Schuwirth 1, Christian Stamm 1, Rik Eggen 1, Ahmed Tlili 1

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Wastewater treatment plant (WWTP) effluents, which contain complex mixtures of micropollutants (MPs), are of major concern regarding their impacts on the quality of receiving water bodies. Periphyton is a complex community of microorganisms composed of bacteria, algae, fungi and protozoa, embedded in an extracellular matrix. Periphyton plays a crucial role in river ecosystem functioning and has been shown to acquire pollution-induced community tolerance (PICT) upon exposure to wastewater in streams. The aim of this study is to unravel the processes underlying PICT by establishing PICT in artificial streams and examining the changes in microbial diversity of periphyton exposed to different dilutions of WWTP effluent.

Periphyton was grown in flow-through channels continuously alimented with stream water and different percentages of urban WWTP effluent (i.e., 0%, 10%, 30% and 80%). After 4 weeks, periphyton was sampled and exposed to increasing dilutions of extract from passive samplers that were immersed in the WW effluent. Tolerance of periphyton communities to the passive sampler extract was then determined according to the PICT concept, focussing especially on the inhibition of algal primary production and photosynthetic efficiency. Periphyton was also characterized functionally and structurally by a set of biological endpoints, including 16S and 18S rRNA gene sequencing. Moreover, 51 MPs, including artificial sweeteners, pesticides and pharmaceuticals, were analysed in composite and grab water samples, in biofilms from all treatments, as well as the passive sampler extracts.

Overall, 37 among the 51 substances analysed could be quantified in grab water samples and their concentrations increased with increasing WW percentage, confirming WWTP effluent to be the primary MP source. All the 24 pharmaceuticals analysed were quantifiable ($\leq 4.5 \ \mu g \ L-1$) while this was true for only 8 of the 20 analysed pesticides ($\leq 1.5 \ \mu g \ L-1$). Artificial sweeteners (acesulfam, cyclamate and sucralose) and corrosion inhibitors (benzotriazole and 5-methylbenzotriazole) were found at relatively high concentrations ($\leq 41.4 \ and 2.7 \ \mu g \ L-1$, respectively). These results are in accordance with the type of effluent treated in the WWTP, which comes mainly from an urban area.

Microbial communities exposed to 30 and 80% WW were more tolerant to the MP extracts than the control communities. This might be due to physiological adaptations and/or the selection of tolerant species to MPs within the community. Indeed, analyses of the microbial diversity showed a change in bacterial and algal community composition in response to wastewater. Some phyla were more abundant in the presence of WW (e.g. Chloroflexi and Cyanobacteria) while the abundance of other phyla, such as Gemmatimonadetes and Planctomycetes, decreased with increasing WW. In addition, we observed a change in abundance of Diatom genera in response to WW.

Overall, we confirmed the link between WWTP effluent and induced-tolerance in artificial streams and describe the effects on microbial communities. The comprehensive integration of functional and structural responses of periphyton communities exposed to effluents, along with a detailed chemical characterization, is crucial to link exposure to community-level effects in multi-contaminated ecosystems.

Dynamics and main players of the N cycle in microbial mats are affected by chronic hydrocarbon contamination

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Microbial mats are peculiar ecosystems found in a huge diversity of extreme environments. Whereas diazotrophy was the object of numerous studies, other steps of the N cycling are very few documented. Using metagenomics and metatranscriptomics, we assessed concomitantly the taxonomic composition and the metabolic potential related to all the pathways of the N cycle in 2 microbial mats differing by their level of hydrocarbons contamination. We analyzed 3 samples per mat during 3 sampling events throughout one year. Metagenomics data showed that marker genes linked to N mineralization and assimilation were quantitatively the most dominant but without any differences between sites or season. Differential metatranscriptomics analysis revealed a high potential of N2 fixation and temporal patterns strongly different between both sites. In the reference site, N2 fixation transcripts peaked in spring whereas in the contaminated site they decreased throughout the study period. Taxonomic affiliations revealed that Proteobacteria and not Cyanobacteria were the main players involved in the potential N2 fixation. In spring, transcripts affiliated to Sulfate-Reducing Bacteria (SRB) increased more intensely in the reference than in the contaminated site. Given that SRB are heterotrophs, we hypothesized this absence of seasonal pattern in the contaminated site is due to the organic matter of lesser quality. This work highlights that considering the overall N cycle, whatever the level of hydrocarbon contamination microbial, mats have the same genetic potential. However, this apparent robustness should be softened by the metatranscriptomics date, showing differences in functioning especially on N2 fixation, which can jeopardize persistence over years in the contaminated site

Metabolic response of Microbacterium sp. C448 exposed to environmental and medicinal concentrations of sulfamethazine antibiotic

Laurianne Paris 1, 2, Muriel Joly 1, 2, Marion Devers-Lamrani 3, Nadine Rouard 3, Didier Viala 4, Christophe Chambon 4, Michel Hébraud 4, Pascale Besse-Hoggan 2, Fabrice Martin-Laurent 3, Isabelle Batisson 1

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3 : Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement Agrosup Dijon, Agroécologie

4 : Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement Plate-forme d'exploration du métabolisme, INRAe Clermont-Ferrand-Theix

Pharmaceuticals, including antibiotics, have a wide application range in human and veterinary medicines. Due to their widespread use in livestock production and their recalcitrance to biodegradation after ingestion, the veterinary antibiotics are frequently released into the environment. In France, 20% of veterinary antibiotics belong to the sulfonamide family. Their presence in the environment represents a potential risk for ecosystems because of the possible selection and dissemination of sulfonamide-resistance genes and -resistant-bacteria.

The aim of our study was to elucidate the metabolic processes involved in sulfonamide biodegradation by using the sulfonamide-mineralizing bacterium *Microbacterium* sp. C448 and the sulfamethazine (SMZ) antibiotic as models. Our starting hypothesis is the following one: at low SMZ concentrations, C448 could mineralize it without activating its resistance pathway, while the bacterium would need to resist to be able to degrade it at high concentrations.

Preliminary experiments allowed us to select the culture medium, the concentrations of SMZ (low 10 mg/L; high 250 mg/L) and the sampling times to be used to test this hypothesis by studying the response of C448 by proteomic approach. Three protocols of protein extraction were compared in order to optimise the quantity of proteins extracted and the characterisation of surfaceome and intracellular proteins of *Microbacterium* C448.

Results, obtained using the best extraction method, will be presented with a specific focus on the expression of C448 biodegradation and resistance genes in response to low and high SMZ exposure. These data will be completed by a transcriptomic analysis.

How to anchor metabolic responses of microbial communities to community functioning?

Stefan Lips 1, Floriane Larras 1, Mechthild Schmitt-Jansen 2

1 : Helmholtz Centre for Environmental Research - UFZ Department Bioanalytical Ecotoxicology, Permoserstr. 15, DE-04318 Leipzig, Germany - Germany

Benthic microbial communities (=biofilms) are composed of diverse consortia of microorganisms such as algae, bacteria, fungi and protozoa which are embedded in an adherent matrix. Biofilms play a vital role in the matter and energy cycling of small streams. Hence, understanding stress effects on the physiological state of biofilms is essential to safeguard ecosystem functioning. Molecular tools allow structural and functional insights into biofilm communities and among those techniques metabolomics provides a snap-shot of the small molecule pools. These pools are modified under stress conditions due to physiological adaptations. While changes in the metabolism of communities may be closely related to their functions like primary production or respiration the challenge remains how to anchor molecular responses to phenotypic functional observations.

Towards this aim we grew environmental biofilms for 4 weeks in control microcosms and under exposure of 4 µg L-1 diuron. Afterwards we exposed them to the herbicide diuron for one hour in a concentration-dependent design. To be able to relate metabolic responses to functional changes in biofilms and to assess responses in community tolerance after exposure the inhibitory effect of diuron to the photosystem was measured by pulse amplitude modulation fluorometry. Further, dissolved oxygen concentrations were monitored for 12 hours. Metabolic responses were analysed by shock-freezing the biofilm and measuring polar extracts on a GC-TOF/MS. Differences in the pool sizes of metabolites were explored by multivariate statistics and the responsive metabolites were annotated and related to their corresponding pathways via the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database. The applied workflow enabled sensitivity analysis of the pathways, ranking in a pathway sensitivity distribution (PSD) and therewith integration of metabolic responses and functional observations.

Session 2: Microorganisms in complex systems: biological interactions, pollution & climate change

Wednesday, October 7th

- 9:40-10:00 am Bacterial transfer from plastic to deep-coral microbiomes: harmful effect and holobiont concept <u>Anne-Leïla Meistertzheim</u>, Leila Chapron, Erwan Peru, Pierre Galand, Franck Lartaud, Jean-François Ghiglione
- 10:00-10:20 am A disturbed environment impacts the gut microbiota of the Mediterranean pond turtle Mauremys leprosa (Schweigger, 1812) <u>Carmen Palacios</u>, Lucie Grecias, Raphael Forne, Mégane Noyer, Olivier Verneau
- 10:20-10:40 am Fish gut microbiome and metabolome responses after an exposure to toxic cyanobacterial blooms <u>Alison Gallet</u>, Benjamin Marie, Hélène Huet, Charlotte Duval, Sébastien Duperron
- 11:00-11:20 am Resistance and resilience of soil N cycle-related bacterial processes to drought and heat stress in rehabilitated urban soils <u>Mehdi Fikri</u>, Catherine Joulian, Mikael Motelica-Heino, Marie-Paule Norini, Jennifer Hellal
- 11:20-11:40 am Toxic bloom-forming Planktothrix (cyanobacteria) stressed by high light and high temperature

<u>Sandra Kim Tiam</u>, Katia Comte, Caroline Dalle, Marine Delagrange, Chakib Djediat, Bertrand Ducos, Charlotte Duval, Kathleen Feilke, Sahima Hamlaoui, Séverine Le Manach, Pierre Setif, Claude Yéprémian, Benjamin Marie, Diana Kirilovsky, Muriel Gugger, Cécile Bernard

- 11:40-12:00 am Microbial production of geosmin within freshwater biofilms: driving factors in a global change scenario *Carmen Espinosa, Meritxell Abril, Helena Guasch, Laia Llenas, Marc Ordeix, Lidia Vendrell,* <u>Lorenzo Proia</u>
- 12:00-12:20 am Particle-attached riverine bacteriome shifts in a pollutant-resistant and pathogenic community during a Mediterranean extreme storm event <u>Mégane Noyer</u>, Brice Reoyo-Prats, Dominique Aubert, Maria Bernard, Olivier Verneau, Carmen Palacios
- 12:20-12:40 am Metal resistance genes rich biofilm communities selected by biocidecontaining surfaces in temperate and tropical coastal environments <u>Jean-François Briand</u>, Elisa Catao, Nicolas Galois, Thomas Pollet, Raphaëlle Barry-Martinet, Fabienne Fay, Dalyal Copin, Alina Tunin-Ley, Jean Turquet, Christine Bressy, Benjamin Misson
- 2:00-2:20 pm Does pesticide pressure affect the grazing behaviour of chironomids on microalgae? Julie Neury-Ormanni, Caroline Doose, Nabil Majdi, Jacky Vedrenne, Walter Traunspurger, <u>Soizic</u> <u>Morin</u>
- 2:20-2:40 pm Ecotoxicity of graphene-based materials toward a diatom-bacteria biofilm <u>Lauris Evariste</u>, Paul Braylé, Jérôme Silvestre, Emmanuel Flahaut, Laury Gauthier, Eric Pinelli, Maïalen Barret
- 2:40-3:00 pm Is plastic a real threat to freshwater detrital ecosystem? A trophic approach. <u>Daniela Batista</u>, Arunava Pradhan, Claudia Pascoal, Fernanda Cassio
- 3:00-3:20 pm Effects of thorium on periphytic biofilm community, taxonomic structures, nutritional quality, and its trophic transfer to the snail Lymnaea sp. <u>Caroline Doose</u>, Soizic Morin, Fadhlaoui Mariem, Laura Malbezin, Jacky Vedrenne, Claude Fortin

Bacterial transfer from plastic to deep-coral microbiomes: harmful effect and holobiont concept

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The harmful effects of plastic released in the environment have been demonstrated at almost all trophic levels in the Oceans, from plankton to whales. However, the microbial transfer from plastic to the animal host microbiomes have been poorly investigated. In the last decade, the 'holobiont concept' provoked a paradigm in Biology where individual phenotypes are now seen as a result of the combined expression of the host and associated microbe genomes. At the same time, a growing literature described the abundant and highly diverse microorganisms living on plastic, the so-called "plastisphere". Here, we designed a long-term experiment on the effect of plastic and its attached microorganisms on 2 deep coral species (*Lophelia pertusa* and *Madrepora occulata*), recognized as sentinel species for deep marine ecosystems.

First, we revealed strong physiological alterations with various impact on coral growth, feeding and behavior depending on the size of the plastics, i.e. micro- (500mm PE beads) vs. macro- (10cmx10cm PE film) plastic (see Chapron et al. 2018, *Sci Reports 8:15299*). Second, we proved for the first time a profound modification of the microbiome specific to these 2 coral species, as directly linked to the transfer of bacteria colonizing the microplastics (500mm PE beads). Specific OTUs from the plastisphere were identified as influencing the coral microbes, with distinct effect depending on the coral species. Such transfers are in accordance of the idea of plastics that may serve as rafts for pathogen species at large global scales. It is noteworthy that deep corals serve as shelters for a rich biodiversity of animals, including heritage and commercial species. This study provides a multidisciplinary approach to give a new proof of the effect of microplastic on marine organisms and open new warning questions on the importance to consider the holobiont concept in further studies aiming to evaluate the impact of plastic in marine ecosystems.

A disturbed environment impacts the gut microbiota of the Mediterranean pond turtle Mauremys leprosa (Schweigger, 1812)

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Context

The host-microbiota association, called holobiont, seems to be essential in the organism's response to environmental changes. Located at the interface between the host and its environment, the microbiota influences many processes within its host. The gut microbiota is especially important for host fitness as it is involved in host digestion, nutrient synthesis and protection against pathogens. Nevertheless, and even in the current context of global changes, environmental studies on the response of host microbial communities to disturbed ecosystems by pollutant mixtures are scarce in the literature, especially in wild animals. In freshwater ecosystems, where biodiversity is being lost due to anthropogenic water pollution, monitoring the impact of these particular stressors on animal species is essential. Indeed, pollutants bio-accumulate and, once in the organism, they have a negative impact on animal's physiology. We hypothesize that risk assessment should consider monitoring the consequences of xenobiotics use on the holobiont as a whole. The Mediterranean basin, which is recognized as one of the top biodiversity hotspots, houses a great number of essential freshwater habitats and endemic species among which the Mediterranean pond turtle, Mauremys leprosa, which is classified as "Vulnerable" by the IUCN. The two main economic activities in the Mediterranean basin are agriculture and tourism, making water a highly demanded, though scarce resource. These activities lead to a high level of contamination in watercourses, which reach surface waters for the most part from wastewater treatment plants (WWTPs).

Objective

The present study aimed to reveal new insights on the impact of anthropogenic pressures on the freshwater turtle species *M. leprosa* through a comparative 16S rRNA metabarcoding study of the cloacal microbiota of turtles captured upstream and downstream of a WWTP. The cloacal microbiota has already been used as a non-invasive proxy to the gut microbiome.

Results and Discussion

From all variables tested for inference on their importance to drive bacterial community diversity and composition using constrained multivariate analysis, only sex and location (upstream or downstream of the WWTP) were significant. WWTP effluents had a negative impact on the bacteriome diversity of males, notably by lowering their equitability. The composition of communities was also affected, both in their abundance and in the identity of the taxa observed. In particular, the structural diversity of the bacteriome of female turtles was more impacted by the WWTP than that of males, with a downstream microbiota highly populated by fecal bacteria of the Enterobacteriaceae family with respect to females upstream more populated by Actinobacteria and Chloroflexi phyla. The Deinococcaceae family, which is known to be tolerant to multiple stressors was part of the core microbiome of *M. leprosa*. It became particularly abundant in downstream individuals and even more so in males. Results presented in this study, together with the relative sensitivity of this pond turtle to oxidative stress induced by pollution, as well as its ubiquitous presence in the Mediterranean basin, make this species a new holobiont model to assess water quality in this region.

Fish gut microbiome and metabolome responses after an exposure to toxic cyanobacterial blooms

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Cyanobacterial blooms are freshwater natural events enhanced by climate change and eutrophication, thus starting to be increasingly frequent, abundant and persistent worldwide. They appear to widely disturb aquatic ecosystems and to pose real public health risks by causing anoxic waters or producing potentially toxic bioactive metabolites then released in the environment. Cyanotoxins are among the most studied metabolites due to their toxic effects on animals including humans. So far, althought effects of metabolites on fish physiology have been well investigated, studies of effects on fish microbiota are still lacking despite potential antimicrobial activities of some of those compounds. Here, we assess microbiome and metabolome responses in the gut of a model fish, to an exposure of the most encountered toxin-producing cyanobacteria *Microcystis aeruginosa*. Medaka fishes (*Oryzias latipes*) have been exposed during 28 days in microbiome (16S rDNA) and metabolome have been analysed from gut samples to study, respectively, the composition of associated bacterial gut communities and the bioactive metabolite diversity. Preliminary results for the microbiome revealed a shift in the composition of bacterial communities for fishes exposed to the highest concentration of *M. aeruginosa*, suggesting the existence of a tipping point.

Resistance and resilience of soil N cycle-related bacterial processes to drought and heat stress in rehabilitated urban soils.

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In the context of global change, drought and heat periods may alter biogeochemical cycles of major elements in terrestrial ecosystems, including the N cycle. The aim of this study was to evaluate the resistance and resilience of restored urban soils' functions related to N cycling. A laboratory experiment was conducted in microcosms with four soil-types collected from a rehabilitated brownfield; an initial soil (IS), a Technosol with a high organic matter level (HO) and two Technosols with less organic matter (LO1 and LO2). Changes in potential denitrification (PDR), nitrification and Nmineralization rates and their interactive relationships with soil microbial activity and diversity, together with soil physicochemical properties were examined for 3 months following a heat (40°C) and drought stress period of 21 days. Immediately after the stress, the PDR decreased significantly for all soils compared to their respective controls (no stress), which demonstrates an impact of the stress on the resistance of denitrifying microorganisms for all soils. HO was the most resistant to stress as showed by significantly higher PDR than for LO1 and LO2. Five days after stopping the stress, all soils had recovered their PDR activity. Similar results were obtained after 30 days, indicating a quick recovery of PDRs and therefore a good resilience of the PDR on short and medium term. However, 92 days after stress, LO1 and LO2 PDR were still significantly lower than their controls, which indicates an incomplete resilience on the long term for these two soils. Additionally, the evolution of several indicators of soil microbial activity, *i.e.* basal respiration (BR), enzymatic activities and carbon metabolism profiles, were monitored along the experiment. LO1 and LO2 treatments' basal respiration was impacted by the stress, as their BRs were significantly lower than their controls; HO's BR was found to be the highest and no difference was found between IS and its control at the end of the stress. At the end of the experiment (92 days) LO1 and LO2 recovered and their BRs reached the same order of activity as the controls. Last, abundance of functional genes of the N cycle (amoA, narG, nirK, nirS and nosZ) were assessed using qPCR. Just after the stress, no significant differences were found between treatments and controls, suggesting no effect of stress on genes abundances. HO had the highest narG and amoA genes abundances compared to other treatments, as well as the highest basal respiration, suggesting a higher microbial activity and a more pronounced denitrification capacity. The correlations between functional genes and nitrification, denitrification processes will be investigated, to highlight relationships between functional diversity and soil functions. Measuring the resistance and resilience of soil functions can help design choose the best ecological restoration trajectory.

Toxic bloom-forming Planktothrix (cyanobacteria) stressed by high light and high temperature

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Cyanobacteria (photosynthetic prokaryotes) play key roles in aquatic ecosystems as there are at the base of the food chain and contribute significantly to primary production. However, over the past three decades, the increasing anthropic pressures, exerted on natural aquatic environment have caused massive cyanobacterial developments, including those containing toxin-producers (i.e. cyanobacteria Harmful Algal Blooms; cHAB). The increasing occurrence of these blooms is a source of concern since blooms can modify the dynamic of ecosystems and have deleterious consequences for the production of drinking water, or recreational aquatic activities as well.

In this study we investigated the short-term response of four *Planktothrix* genotypes to high light or high temperature, and how ecotype and MCs production capability affect the ability of cyanobacteria to cope with such stresses. We employed targeted (high throughput qPCR, *in vivo* fluorescence measurements and transmission electron microscopy) and global (metabolomic) approaches to understand the mechanisms enabling these cyanobacteria to cope with short-term stresses. We focused on cellular functions related to photosynthesis, photoprotection, heat shock response and metabolites synthesis (including cyanotoxins).

Our results show that high light modifies the expression of genes involved in the regulation of photosynthesis and photoprotection, strongly impacts photosynthetic capabilities and modifies thylakoid ultrastructure. High temperature has strong effects on the expression of genes related to general stress responses and induced important ultrastructural modifications including those on thylakoids. Besides the contrasted responses of the cyanobacteria to either light or temperature, our results highlighted some strain specificities regarding photosynthetic activities and metabolomic profiles. This contributes to explain the success of *Planktothrix* blooms i/in lakes with different environmental characteristics (Alpine lakes, big lakes, urbans lakes) and ii/ over a large period of time where environmental conditions change. According to our results, short-term stresses will not increase MCs content or favour MC producers contrary to more gradually changes related to global warming.

In addition, our study highlighted the need of considering genetic diversity in order to understand blooms dynamics. Future research should not only include genetic diversity within a genus but also should be more integrative by considering the entire microbial community and the effects of environmental factors on its structure and functions. Moreover, we believe that the combination of integrative studies and global approaches such as OMICS will permit to better understand the effects of abiotic stresses on bloom dynamic regulation in an anthropized world.

Microbial production of geosmin within freshwater biofilms: driving factors in a global change scenario

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Geosmin is a semi-volatile and microbially produced molecule, responsible for earthy and musty odor in surface waters, fact that is causing important issues to drinking waters facilities because a bad taste is detected by humans at very low concentrations (5-10 ng/L). Cyanobacteria are, among others (i.e. fungi, amoeba, filamentous bacteria), the most important geosmin-producing microorganisms and its lifestyle include both planktonic and benthic species growing within biofilm communities. Several geosmin-producing cyanobacteria have been identified, being Oscillatoria sp. and Anabaena sp. the most commonly found in freshwater ecosystems. It has been described that different environmental factors related to global change (i.e. temperature, eutrophication, droughts, nutrients imbalance) may influence cyanobacterial growth within biofilms communities and eventually geosmin production. Nevertheless, most of the studies were carried out with single specie cultures under controlled conditions and there are very few studies dealing with geosmin occurrence drivers under natural environmental conditions in complex microbial communities. It is therefore essential to understand how the variation of multiple environmental variables can affect biofilm community structure-function and subsequently geosmin production and release. This talk will present the results of studies carried out at different spatial and temporal scale to figure out the role of several potential environmental factors related with global change as geosmin production drivers within biofilm communities. A microcosms experiment was focused on the independent and combined effect of nutrient concentrations and nitrogen to phosphorus ratio (N:P) on geosmin production and release to the water, whereas a mesocosm approach in experimental flumes receiving water continuously from the river, was used to understand the role of water flow and light availability in the intracellular geosmin production of biofilm. The main hypotheses and conclusions reached with controlled experiments have been contrasted against the results obtained through the monitoring of different variables carried out during 3 years at 5 sampling sites along the Ter River catchment (NE Spain). Overall, these studies highlighted that the dominant geosmin producing microorganism in the Ter River biofilms is the cyanobacteria Oscillatoria sp. The microcosm experiment confirmed that geosmin production and release is favored by decreased N:P, especially under higher nutrient concentrations. This result was confirmed by the field monitoring in which the concentration of geosmin in water proportionally increased while the N:P was gradually decreasing among sampling dates, starting from late winter. The mesocosm experiments evidenced that lower water flows are optimal to favor the growth of Oscillatoria sp. in river biofilms triggering the intracellular production of geosmin, especially under lower light conditions. The long-term field monitoring results agreed with these conclusions and highlighted that geosmin episodes in the Ter River are influenced by the hydrological regime being the year with the lowest water flow when the highest geosmin concentrations were detected (249 \pm 33 ng/L). Moreover, field observations confirmed greater abundances of Oscillatoria sp. within biofilms in the zones close to the riverbank where light availability is usually reduced by riparian vegetation. Overall, these studies highlighted the set of co-occurring events needed to trigger geosmin episodes in fluvial ecosystems.

Particle-attached riverine bacteriome shifts in a pollutant-resistant and pathogenic community during a Mediterranean extreme storm event

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State of the art and scope

Rivers are representative of the overall pollution of their catchment area, while contaminants presence and concentration in watercourses depend on different factors such as land use and rainfall intensity. Rainstorms and urban sewer systems have been described as major contributors to mixtures of xenobiotics in watersheds, which have been coined multipollution events. In Mediterranean climate regions, saturation of the urban sewer system leads to Combined Sewer Overflows (CSO), which occur very often because intense rainfalls are the common rule under this climate. Rivers in these regions are therefore recurrently impacted by pollutant mixtures from river sediments and in-sewer solids resuspension due to CSOs and floods. Currently, the impacts of the release of multiple pollutants linked to extreme weather events on river ecosystems have not yet been adequately addressed.

In this context, microbial communities provide critical ecosystem services to the maintenance of water quality as they are the only biological compartment capable of degrading or transforming pollutant substances. Few studies, however, have examined the *in situ* effect of pollutants mixtures on the riverine microbiome or assess shifts in microbial community composition along rainstorms, highlighting the need for research in these fields. Because suspended particles act as both sources and sinks for nutrients and contaminants, playing a fundamental role in the transfer of chemical substances during storm events, we concentrated on the particle-attached microbial compartment to investigate the evolution of the environmental microbiome under these circumstances.

<u>Aims</u>

Our first objective was to determine the impact of multipollution phenomena on the particle-attached fluviatile bacteriome evolution along a typical Mediterranean heavy rainfall and flood. Secondly, through the study of specific ecotypes significantly related to multipollution events, we aimed to propose key players of multiple stress.

Major results and discussion

During an extreme storm event in the Têt River, a typical Mediterranean coastal watercourse, we show that large loads of urban-associated microorganisms, including gastrointestinal tract microorganisms, as well as highly pollutant-resistant and/or pathogenic bacteria, were released from CSOs and during the flood simultaneously to multipollution phenomena. Given the significant and rapid response of the particle-attached riverine bacteriome to pollutant mixtures, we were able to propose key players derived from multivariate statistical modelling combined with network module eigengene analysis.

We will further discuss how public health – as well as ecosystems – are at risk in this scenario of continuous disturbance of river resident bacterial communities subjected to multipollution phenomena associated to CSOs and floods. We will also highlight why urges improving certain urban management practices.

Metal resistance genes rich 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 both the immersed surface and the seawater characteristics. Our aim was the study of microbial colonization of biocides-containing surfaces, namely antifouling coatings. Cell attachment directly on toxic surfaces suggests a selection of tolerant (or resistant) organisms with characteristics conferring adaptative advantages. Biofilms were sampled from three types of surfaces (a plastic reference and two antifouling coatings) deployed in three coastal waters with dissimilar environmental conditions: North-Western Mediterranean Sea and the Atlantic and Indian Oceans. The two coatings differed in terms of biocides composition – either Cu thiocyanate and Zn pyrrithione (A3) or only Cu2O (Hy). Metal resistance genes (MRG) specific to copper (cusA, copA, cueO) or other metals (czcA and pbrT) were monitored with qPCR and compared to the microbial community structure, using 16S rRNA gene metabarcoding. After treatment with Dada2 pipeline, the amplicon single variants (ASV) suggested a lower α -diversity in A3 or Hy than in PVC whatever the site. Weighted Unifrac suggested segregation of communities primarily by surface, with lower site effect, and greater distinction in Hy biofilm communities. Metacoder log2 fold change ratio and LeFSe discrimination suggested Marinobacter to be specific of Hy and specific genera as Altererythrobacter, Erythrobacter and Sphingorhabdus to 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 in Toulon. Hy surface presented the highest relative abundance for copA, specifically at Lorient. These relative abundances were correlated (Spearman) with LeFSe discriminant taxa. Dasania correlated positively with all MRG except cueO, Marinobacter found in Hy highly correlated with the abundance of copA, and czcA appeared associated to Roseovarius. Certain taxa and genes were only significantly found in each surface in one or the other site. These results confirm the selection of specific communities with abilities to tolerate metallic biocides forming biofilms over antifouling surfaces and depending on their environmental source.

Does pesticide pressure affect the grazing behaviour of chironomids on microalgae?

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Generalized pesticide pressure in freshwater environments is likely to drive direct and indirect effects towards aquatic organisms, depending on their targets. It may also impact biological interactions within communities.

In this study, we assessed the effect of the herbicide diuron and of the insecticide imidacloprid, alone and in combination, on the feeding behaviour of chironomid larvae. Two complementary experiments were performed, under environmental concentrations (5µg L-1) of each pesticide, to investigate their impact on the responses of food (algae growth rate) and biological (mortality) and behavioural (mobility, food selection) responses of chironomid larvae.

The first experiment aimed at determining the grazing rate of chironomids on three microalgae species, independently, under control and contaminated conditions. To that aim, two diatom species, *Gomphonema gracile* (two different morphotypes: normal and teratogen) and *Planothidium lanceolatum*, and one green alga *Desmodesmus* sp. were offered as food, during 24h. The grazing rates of chironomids varied according to the pesticide and algae species. Diuron impacted algae and likely reduced their palatability, leading chironomids grazing rates to increase as a way to compensate for a loss of nutritional quality. In contrast, imidacloprid directly targeted insect larvae, which modified their grazing behaviour depending on the food source.

The second experiment followed a cafeteria design where the food selectivity of chironomids on previous algae, provided simultaneously, was determined over 4h. Under diuron exposure, larvae switched equally between the microalgae and were as mobile as in the control, while imidacloprid and the pesticide mixture condition altered chironomid movements and grazing behaviour.

This study demonstrated a significant impact of all pesticide exposure conditions on grazing rates and selective grazing behaviour of chironomid larvae, providing new insights on the direct and indirect effects of pesticide contamination on a simplified trophic web.
Ecotoxicity of graphene-based materials toward a diatom-bacteria biofilm

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Graphene-based nanomaterials (GBMs) such as graphene oxide (GO) or reduced graphene oxide (rGO) possess unique properties triggering high expectations for development of new technological applications and are forecasted to be produced at industrial-scale. This raises the question of potential adverse outcomes on living organisms and especially toward microorganisms constituting the basis of the food chain in ecosystems. However, investigations on GBMs toxicity were performed on various microorganisms using single species that are helpful to determine toxicity mechanisms but often fail to predict the consequences of the observed effects at a larger organization scale. Thus, this study focuses on the ecotoxicological assessment of GO and rGO toward a reconstituted biofilm composed of the diatom *Nitzschia palea*, alone or associated to a bacterial consortium.

After 144 hours of exposure to these GBMs at 0, 0.1, 1 et 10 mg/L, their effects on the diatom physiology and on the structure and the metabolism of bacterial communities were measured through the use of flow cytometry, 16S amplicon sequencing and Biolog ecoplates. The results obtained indicated that the exposure to both of these GBMs stimulated the diatom growth while opposite effects were observed when the diatom was exposed alone. This suggests that the diatom benefited from diatom-bacteria interactions when exposed to GBMs. In addition, the GO exerted strong growth inhibition as from 1 mg/L, modified the taxonomic composition of diatom-associated bacterial consortium, and increased bacterial metabolism related to carbon cycling with weak toxicity toward the diatom. On the contrary, rGO was shown to exert a weaker toxicity toward the bacterial consortium but influenced more strongly the diatom physiology.

Overall, this study highlights a higher toxicity of GO when compared to rGO. The reduction of GO may constitute an appropriate "safer-by-design" strategy for the development of a safer nanomaterial for microorganisms. Further research is being conducted to investigate more thoroughly the ecotoxicity of GO. GO effects observed in this study will be confronted to results derived from a mesocosm experiment in which such biofilm is part of a reconstituted trophic chain.

Acknowledgement - The authors thank the European Union's Horizon 2020 research and innovation programme under grant agreement No 696656. This research was also supported by the French Ministry of National Education, Higher Education and Research.

Is plastic a real threat to freshwater detrital ecosystem? A trophic approach.

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Plastic pollution is a global problem with major impacts on the environment and the human health. Freshwater ecosystems are being subject to extensive pollution by plastics as they are the direct link between plastic wastes and marine ecosystems. However, the research on the impacts of plastics on freshwater detrital ecosystems and the organisms involved in this key process is limited. We assessed the impacts of polyethylene microplastics (MP, <5 mm) that were extracted from health care products (foot scrub) and polyethylene trash plastic bags (PB) on stream-dwelling microbial decomposers involved in leaf litter decomposition by exposing leaf associated microbial assemblages to concentrations of MP (0.5 and 1.5 g L-1) and to PB. Further we conducted a feeding preference experiment with the invertebrate shredder Limnephilus sp., by exploring its capacity to distinguish: (i) leaves treated and non-treated with MP or/and PB; and also (ii) leaves from PB. Leaf decomposition and fungal sporulation were inhibited when exposed to increased concentrations of MP and especially in the presence of PB. Even so, invertebrate shredders preferred leaves treated with MP but totally avoided those exposed to PB. Our results showed varied impacts of polyethylene MP from healthcare products and polyethylene macro-plastics to the organisms involved in plant litter decomposition, highlighting the importance and need to study the plastic transfer along trophic chain in freshwater ecosystems.

Effects of thorium on periphytic biofilm community, taxonomic structures, nutritional quality, and its trophic transfer to the snail Lymnaea sp.

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Very few ecotoxicity data are available for thorium (Th) which reduces our ability to evaluate its ecotoxicological risk. In this work, the effects of Th on microorganism communities of periphytic biofilm were investigated. Biofilms host a diverse ecosystem comprising bacteria, fungi, algae and micromeiofauna. In this study, biofilms were grown on ceramic tiles for one month in the laboratory using as an inoculum of biofilm sampled in natural freshwater. After colonization, tiles were randomly placed in channels containing culture media representing three different exposure conditions: a control condition (C0; background Th concentrations of 0.004 \pm 0.002 nM), a low Th concentration condition (C1; 0.19 \pm 0.09 nM Th) and a moderately high Th condition (C10; 8.7 \pm 3.5 nM) for up to 4 weeks.

In a first part, we observed taxonomic structure effects by optical microscopy (diatoms and micromeiofauna) and 16S rRNA analysis (bacteria). The presence of Th modified the diatom community by changing the taxonomic structure, reducing the diversity, and increasing the cell density. The taxonomic structure of the bacterial community was also affected. No direct toxic effect of Th was observed on counted micromeiofauna but the changes in diatom and bacterial communities could explain the higher individual number of diatoms and micromeiofauna observed in Th-exposed conditions.

In a second part, we determined the biofilm nutritional quality with total protein and polysaccharide measurements as well as fatty acids (FA) composition. A trophic transfer experiment of Th from the C10 exposed biofilm to the snail *Lymnaea sp.* was performed and FA composition was also determined in the snails. The Th concentrations were significantly higher in snails fed with the C10 exposed biofilm to he control biofilm.

In the biofilm, no significant effect was observed on the polysaccharides but the total protein concentration in the C10 condition was significantly lower after 2 weeks of exposure compared to the control exposure. The FA composition of the control biofilm was coherent with the microscopy observations, showing a dominance of cyanobacteria and green algae. The FA composition was affected by Th after 4 weeks of exposure, notably by a decrease of saturated FAs (SFA, 15:0 and 17:0) and polyunsaturated FA (PUFA, 20:2n6) in the C10 exposed biofilm compared to the control. Snails fed with the Th exposed biofilm had also a different FA composition compared to those fed with the control biofilm. Monounsaturated FA (MUFA, C20:1n9) and PUFA (20:2n6, 20:4n6 and 20:5n3) were significantly lower in the snails fed with Th-laden biofilms than in the control. This work shows that low concentrations of Th can modify biofilm taxonomic structure and nutritional quality which in turn could disturb its ecological key functions.

Abstracts – Oral communications (3/4)

Session 3: Microorganisms role in contaminants (eco)dynamics & application to bioremediation

Thursday, October 8th

- 3:00-3:20 pm Dichloromethane degradation at contaminated sites Insights from biomolecular and dual-element isotope analysis approaches in model aquifers *Maria Prieto, Carmen Lázaro-Sánchez, Emilie Muller, Jeremy Masbou, François Lehmann, Françoise Bringel, Raphaël Di Chiara, Benjamin Belfort, Sylvain Weill, Gwenaël Imfeld, <u>Stéphane</u> <u>Vuilleumier</u>*
- 3:20-3:40 pm Nutritional inter-dependencies and a carbazole-dioxygenase are key elements of a Sphingomonas dependent consortium for thiabendazole degradation (Sotirios Vasileiadis) <u>Sotirios Vasileiadis</u>, Chiara Perruchon, Benjamin Scheer, Lorenz Adrian, Nicole Steinbach, Marco Trevisan, Patricia Plaza-Bolaños, Ana Agüera, Antonis Chatzinotas, Dimitrios G Karpouzas
- 3:40-4:00 pm Elucidating the micropollutant biotransformation potential of natural stream biofilms <u>Werner Desiante, Kathrin Fenner</u>
- 4:20-4:40 pm Adaptability of iron-oxidizing bacteria on printed circuit boards bioleaching: effects of a metal-rich leachate. (Juan Anaya) Juan Anaya, Agathe Hubau, Catherine Joulian, Vincent Semetey, Anne-Gwenaelle Guezennec
- 4:40-5:00 pm Antibiotics favor the establishment of antibiotrophic bacteria in agricultural soil microbial communities, but are not always sufficient to enhance antibiotic-degradation: manure spreading can help.

Loren Billet, Stephane Pesce, Nadine Rouard, Aymé Spor, Fabrice Martin-Laurent, Marion Devers-Lamrani

5-00-5:20 pm Organic micropollutant degradation in a membrane bioreactor inoculated with activated sludge.

<u>Ana B Rios Miquel</u>, Tamara J H M Van Bergen, Rosalie Van Zelm, Jan Hendriks, Mike S M Jetten, Cornelia U Welte

5:20-5:40 pm Emerging micropollutants distribution and degradation in marine Capbreton Canyon (North Altantic Ocean) : from sediments to pure strains *Alyssa Azaroff, Mathilde Monperrus, Carole Miossec, Claire Gassie, <u>Rémy Guyoneaud</u>*

Dichloromethane degradation at contaminated sites - Insights from biomolecular and dualelement isotope analysis approaches in model aquifers

Maria Prieto 1, Carmen Lázaro-Sánchez 2, Emilie Muller 2, Jeremy Masbou 1, François Lehmann 1, Françoise Bringel 2, Raphaël Di Chiara 1, Benjamin Belfort 1, Sylvain Weill 1, Gwenaël Imfeld 1, <u>Stéphane Vuilleumier</u> 2

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Dichloromethane (DCM) is a genotoxic, major industrial solvent frequently detected in multicontaminated aquifers. DCM is often found in complex mixtures with other contaminants, posing challenges to predict its fate in groundwater. Variations in hydrochemistry and redox conditions in groundwater due to fluctuations in the water table also affect pollutant biodegradation extent and associated degradation pathways. Several distinct modes of bacterial DCM degradation under both aerobic and anaerobic conditions have been described. Little is yet known about the diversity of degradation mechanisms involved. Also, the relationships between hydrogeochemical variations caused by water table fluctuations and associated bacterial communities and prevailing DCM degradation processes have not been investigated in detail. In this context, compound-specific isotope analysis (CSIA) may help define the dominant modes of contaminant dissipation at contaminated sites. In this study, the impact of hydrological fluctuations on DCM biodegradation was examined in two laboratory aquifers with contaminated groundwater from the former industrial site of Themeroil (France) (Hermon et al., 2018), using both DNA-based approaches and dual-element carbon and chlorine CSIA.

High-resolution sampling and monitoring of pore water allowed to examine, under steady-state and fluctuating conditions, the aquifer response with respect to bacterial composition and hydrochemistry. Bacterial communities did not significantly vary over time. Nevertheless, the presence of Geobacter in significant proportions under all investigated conditions supports the hitherto poorly documented notion that significant DCM degradation may occur under anaerobic, iron-reducing conditions. A dual C-Cl stable isotope approach (Λ C/Cl = $\Delta\delta$ 13C/ $\Delta\delta$ 37Cl) using GC-IRMS (C-DCM) and GC-MS (Cl-DCM) was developed to investigate the extent of DCM degradation, as well as prevailing bacterial and abiotic modes of DCM degradation in the light of recently discovered novel pathways for DCM biodegradation. Larger carbon isotope fractionation of DCM, associated with more extensive DCM mass removal (>90%), was observed under fluctuating conditions, as compared to steady-state conditions (mass removal of 35%). Dual C-Cl isotope plots also indicated distinct predominant reactions of C-Cl bond cleavage under fluctuating and steady-state conditions, with Λ C/Cl values of 3.41 ± 0.50 and 1.68 ± 0.26 respectively. Under fluctuating conditions, carbon enrichment factors (EC) became larger over time, ranging from -18.9 \pm 3.4‰ to -33 \pm 0.3‰, whereas chlorine enrichment factors (ϵ Cl) remained constant (-3.6 \pm 0.7%). In contrast, constant ε C values (-20 \pm 3.5%), but larger ε Cl values (-10.8 \pm 2%), were observed under steady-state conditions.

Taken together, the obtained results highlight the potential role of water table dynamics in DCM biodegradation. Obtained data are also being used to help develop and test a predictive model of DCM dissipation processes. This model may prove useful for improved assessment of natural attenuation of DCM at contaminated sites.

Reference

Hermon L, Denonfoux J, Hellal J, Joulian C, Ferreira S, Vuilleumier S, Imfeld G (2018) Dichloromethane biodegradation in multi-contaminated groundwater: Insights from biomolecular and compound-specific isotope analyses. *Water Research* **142**, 217-226.

Nutritional inter-dependencies and a carbazole-dioxygenase are key elements of a Sphingomonas dependent consortium for thiabendazole degradation

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Thiabendazole (TBZ), used as a fungicide and anthelminthic, is persistent (soil DT50> 1-2 years) and particularly toxic to non-target organisms, posing a serious environmental threat. Without previously known biodegraders, we enriched from soil a bacterial consortium rapidly degrading TBZ, with a recalcitrant to isolation Sphingomonas being the sole TBZ-degrader. Using multi-omics and stable isotope probing (SIP), we opted to elucidate the underlying interactions that retain the consortium TBZ-degrading capacity and integrity. Metabolomics showed that TBZ biodegradation occurs through the benzimidazole ring cleavage, with thiazole-4-carboxamidine being the single dead-end product. 16S rRNA gene amplicon sequencing demonstrated the community stability in time during TBZ degradation. SIP coupled with amplicon sequencing verified the key role of Sphingomonas in TBZ degradation, assimilating most of the 13C-labelled TBZ phenyl moiety. Time series metagenomic analysis of TBZ or succinate supplemented consortium led to the assembly of 18 high quality metagenome-assembled genomes (MAGs), with six (the Sphingomonas and a Hydrogenophaga among others) being dominant. Functional annotation of the extracted MAGs revealed a versatile toolset for aromatic compound degradation throughout the assembled bins. Gene expression analysis of the consortium while supplied with TBZ as sole carbon source, showed the activation by the Sphingomonas annotated MAGs of an operon coding for proteins with homologs previously shown to participate in the degradation of the structurally similar to TBZ, carbazole. Sphingomonas mobilizes a carbazole dioxygenase (car) operon during TBZ cleavage to thiazole-4-carboxamidine and catechol, and its catechol ortho-cleavage (cat) operon. In silico docking analysis showed high affinity of the Sphingomonas CarAa enzyme for TBZ and carbazole (ΔG values of -6.8 and -7.5 kcal mol-1 respectively), with the carbazole affinity being in the same range with that of the enzymes of previously characterized degraders like Sphingomonas sp. KA1 and Janthinobacterium sp. J3, reinforcing the scenario of its suspect role. Gene expression network analysis revealed strong interactions between the Sphingomonas and a Hydrogenophaga MAG, with Hydrogenophaga activating its cobalamin biosynthetic pathway and Sphingomonas its cobalamin salvage pathway along TBZ degradation. Our findings suggest that the TBZ-degrading consortium fits to the black queen hypothesis: Sphingomonas detoxifies TBZ (helper) and relieves Hydrogenophaga and other consortium members from its toxicity (beneficiaries), while Hydrogenophaga provides cobalamin (helper) to auxotrophic Sphingomonas (beneficiary).

Acknowledgments: This work was carried out within the frame of the projects (a) MINOTAUR (MIS 5030360, call RESEARCH – CREATE – INNOVATE, project code: T1EDK-02566) and OMIC-ENGINE (MIS5002636) co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation (b) the MSCA-IF-H2020 project EMIGRATE.

Elucidating the micropollutant biotransformation potential of natural stream biofilms

Werner Desiante 1, Kathrin Fenner 1

1 : Eawag

Micropollutants are released through various anthropogenic sources and can end up in complex mixtures in the aquatic environment. Such mixtures pose a potential risk to aquatic organisms. Stream biofilms, consisting of algae, bacteria and other microorganisms, play a key role in nutrient cycling and have been demonstrated to potentially act as natural sinks for micropollutants. While evidence for increased tolerance to micropollutants upon exposure to treated wastewater has been reported, little is known about the potential of stream biofilms to degrade micropollutants, especially when released as complex mixtures as in wastewater.

In our research we explored the ability of differently adapted natural river biofilms to biotransform micropollutants at environmentally relevant concentrations. In a field experiment, we investigated stream biofilms cultivated up- and downstream of two wastewater treatment plant outfalls. The main aim of this study was to investigate the biotransformation potential of natural river biofilms towards a large mixture of environmentally relevant micropollutants. Our experiments consisted of suspended biofilms in batch reactors with adequate controls. A mixture of 55 compounds was spiked into the reactors and concentrations were monitored over time by means of high-resolution mass spectrometry.

In general, we found a broad range of micropollutants to be biotransformed. About fourty compounds showed a concentration decrease, that could be attributed to biotransformation. Of these, five compounds were biotransformed only by the downstream communities of one site. Those findings lead to the conclusion that the respective downstream communities underwent adaptive changes that lead to an increased potential to biotransform those compounds, whereas the upstream communities did not. Interestingly, those five compounds typically occur at elevated concentrations in wastewater and are typically removed by activated sludge communities to a large extent. This lead us to the hypothesis that either the compounds themselves lead to adaptive changes within biofilm communities or that already adapted microorganisms are released in wastewater and integrate into the biofilms, thus enhancing their biotransformation potential.

To test the hypothesis we conducted another series of experiments, involving biofilms cultivated in a flume system. Biofilms were grown under four different conditions: river water only, and 10%, 30% and 80% treated wastewater blended with river water, respectively. Batch experiments were conducted as described above. We also observed a broad range of micropollutants that were generally biotransformed. Additionally, we found that the biotransformation potential towards those five compounds mentioned above increased with increasing amount of treated wastewater the biofilms were exposed to during cultivation. Those findings confirmed our hypothesis that the increased biotransformation potential of downstream biofilms is caused by wastewater treatment plant effluents. We further explored the underlying mechanisms by comparing exposure to filtered and unfiltered wastewater. The results of these experiments are still to be analyzed.

In summary, our results demonstrate the relevance of stream biofilms in biotransforming micropollutants at environmentally relevant concentrations. They further confirm an increased biotransformation potential towards specific groups of wastewater contaminants upon exposure to wastewater. This leads to some interesting open questions regarding the origin of the increased biotransformation potential which are currently being explored further.

Adaptability of iron-oxidizing bacteria on printed circuit boards bioleaching: effects of a metal-rich leachate

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Bioleaching is a bio-hydrometallurgical process that uses microorganisms, generally acidophilic, ironand sulfur-oxidizing Bacteria and Archaea, for mineral dissolution and recovery of valuable metals. The bioleaching of spent printed circuit boards (PCBs) is an emerging and promising bioprocess for precious and strategic metals recycling. Microorganisms must catalyze efficiently the chemical leaching of metals from PCBs and be resistant to the leachate containing a mixture of highly concentrated dissolved metals. This study aimed to investigate the adaptability of the well-known acidophilic and iron-oxidizing BRGM-KCC consortium towards metals-rich PCBs' leachates. BRGM-KCC was mainly composed of Leptospirillum ferriphilum according to molecular fingerprints of the 16S rRNA gene. This strict aerobic, extremely acidophilic and chemolithoautotrophic bacterium fixes carbon from CO2 and oxidizes ferrous iron for cell growth and energy production. Leachates were obtained by dissolving PCBs from a 10% solid content reactor operated in abiotic conditions, with H2SO4 as leaching agent and Fe(III) as oxidizing agent. The consortium, at first adapted to an environment with 1% solid PCBs content and pH 1.2, was inoculated in triplicates into shake flasks in a minimal mineral medium with no leachate or quantities of leachates equivalent to 2%, 4%, 6% and 8% PCBs content. Each flask was amended with ferrous iron to have a total amount of 8 g.L-1 Fe(II). The flasks were incubated in agitated and aerobic conditions at 36°C, with a pH daily controlled and adjusted at 1.2. The indicators of adaptability to increased metals concentration were the microbial growth and maintenance, followed by microscope counting and diversity fingerprints, and the kinetics of ferrous iron biooxidation, estimated from the redox potential evolution. The biomass concentration, initially of 1x107 cells per mL, increased by a factor of 10 during the exponential growth phase to reach a stationary phase in experiments with no leachate and leachates concentrated up to 6% PCBs content. However, the lag phase increased with the leachate concentration, lasting from two (no leachate) to seven days (6% PCBs content leachate). At 8% PCBs content leachate, the biomass remained in lag phase and the bio-oxidation of Fe(II) took place after 29 days. All the experiments reached a redox potential above 900 mV with respect to the standard hydrogen electrode, confirming the total bio-oxidation of ferrous iron. A maximum bio-oxidation rate of 241 mg.L-1.h-1 Fe(II) was estimated during the exponential growth phase of microorganisms in 2% PCBs content leachate, which is a usual value for bioleaching processes with Leptospirillum ferriphilum. Based on the findings, the consortium successfully adapted to the 6% PCBs content leachate containing 8.5 g.L-1 Cu, 8 g.L-1 Fe, 1 g.L-1 Zn, 92 mg.L-1 Ni, 12.6 mg.L-1 Pb, and 4.4 mg.L-1 Co, among other metals. This opens an interesting route towards PCB bioleaching at higher solid loads, a key parameter for optimizing the bioprocess, as well as perspectives for genomic studies on the adapted consortium to help elucidating metal resistance mechanisms.

Antibiotics favor the establishment of antibiotrophic bacteria in agricultural soil microbial communities, but are not always sufficient to enhance antibiotic-degradation: manure spreading can help.

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During the past decades, environmental concentrations of antibiotics have largely increased, resulting in a risk of ecosystem disturbance. However, because their composition is often rich in nitrogen and carbon, antibiotics are of nutritional interest for microorganisms, as long as their biocidal character is not considered. Antibiotic-degrading bacteria have therefore emerged amongst strains that were resistant to antibiotics. Called antibiotrophs, they are able to use selected antibiotics as nutritive sources for their growth. While several antibiotrophs have been isolated from different agroecosystems, little is known about their ecology. In particular, their dispersal capacity is poorly evaluated, even though manure spreading is a suspected source of antibiotrophs for agroecosystems. Also, their antibiotic degradation ability in complex agricultural soil communities remains insufficiently studied.

In this context, a microcosm experiment was set up by inoculating the sulfonamide-degrading and resistant bacterium, Microbacterium sp. C448, in four different soil types supplied or not with sulfamethazine and/or swine manure. After one month of incubation, qPCR analyses and 16S rDNA sequencing were performed to respectively quantify the inoculated strain, its antibiotrophic gene sadA, and to characterize the structure of bacterial communities. In parallel, a similar experiment was carried out with the addition of radiolabeled sulfamethazine, in order to monitored antibiotrophy activity during incubation by radiorespirometry.

Quantitative PCR results showed an effective establishment of the strain and its sulfamethazinedegrading gene (sadA) only under sulfamethazine selection pressure. This reflects the low competitiveness of the strain, which possesses a low invasion potential under non-antibiotic contaminated conditions. Sulfamethazine treated soils differed in their capacity to mineralize the antibiotic. Indeed, in absence of manure and despite the presence of Microbacterium sp. C448, only one of the four tested soils exhibited slight mineralization capacities. Whatever the soil type, radiorespirometry analyses showed that manure addition significantly enhanced sulfamethazine mineralization. These results confirm that the presence of functional genes does not necessarily ensure functionality. Moreover, they suggest that sulfamethazine does not necessarily confer a selective advantage to the degrading strain, as a nutritional source. In addition, 16S rDNA sequencing analyses strongly suggest that sulfamethazine has released trophic niches by biocidal action. Accordingly, manure-originating bacteria and/or Microbacterium sp C448 could have access to low competition or competition-free trophic niches. However, simultaneous inputs of manure and of the strain could induce detrimental competition for Microbacterium sp. C448, thus forcing it to use sulfamethazine as a nutritional source. Altogether, these results suggest that the studied antibiotrophic strain can modulate its sulfamethazine-degradation function depending on microbial competition and resource accessibility, to establish in an agricultural soil. Most importantly, this work highlights an increased dispersal potential of antibiotrophs in antibiotic-polluted environments, as antibiotics can not only release existing trophic niches but also constitute new ones.

Organic micropollutant degradation in a membrane bioreactor inoculated with activated sludge

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Background. In the past years, sensitive mass spectrometry methods have enabled the detection of pollutants at very low concentrations in nearly all water bodies globally. These organic micropollutants (OMPs) including pharmaceuticals, pesticides, and personal care products may cause toxicity to many organisms. Current wastewater treatment plants (WWTPs) are not very effective at removing all these OMPs and are thus a major source of surface water contamination. Microorganisms are still not fully adapted to converting these OMPs and evolution seems to be slow due to low concentrations and complexity in chemical structures. In this study, the microbial removal of selected OMPs was studied in a membrane bioreactor at increasing concentrations.

Methods. The membrane bioreactor (5L) was inoculated with activated sludge from a municipal WWTP in Groesbeek, the Netherlands. The bioreactor was fed with synthetic wastewater containing ammonium, acetate and methanol as main energy sources. The OMPs (Acetaminophen, diclofenac, metoprolol, metformin, carbamazepine, and fluoxetine) were added after dissolution in methanol. After an acclimatization period of 180 days at a hydraulic retention time (HRT) of 3.5 days and a solid retention time (SRT) of 15 days, the OMP concentration in the influent was increased every two weeks from 4nM to 800 nM. During the process OMPs were monitored by LC-MS/MS and the microbial community was analysed by 16S rRNA gene amplicon sequencing.

Results. Acetate, and methanol were fully consumed, and ammonium was converted to nitrate. Total suspended solids (TSS) were stable at 0.2-0.3 g/L during the whole experiment. Each OMP had a different removal value. Acetaminophen was the only OMP removed at 100% . Metoprolol and diclofenac removal was between 20% and 40%, while metformin and carbamazepine removal values varied between 10% and 20%. Removal of fluoxetine could not be determined accurately. The absolute amount of nmols removed increased proportionally with the concentration, indicating that enzyme saturation was not yet achieved. However, the removal % of each OMP did not change along with the concentration. In a second step, the HRT was increased to 5 days to prolong the reaction time of the OMP with the microbial community. Only metformin was removed at a higher degree. Finally, the microbial community was profiled using 16S rRNA gene amplicon sequencing during different time points/OMPs concentrations, and we observed a clear shift in the community composition: Bacteroidetes and Acidobacteria increased in abundance with increasing concentration, while Nitrospirae and Planctomycetes became less abundant. Furthermore, the genus Dokdonella (Proteobacteria), previously suggested to be responsible for degradation of acetaminophen and other OMPs, significantly increased in abundance.

Conclusion. Overall, the here presented results suggest a constrain in the removal of several OMPs under activated sludge conditions and inhibitory effects on specific taxonomic groups. The observed abundance shifts could also point out several microbial groups potentially involved in degrading OMPs. Further experiments are needed to better understand the removal constrains and to elucidate the specific microorganisms able to degrade OMPs in WWTPs.

Emerging micropollutants distribution and degradation in marine Capbreton Canyon (North Altantic Ocean) : from sediments to pure strains

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Since few decades, emerging substances such as personal care products and pharmaceuticals are becoming of high concern since only scarce information about their occurrence, reactivity, impact and degradation are available, especially in the marine environment.

A large screening of several micropollutants has been achieved in sediments collected in the Canyon of Capbreton (North Atlantic Ocean) in order to assess their occurrence and their concentrations. Twenty-four stations have been sampled within the first 30 km of the canyon in July 2017. Emerging micropollutants were detected and measured for instance for musk compounds (HHCB, AHTN and MK) with maximum concentrations at 3.6, 2.3 and 7.0 ng.g-1 or for sunscreens (3-BC, 4-MBC, EHMC and OC) with maximum concentrations at 6.2, 9.2, 31.8 and 29.2 ng.g-1, respectively. Highest concentrations of these pollutants were observed from offshore stations and in sediments sampled on terraces or slopes of the canyon. These results suggested emerging micropollutants released in the marine environment are transported through the Capbreton Canyon until the deep sea sediments due to their high persistence.

Degradation of synthetical musks (galaxolide and tonalide), UV filters (O padimate and octoctrylen) and a pharmaceutical compound (carbamazepine) was then studied in marine sediment samples, under laboratory conditions. After slurry incubation, half-life times of under biotic conditions were 16 days, 119 days and 231 days for O padimate, galaxolide and carbamazepine, respectively. Enrichments conducted under both anoxic and oxic conditions demonstrated, that under anaerobic conditions, degradations after one month of incubation under both biotic and abiotic conditions were limited compared to aerobic conditions. Novel aerobic bacteria, able to degrade synthetical musks and UV filters could be isolated. These novel strains were mainly related to *Bacillus megaterium*. Based on these results, the isolated strains able to degrade such emerging substances, can have a strong implication in the natural resilience in marine environment, and could be used in remediation processes.

The overall results indicate that emerging micropollutants should be included in the Water Framework Directive and Marine Strategy Framework Directive in order to assess the environmental quality.

Abstracts – Oral communications (4/4)

Session 4: Microorganisms for environmental risk assessment Session 5: Microbial ecotoxicology: from research to end-users

Wednesday, October 7th

4:20-4:40 pm Characterizing the effects of a complex contamination by pesticides: Preliminary results on the Cleurie River (France), a pilot site for an interdisciplinary approach based on phototrophic biofilms.

<u>Martin Laviale</u>, Sarah Chéron , David Heudre, Chloé Bonnineau, Soizic Morin, Marie-Noëlle Pons, François Guérold, Vincent Felten

- 4:40-5:00 pm Test of biodegradable polymers to replace the European-banned conventional plastic sticks : from research to end-users (Jean-François Ghiglione) Justine Jacquin, Nolwenn Callac, Jingguang Cheng, Charlène Odobel, Carolane Giraud, Clément Denoual, Mireille Pujo-Pay, Pascal Conan, Anne-Leila Meistertzheim, Valérie Barbe, Stéphane Bruzaud, Jean-François Ghiglione
- 5:00-5:20 pm Application of toxicophenomics in ecotoxicity tests using marine phototrophs: merging bio-optics and artificial intelligence. <u>Bernardo Duarte</u>, Eduardo Feijão, Ricardo Cruz De Carvalho, Irina Duarte, Silva Marisa, Jorge Marques Da Silva, Ana Rita Matos, Maria Teresa Cabrita, Rui Rosa, Andreia Figueiredo, Ana Marques, Marta Sousa Silva, Carlos Cordeiro, Pedro Mariano, Sara Silva, Andrei Utkin, João Carlos Marques, Isabel Caçador, Patrick Reis-Santos, Vanessa Fonseca

Characterizing the effects of a complex contamination by pesticides: Preliminary results on the Cleurie River (France), a pilot site for an interdisciplinary approach based on phototrophic biofilms.

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In France, many rivers face complex cocktails of contaminants at concentrations sometimes close to environmental quality standards (EQS). This type of complex pollution is typically encountered in the case of pesticides, and is usually poorly reflected by the bioindicators currently implemented within the European Water Framework Directive (WFD). In fact these indicators are not always able to reveal the subtle effects of this type of pollution, i.e. direct episodic and chronic or indirect (via trophic transfer, etc.) effects. It is therefore essential to develop indicators able to detect early signs of changes in water quality under natural conditions, but also to assess the possible associated indirect effects (e.g. propagation of effects in the food chain, and from upstream to downstream) and their consequences on the functioning of ecosystems. For this purpose, phototrophic biofilms present a wide range of structural and functional descriptors that could be of interest.

The contamination that has been observed for several years in the Cleurie River (Eastern France, Vosges) appears to be an ideal case study site for testing such descriptors. Due to industrial activities (textiles), this small headwater forested stream exhibit a complex cocktail of molecules, chronic and episodic (from daily to seasonal basis) but below EQS. This cocktail is characterized by high concentrations of glyphosate and AMPA as well as a high load of dissolved organic matter (OM) dominated by optical brighteners/dyes. The current media coverage of glyphosate associated to frequent changes in the color of the water feed a local environmental controversy, which is part of the more general and old one on the toxicity of pesticides.

In this context, an in situ monitoring, solicited by stakeholders, has been recently initiated in order to identify global indicators allowing studying this contamination. Our objectives are to fingerprint the chemical signature of the toxic pressure (e.g. using integrative passive sampling and spectrometry) and to characterize its effects on biofilms, a pivotal ecological player in these ecosystems. This presentation will focus on the first results: traditional biofilm-based descriptors (primary production, biodiversity) will be compared to others currently under development, either structural (the occurrence of deformities displayed by diatoms) or functional (acquisition of tolerance to glyphosate via a PICT-Pollution Induced Community Tolerance approach).

These results will be further used within the framework of a broader interdisciplinary approach aiming at studying the circulation of scientific knowledge between the different actors (researchers, end-users, stakeholders...) involved in this controversy.

Test of "biodegradable" polymers to replace the European-banned conventional plastic sticks : from research to end-users

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The European parliament voted to ban single-use plastic items in 2021 such as plates, cutlery, straws, food containers, expanded polystyrene cups and sticks used for cotton bud and plastic balloon. Transition to bio-economy includes the substitution of these banned products by biodegradable polymers. Here, we tested the colonisation (40 days) and the biodegradability (90 days) under marine conditions of 6 plastic slicks types, including petrochemical-based (PBS), bio-based (PBS, Bioplast, PHA) or mixed (MaterBi) polymers, which may be used as substitutes to conventional (PP) plastic sticks. Scanning electron microscopy, bacterial activity (3H Leucine incorporation) and diversity (Illumina sequencing) were followed during the course of the experiment. As we previously observed for plastic films under marine conditions, the bacterial colonisation followed a succession of primo-colonisation, growth and maturation of the biofilm (Dussud et al. 2018, Front. Microbiol. 9:1571). Each polymer together with its mature biofilm was then transferred in a minimum medium with no other carbon source than the polymers. Bacterial activity decreased drastically for polymers that did not show biodegradation potential (PP, PBS and PLA). Other polymers have a greater biodegradation potential (PHA, Bioplast, Mater-Bi) as depicted by high remaining bacterial activity after 3 months incubation in a minimum medium without any other carbon source. Different trends were observed in bacterial diversity for biodegradable vs. non-biodegradable polymers. In the case of PP, bacterial community structure of the mature biofilm remained stable during the course of the experiment, whereas some OTUs became rapidly specific for other polymer types. It allowed us to describe OTUs as good candidate to decipher the polymer degradation mechanisms under natural conditions. As mentioned in a recent paper (Jacquin et al. 2019, Front. Microbiol. 10:865), such study emphasises the great potential of microbial ecotoxicology to move from research to end-users in the context of circular economy.

This work has been founded by the French ministry of the ecological and united transition.

Application of toxicophenomics in ecotoxicity tests using marine phototrophs: merging biooptics and artificial intelligence.

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Based on the biophysical mechanisms of the photosynthetic metabolism, where the energy from photons is conveyed to electrons, ultimately producing chemical energy, this work provides a different approach to ecotoxicological assessments. These biophysical processes can be assessed by remote sensing, using different bio-optical tools such as diverse types of fluorometry and spectroradiometry. These techniques provide complementary information, regarding the stress level of the target organism, without disturbing any occurring test and are entirely non-invasive. Considering this, biooptical tools arise as possible and efficient instruments for ecotoxicology of marine primary producers, alternative to the traditional, expensive and time-consuming biochemical biomarker assays. Over the last decades, the impact of human activities in marine systems has increased exponentially, due to uncontrolled development. Beyond the classical contaminants, we are currently observing an increase in the appearance of what are normally known as Emerging Pollutants (EPs), such metal nanoparticles, pesticides, pharmaceuticals, and personal care products (PPCPs). All these EPs are being continuously released from wastewater treatment plant purification mechanisms to the marine environment. Therefore, the development of new ecotoxicological methodologies for the evaluation of the impacts of these new EPs in marine organisms is of paramount importance. Within this context, diatoms arise as excellent test organisms for ecotoxicity assays. The information gathered from the exposure to different EPs at different concentrations, using a variety of bio-optical tools allied to state-of-the-art metabolomics, will provide comprehensive information on the physiological stress imposed by the exposure to contaminants. All this data is given as input to highly efficient artificial intelligence and machine learning methods that discover the combinations of bio-optical variables that more efficiently detect dose-related stress for each organism and contaminant. This information will then be integrated into ecotoxicological indexes, for better communication to stakeholders and management entities. Summing up, this multi-disciplinary approach will fill the knowledge gaps existent on the ecotoxicology of EPs, while providing high-throughput screening tools passible to be applied in: i) impact assessment studies and ii) tests of new substances passible to be released in the environment.

Abstracts – Posters

(presented in alphabetical order of first author)

1. Alexandrium minutum from the Gulf of Gabès (Southeastern Mediterranean): Morphology, toxicity and molecular characterization towards risk assessment for Paralytic Shellfish Poisoning

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The dinoflagellate species *Alexandrium minutum* produces paralytic shellfish Poisoning (PSP) which can severely affect fisheries, aquaculture activities and humans through the consumption of contaminated shellfish. This harmful species proliferates regulatory on the coasts of the Gulf of Gabès (Tunisia, Southeastern Mediterranean) and may threaten shellfish harvest especially that this area is in important for natural stocks of the grooved carpet shell *Venerupis decussata* (*Ruditapes decussatus*) (Linnaeus, 1758).

In this study, morphological and molecular characterization as well as toxicity evaluation of *A. minutum* strains isolated from the Gulf of Gabès, during former (January 2006 and 2009) and recent outbreaks (June 2019), were investigated. Examination of the thecal plate morphology by light, epifluorescence and scanning electron microscopy revealed no significant morphological differences between the Tunisian strains and other geographically distant strains of *A. minutum*. The Sequence analysis of the internal transcribed spacer region (ITS- 5.8S rDNA) and the D1/D3 region of the large subunit (LSU rDNA) confirmed the morphological identification of *A. minutum*

The results regarding the mouse bioassay revealed that all *A. minutum* strains were toxic to mice after i.p. injection. Characteristic symptoms of PSP were observed in all the mice tested such as decreased locomotion, stretching of hind limbs and lower back, ataxia, creeping paralysis and dyspnea. A dose level 5.49 × 108 cells was found to be lethal to mice.

2. Interaction between glyphosate and dissolved phosphorus on bacterial and eukaryotic communities from river biofilms

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Since the capacity of river biofilms to degrade glyphosate has been proven to increase when the availability of dissolved phosphorus (P) in water decreases, the present study investigates the diversity responses of bacterial and eukaryotic microbial communities from biofilms in a search for glyphosate-degrader candidates. Glyphosate and P interactions were observed for eukaryotic communities, the highest community richness and diversity being preserved at low concentrations of glyphosate and P. However, species richness and diversity in bacterial communities were not affected by glyphosate, though shifts in the structure of these communities were concomitant with the degradation of the herbicide. Bacterial communities capable of using glyphosate as P source were characterized by increases in the relative abundance of certain Bacteroidetes, Chloroflexi, Cyanobacteria, Planctomycetes and alpha-Proteobacteria members. Glyphosate-degrader candidates found in natural river biofilms can be further isolated for better understanding of glyphosate degradation pathways, and used as bioremediation strategies in heavily contaminated sites.

3. Soil microbial community responses to pesticides

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Assessment of the effect of plant protection products on the composition and activity of the microorganisms is a classical problem of microbiology. Although the impact of pesticides is considered to be negative, the scope of this phenomenon, presence and duration of its chronic effects, consequences of regular pesticide application for a microbial community are not determined. These problems can be solved only with a help of modern molecular-genetic methods in conjunction with the traditional methods of soil microbiology.

The impact of three types of pesticides (herbicide metribuzin, insecticide imidalocloprid, and fungicide benomyl) has been assessed using the method of next-generation sequencing (NGS). The pesticides have been applied both separately and together at one- and ten-fold rates on the structure of microbial complexes and indicators of biological activity of Albic Retisols (the Moscow region). The carbon of soil microbial biomass (Cmic) was quantified using the substrate-induced respiration method. The rate of substrate-induced respiration was estimated according to the C-CO2 production in 3 h after 10% glucose solution (0.2 mL) was added to the soil. Nitrogenase activity was determined by the acetylene to ethylene reduction test with recalculation of the formed ethylene to the amount of fixed nitrogen at a ratio of 1:3. The rate of nitrous oxide formation was assessed under anaerobic conditions (after blowing vials with argon) with addition of acetylene as a nitrous oxide reductase inhibitor. All measurements were made in five replicates. It has been discovered that pesticides have a greater impact on a fungal community compared with a prokaryotic one where only the changes of phyla of Actinobacteria and Proteobacteria occurred. The research of fungal communities based on the results of molecular-genetic analysis has revealed two dominant divisions of fungi in all soil samples -Ascomycota (72.6 ± 8.0%) and Basidiomycota (26.0 ± 7.7%). Ascomycota and Basidiomycota are important destructors in the soil carbon cycle and secrete the enzymes that destroy organic polymers. Moreover, the samples with ten-fold pesticide application rate (both in a mixture and separately) have been revealed to have the amplification of the *Basidiomycota* division. Application of pesticides at a tenfold rate without starch-mineral mixture causes an increase in the share of Mortierellomycota representatives. It is shown that application of pesticides has a statistically significant effect (p < 0.05) on the structure of fungal community. An increase in the shares of Mortierellomycota and Basidiomycota following the application of a tenfold rate of the pesticides independently of the presence or absence of additional carbon source is explained by the fact that these divisions contain certain species known to have a high hydrolytic activity, in particular, able to utilize certain constituents of xenobiotics as a nutrient substrate. Pesticide application has a short-term stimulating effect on the carbon content of microbial biomass. It has been shown that insecticide imidalocloprid stimulates nitrogen fixation whereas other types of pesticides do not affect this factor.

The study was funded by RFBR according to the research projects No 18-316-00054 and No 18-016-00130.

4. Spatio-temporal variation of microbial communities of the Seine River surface waters at the scale of the Parisian agglomeration

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Water resource management and environmental protection are two big challenges worldwide. Toxic and hazardous substances from wastewater treatment plants (WWTPs) continue to be discharged into the receptor-based water. The Water Framework Directive (WFD) has been implemented in October 2000 in order to ensure a better sustainable water management. A list of priority and hazardous substances presenting a significant risk to the aquatic environment was established by WFD. According to this directive, the discharges, emissions and losses of these substances need to be reduced and/or eliminated according to their toxicity.

Moreover, this WFD requires the member states to protect, enhance and restore the surface waters with the aims of achieving a good ecological status. To assess the ecological status of rivers, it is necessary to set up biological tools. First, bioassays have been set up to determine the effect of a substance on living organisms. Then, different techniques will be used to characterize the abundance, activity and diversity of microbial communities.

In the frame of MeSeine Innovation program coordinated by the Innovation Direction of SIAAP, the aim is to monitor the knowledge of the ecological status of the Seine River. Until now, the microbial communities of the Seine River has been poorly studied, the toxic effect of the priority and hazardous substances on these communities is even less.

The objective of this study is to investigate the impact of the priority substances from the anthropogenic activities on the microbial communities in terms of diversity, activity and abundance for the Seine River. Seven sampling points (Juvisy-sur-Orge, Choisy-le- Roi, Vitry-sur-Seine, Champigny-sur-Marne, Bougival, Conflans-Sainte-Honorine, Triel-sur-Seine) were selected along the Seine River from upstream to downstream of the Paris conurbation over one hundred kilometers. These sites will make it possible to study the influence of (i) the main tributaries of the Seine River (from upstream to downstream: Orge River, Marne River, Oise River), (ii) dry weather discharges from WWTPs located on the Seine and Marne Rivers. Five campaigns have been planned (2 in 2020 and 3 in 2021).

For each sample, the microbial diversity, abundance and activity were monitored using Illumina high throughput sequencing technique, flow cytometry and enzymatic assay (C, N, P cycles), respectively. The results (currently in progress) will be presented in the poster and should help us understanding the impact of the anthropogenic pressure on the quality of the Seine River.

5. Agricultural adjuvants: impact on the microbial toxicity and biodegradation of the pesticide active substance?

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When a farmer spreads pesticides on his fields, he applies not only one or several active substances but also a number of other ingredients. These supplementary ingredients, described as adjuvants, co-formulants or inerts - as they are assumed to be biologically inactive -, are added to stabilize and enhance the efficiency of the active substances. Although surfactants and solvents make up the largest group of adjuvants the chemical families used can be diverse. The adjuvants are present as mixtures and the exact composition of a given formulation is often protected by the industrial secret. All these chemicals can be found in the soil at the same time as a multi-contamination. Therefore the "cocktail effect" can drastically modify the fate and toxicity of each individual molecule, in particular those of the active substance.

The objective of our research work was to investigate the impact of formulations on the biodegradation capacity of active substances by pure bacterial strains and on their toxicity in terms of bacterial growth and survival. Pure and formulated herbicides used on corn crops (mesotrione, nicosulfuron) and a fungicide used on wheat (tebuconazole) were studied.

The comparative studies on active ingredient/formulation showed a major inhibitory impact of adjuvants on bacterial growth, particularly on Gram positive bacteria and an increased cell death. Their effects can also significantly modulate the degradation kinetics according to the bacterial pure strain tested, from an inhibition to an increase of the biodegradative activity. The monitoring of the overall adjuvant concentrations by 1H NMR during the active ingredient degradation showed that some of them were degraded. This could stem from either an adjuvant competition towards the substrates (negative impact on the active ingredient biodegradation) or a co-metabolism phenomenon (positive effect).

All these results emphasize the necessity to study formulated pesticides in order to assess their actual eco-toxicological profiles.

6. Are aquatic fungi able to evaluate microplastic impacts on leaf litter decomposition process?

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Microplastics (MPs) have been recognized as a threat, and an ecotoxicological risk for aquatic ecosystems. MPs toxicity is determined by their physical and chemical properties, including particle size, shape, surface area or polymer type. Aquatic hyphomycetes are a group of fungi that have the greatest ecological effects on freshwater ecosystems, as they are involved in a key process on these ecosystems, the decomposition of plant litter in streams. These fungi have the ability to degrade and consequently transform leaf material into a more suitable food source for stream detritivores, being important mediators in the energy and nutrient transfer to higher trophic levels. The knowledge on the impacts of MPs on aquatic fungi and the processes they driven is very scarce, so the main goal of this study was to assess the impacts of different sizes of MPs (fragments, FR; and pellets, PE) on the leaf litter decomposition, measuring functional aspects such as leaf mass loss, fungal reproduction and activity of plant litter degrading enzymes. A microcosm experiment was used with monocultures and combinations up to three aquatic hyphomycetes species (Articulospora tetracladia, Tricladium splendens, and Heliscus lugdunensis), where concentrations of FR (0.5 and 2 g L-1) and PE (2 g L-1) were added. We expected that: i) the presence of FR could negatively affect all functional aspects; ii) the impacts of the PE could not be so strong as FR impacts, as PE has a bigger size than FR; iii) the traits of the fungal species would matter to face MP exposure because the traits of certain fungal species may be of greater importance than species number to maintain ecological processes. FR or PE led to a stimulation of leaf mass loss especially in combinations of two aquatic hyphomycetes. Consequently, the activity of degrading enzymes increased in the presence of 0.5 g L-1 FR and PE. Fungal sporulation rate increased in the presence of FR, especially for monocultures and combinations of three aquatic hyphomycetes, while the presence of PE decreased fungal sporulation in combinations of the three aquatic hyphomycetes. Our results showed the MPs influence in the activity and diversity of microbial decomposers, putting at risk the processes they drive in freshwaters. This suggest the aquatic hyphomycetes as potential microorganisms to be used to evaluate the MPs impacts on leaf decomposition process.

7. Toxicity of PAHs to phytoplankton: an example of legacy contaminants with effects still not adequately addressed

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Polycyclic aromatic hydrocarbons (PAHs) have long attracted scientific interest because of their strong toxicity to living organisms pointed out as early as in the 1940's, and the worldwide contamination of atmospheric, terrestrial and aquatic ecosystems. As a consequence, environmental protection regulations have been implemented in many countries, based on the effects of PAHs on non-target organisms with sixteen PAHs designated as priority pollutants that deserve special attention for aquatic ecosystem management. In this study, the reported effects of PAHs on phytoplankton were collectively analyzed. Phytoplankton fuels the major part of aquatic carbon cycling and drives food webs in marine and fresh waters. We compared different toxicological endpoints including growth, chlorophyll *a*, fluorescence yield, membrane integrity, lipid content, anti-oxidant responses and gene expression for both freshwater and marine species in culture and in natural assemblages exposed to various PAHs. EC50s (half-maximal effective concentrations) reported in the scientific literature were highly variable, ranging over five orders of magnitude. The lowest EC50 was reported for *Chlorella fusca* var. *vacuolata* exposed to benzo(a)pyrene at 0.63 µg/L, the highest at 68 mg/L for *Scenedesmus subspicatus* exposed to naphthalene. The toxicities of PAHs were species-dependent, and influenced by numerous environmental factors such as UV radiation, temperature, and salinity.

To assess the ecological risk linked to phytoplankton PAH exposure, species-sensitivity distribution (SSD) analyses were performed based on EC50s available from the ECOTOX database of US-EPA and published literature. While numerous methods have been used to construct SSD relationships, we chose to use the *ssdtools* web app (https://bcgov-env.shinyapps.io/ssdtools/) based on a dedicated R package to calculate HC5 (harmful concentration for 5% of the species assessed) and confidence limits. According to HC5, the most water-soluble PAHs seemed to be the least toxic to microalgae. Based on a weak relationship between the EC50/HC5 ratio and cell biovolume, larger microalgal cells appeared less sensitive to PAHs. Furthermore, the HC5 for marine species were on average one order of magnitude higher than for freshwater species, suggesting a higher tolerance of marine phytoplankton towards PAHs.

However, available data on PAH toxicity to phytoplankton were often scarce, including those specific to the 16 priority PAHs, and a significant number of studies were flawed by uncertainties surrounding solubilities. Standardized experimental assessment of dose-effect relationships using model phytoplankton is still needed to refine and complement the knowledge about PAHs toxicity thresholds and mechanisms, which is the basis of environmental risk assessment. We assume that this is also the case for other legacy contaminants whose effects are considered as trivial, such as recently pointed out for the herbicide atrazine.

8. Evolution of the biodiversity and interspecific relationships in river biofilms from a preserved environment or exposed to pharmaceuticals

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Microorganisms forming river biofilms, when exposed to various pollutants, could have their taxonomic composition impacted as formerly described in several publications. The aim of this study is to analyse the impact of the effluent of a pharmaceutical factory WWTP (wastewater treatment plant), which was previously associated with an abnormal development of fish gonads and subsequently reinforced by new filtration systems to avoid this problem with success, on the evolution of the biodiversity and interspecific relations indownstream river biofilms during six months.

Different supports for biofilm development and several primer sets were tested in order to describe the microbiological diversity with the most exhaustive manner, and to detect variations in biofilm biodiversity even in presence of an effluent weakly loaded in xenobiotics. The rDNA from prokaryotic and eukaryotic communities have been sequenced upstream and downstream the industrial site producing pharmaceutical compounds during six sampling campaigns. The biofilms were left to grow for one month on synthetic matrix.

Bioinformatical and statistical analyses of data show a similar taxonomic profile upstream and downstream the WWTP effluent for each month, suggesting that the weak concentration of pharmaceuticals into the effluent could not perturb the evolution of the biodiversity. However, more advanced statistical analyses highlight potential perturbations of interspecific relationships into biofilms exposed to this effluent.

9. Bacterial diversity reports contamination around the Ichkeul Lake (Tunisia)

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The anthropogenic pressure in coastal areas increases dramatically with the exploitation of environmental resources. Biomonitoring coastal areas is crucial to determine the impact of pollutants on bacterial communities in soils and sediments, since they provide important ecosystem services. However, relevant biomotoring tools allowing fast determination of the ecological status are yet to be defined. Microbial ecology approaches provide useful information for developing such microbial monitoring tools reporting on the effect of environmental stressors. Chemical and microbial molecular approaches were combined in order to determine microbial bioindicators for assessing the ecological status of soil and river ecosystems around the Ichkeul Lake (Tunisia), an area highly impacted by human activities. Samples were collected along soil/river/lake continuums in three stations around the Ichkeul Lake influenced by different human activities at two seasons (summer and winter). Contaminant pressure indexes (PI), including PAHs (Polycyclic aromatic hydrocarbons), alkanes and OCPs (Organochlorine pesticides) contents, showed significant differences in the contamination level between the stations with seasonal variation. Bacterial communities were characterized by 16S rRNA gene metabarcoding. Although microgAMBI indexes, determined from the sequencing data, were in accordance with contaminant contents, they were not sufficient to fully explain the PI. Therefore, further microbial indicators are still to be defined. The comparison of bacterial communities revealed the specific microbial assemblage for soil, river and lake sediments, which were significantly correlated with contaminant contents and PI. Such observation offers the possibility to define a relevant set of bioindicators for reporting the effects of human activities on the microbial communities structure. Such bioindicators might constitute useful monitoring tools for the management of microbial communities in coastal areas.

10. Study of the recovery of bottom ashes from the combustion of wood-sludge mixtures from wastewater treatment plants in the context of agricultural amendment in France

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Approximately 15 Mt of sludge (1.5 Mt of dry sludge equivalent) is produced in wastewater treatment plants (WWTP) in France each year. The use of mixing of dry sludge with biomass, for boiler feed as a source of energy, appears as a solution for its valorization. If this activity is implemented, the combustion of this material will produce a large amount of ash. In other words, the companies will have a new residual waste to valorize. Ashes can be a source of readily available chemical elements as well as heavy metals, becoming a source of contamination for soils and living organisms in contact with the soil. Thus, the objective of this study on the agronomic valorization of ashes resulting from the combustion of a wood mixed with Thionville WWTP sludge to analyze the impact of the return of these ashes to the soil in terms of agronomic value and innocuitys on vegetal and soil microorganisms exposed to the heavy metal added by the ashes.

Durum wheat was cultivated in mesocosms under controlled conditions. The mesocosms received the application of four types of ashes under fire (i) Forest Pellets (FP) + Wood Pellets (WP); (ii) FP + WP + 12% mud; (iii) FP + WP + 12% mud + 1% CaO; (iv) FP + WP + 20% mud + 1% CaO applied with four different rates (0, 1, 10, 50 Mg ha-1). The plants were grown until the full maturity of the seeds. Analyses were carried out to investigate the effect of the application of the ashes on crop production, nutrient and trace metal concentrations in soils, above-ground biomass, and grains. The effect of the amendment of the soils with ashes on microorganisms was measured by the density and diversity of microorganisms. Ecotoxicity tests on higher plants were also carried out at the beginning and end of the experiment to verify the effect of the ashes on the soils before and after wheat cultivation.

The addition of ashes resulting from the combustion of the wood-mud mix on reconstituted soils shows that the addition of metals is higher than the maximum flow allowed over 10 years for the parameters Ni + Co + Cu + Zn compared to the decree No. 97-1133/98. However, these values are much lower than the natural values of the heavy metals found in the soils of the region (background values). The ecotoxicity tests showed no negative effects on the plants studied, regardless of the type of ash and the dose of ash applied. Positive effects are noticeable at the level of wheat production where growth was visibly strongly influenced by the contribution of ashes. The presence of metals influenced the density and diversity of soil organisms. Thus, the return to the soil of ashes from the wood/mud mix recovered from the fireplace is a promising source of nutrients, but further studies need to be carried out to classify these matrices without any real environmental risk.

11. Bioremediation of agricultural soils: a natural preventive approach to reduce the environmental contamination by pesticides

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A major problem with pesticides is linked to the non-negligible proportion of the active ingredient spread that does not reach their intended targets and contaminate environmental compartments. Various regulations calling for the reduction of pesticide use have been set up at the European and national level but they are far from reaching the quantitative objectives announced. During this period of change in agriculture, alternative solutions such as biocontrol or plant growth promoting agents are the subject of intense research but are still struggling to emerge *in situ* and to demonstrate the same efficiency as pesticides. Before reaching the "zero" pesticide objective, we developed an intermediate solution based on "a new agricultural practice" which relies on a process of preventive agricultural soil bioremediation. This innovative method consists in simultaneously applying a pesticide and the pesticide-degrading microorganisms, providing optimal conditions for microbial degradation of pesticides after their specific action and before their transfer to the surrounding ecosystem.

The aim of our study was to determine the suitability of this concept, with a model herbicide (2,4-D = 2,4-dichlorophenoxyacetic acid) and one of its bacterial mineralizing-strains (*Cupriavidus necator* JMP134).

The 2,4-D biodegradation was studied in microcosms of soils with different physico-chemical characteristics and planted with sensitive and non-sensitive plants. Simultaneous application of formulated 2,4-D (DAM[®], Agriphar) at the recommended agronomic dose and 105 cells.g-1 dw soil of fresh cultivated JMP134 strain was carried out. Residual amounts of 2,4-D in soils, the survival rate of inoculated bacteria and the herbicidal effect on sensitive plants were monitored. We demonstrated that this technique accelerates degradation of the herbicide (persistence reduced by over a factor of three for soils supplemented with the mineralizing bacterium) while maintaining its effectiveness. The concept was also validated for indoor fields using the same protocol, except that a formulated JMP134 strain was inoculated in order to better mimic the real field conditions.

Our studies constitute the first bases for the development of natural preventive bioremediation as a good tool to reduce environmental contamination by pesticides.

12. Passive samplers to highlight the presence of pharmaceuticals and their potential effect on microbial communities

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Freshwater ecosystems are increasingly affected by anthropogenic pressure. One of the main sources of contamination comes from wastewater treatment plant (WWTP) effluents that contain wide range of micro- and macropollutants. Compared to urban treated effluents (TEs), hospital ones may contain more active pharmaceutical compounds (PhC). PhC and their byproducts have become an important environmental issue, because of their high variety, potentially toxic byproducts and bioactivity at low concentrations. However, concentrations of PhC, especially in WWTP effluents, often exhibit large and rapid daily and weekly variations that require extensive composite water sampling, which is highly demanding in physical, logistical and financial aspect. Passive samplers enable time-integrated measurement of PhC and are therefore helpful to detect spot pollution events and record PhC occurrence at very low concentrations.

Here we aim at comparing (i) PhC accumulation in two different passive samplers - polar organic chemical integrative samplers (POCIS) and environmental biofilms exposed to urban (U) and hospital (H) treated effluents and (ii) evaluating the capacity of the two matrices to explain changes in bacterial and diatom biofilm communities. Furthermore, we (iii) determine via POCIS the enrichment of PhC in the recipient river downstream from the WWTP output in order to evaluate the contamination level and suggest which wastewater origin could be the major source of pollution.

Environmental biofilms were colonized and POCIS were installed in TEs and the recipient river up- and downstream from the WWTP output to study PhC in the two passive samplers. Concentrations in the water column for six of the molecules were also recorded and bacterial and diatom biofilm communities in the sampling locations were studied via DNA metabarcoding.

Results showed that the performance of the two passive samplers (POCIS and biofilms) to detect and quantify PhC differs and is compound specific. Some molecules were better quantified in POCIS, and others – in biofilms. However, when comparing concentrations of quantified PhC in U and H, the two passive samplers showed the same trend, validating the location with higher presence for each of the compounds. PhC concentrations measured in the water column showed significantly positive correlation to PhC concentrations measured in POCIS, but not to those measured in biofilms. A Procrustes analysis revealed that concentrations in both passive samplers had a significant correlation with biofilm community changes of bacteria and diatoms. However, PhC concentrations measured in POCIS correlated better with the microbial variations.

Furthermore, in the recipient river downstream from the WWTP, POCIS showed higher accumulation of PhC, especially in colder months. Bacterial and diatom biofilm community changes in the recipient river were also significantly correlated to PhC concentrations as revealed by POCIS.

Finally, we can conclude that PhC measurements in POCIS correlate better to measurements in the water column and to microbial community changes. However, some molecules were better quantified in biofilms. Hence, both methods are complimentary. This topic need further investigation, as robust measurements of PhC in treated effluents are essential to better detect hotspot pollution that may affect recipient freshwater environments.

13. The URBIFUN project – Urbanization effects on the relationship between microbial diversity and ecosystem functioning

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More than a half of the global current population lives in urban areas, and this figure is expected to increase soon. Urban areas are largely recognized as a source of stress for their surrounding water bodies, such as rivers and streams. Despite the acknowledged impact of urbanization on water bodies, there is still much to do in order to fully understand the impact of cities on river and stream ecosystems, particularly regarding the relationship between microbial diversity and ecosystem functioning. In line with this, URBIFUN (Urbanization effects on the relationship between microbial biodiversity and ecosystem functioning) is the 3rd young-AIL collaborative project. URBIFUN addresses this knowledge gap by investigating how a gradient of urban impact alters the structure of the microbial community and how this translates into shifts on the ecosystem functioning in river sediments. First, we will review available studies relating urban impacts on the biodiversity-ecosystem function relationship in river sediments, in order to identify major challenges and knowledge gaps. Second, we will perform a field campaign where river sites spanning a gradient of urban land-use will be sampled (sediments and water). We will use a metabarcoding approach to identify the response of the microbial community to the land-use gradient. We will also analyse how changes in community composition translate into alterations in the ecosystem functioning in situ (i.e. community respiration and primary production). We expect to find shifts in microbial community composition with increasing urban pressure, leading to the identification of potential indicator taxa and community thresholds. We also expect that these community changes will be reflected at the functional level, decreasing production-respiration ratios across the urbanization gradient.

14. Field testing of two filling materials for the bioremediation of arsenic-rich Acid Mine Drainage

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Acid Mine Drainage (AMD) is an undesired product of the weathering of sulfide minerals present in ores deposits and mining wastes. These leachates are responsible for extreme pollution of the freshwater ecosystems. When produced from arsenic-rich tailings, AMD are concentrated in highly toxic arsenic (As) worsening their adverse effects on the environment. It is then essential to develop cost-effective methods to treat this pollution. Biological treatment based on bacterially mediated Feoxidation followed by Fe-As co-precipitation proved to be a promising strategy.

The present study aimed at the optimization of a field-scale system for the treatment of arsenic-rich AMD from the ancient Pb-Zn Carnoulès mine in Southern France. Twenty years of monitoring of this AMD provided a fine comprehension of the biogeochemical processes leading to As attenuation at this site. Two field units were designed with the objective to maximize the surface available for the Fe- and As-oxidizing biofilm growth. Two filling materials were compared: plastic support (PS, specific surface 160 m2/m3) and wood/pozzolana mixture (WP, 80%-20% of the mass respectively, specific surface 400 m2/m3). Forced aeration was provided so that dissolved oxygen was not a limiting factor. The field pilots were installed on July 2019 and fed by As-rich AMD water ([Fe] = 500-900 mg/L, [As] = 50-100 mg/L, pH = 4.3 ± 0.8) during five months at controlled flow rate (hence controlled Hydraulic Retention Time, HRT). Water samples were collected periodically at the inlet and outlet of the systems for chemical characterization, including the determination of dissolved Fe(II) concentrations, total dissolved Fe, As and S concentrations and redox arsenic speciation.

Our results showed that a steady state regarding iron oxidation was reached more rapidly in the WP pilot compared to the PS pilot where a latency phase was observed. The two field pilots showed an average Fe oxidation efficiency of $92\pm3\%$ (PS) and $97\pm1\%$ (WP) for a theoretical HRT of 17.5 h (PS) and 19.3 h (WP). Fe precipitation efficiency reached $38\pm5\%$ (PS) and $42\pm5\%$ (WP). As precipitation efficiency reached $60\pm2\%$ (PS) and $73\pm5\%$ (WP). The removal rates (in mol. L-1.s-1) showed no significant differences between the two pilots over the studied period (p >0.05). The abatements obtained in this study were improved compared to those obtained with a previous field pilot tested on Carnoulès site that was fully passive (without forced aeration) and filled with river sand material.

Further work is needed to characterize the diversity and the activity of the bacterial community colonizing the pilots, and to assess the possible effect of the filling material on these communities. A particular attention will be paid to arsenic oxidation activity since it will contribute to the formation of more stable and less toxic As(V)-rich precipitates. Long term monitoring of the two pilots is necessary to confirm the robustness of the systems. We can hypothesize that the pozzolana-wood system would show a better robustness towards possible environmental and operational fluctuations.

15. Production of biosurfactants and biodegradation of total hydrocarbons (THC) by an endemic microbial consortia and Burkholderia thailandensis

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Petroleum hydrocarbons, used as the main energy source in the world, are persistent contaminants and toxic to the environment (*Liu and al., 2015*). The ecotoxicity of hydrocarbons results in biological alterations in soil organisms such as fungi (*Chiapusio and al., 2007*), bacteria or plants. Elimination of petroleum hydrocarbons present in contaminated soils can be envisaged by different treatments (*Khan, 2005*). Chemical and thermal treatments are often effective, but expensive and destroy soil (structure, composition, flora and/or fauna) (*Dermont and al., 2008*). The biological treatments are therefore presented as an effective and environmentally friendly alternative (*Iwamoto and Nasu, 2001; Khan, 2005*).

Some microorganisms have the capacity to degrade organic contaminants and can be used as a bioremediation technique. One of limiting factors in this process is the lack of hydrocarbons bioavailability; these organic contaminants are fixed on soil particles or organic matter (Ockenden and al., 2003) limiting the access of microorganisms and therefore their degradation. The production of biosurfactants by soil microorganisms (Zajic and Seffens, 1984) could be a solution to increase the bioavailability of organic contaminants.

Two consortium, isolated from contaminated soil, produce biosurfactants from 14 days. The microbial growth of consortium in an environment with hydrocarbons as the only carbon source attested that these consortium are hydrocarbonoclasts and hydrocarbons are potentially degraded. The degradation of the hydrocarbons by the consortia, having been conclusive in previous experiments, is being analysed.

In this context, a 65-day experiment was carried out to assess the potential for degradation of hydrocarbons and the production of biosurfactants by a microbial consortia SHEMS1 isolated from contaminated soil and *Burkholderia thailandensis*. The production of biosurfactants was studied by sensitive rapid methods: the drop-collapse test (*Jain and al., 1991; Tugrul and Cansunar, 2005*) and the oil spread method (*Varjani and al., 2007*). Spectrophotometric measurements at 620 nm were realised to control microbial growth following a calibration range between the absorbance measurements at 620 nm and the microbial concentration (CFU / mL). The degradation of hydrocarbons by microorganisms was defined by GC-FID analysis following liquid-liquid extractions with dichloromethane.

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16. Microbiome-aware ecotoxicology of organisms: relevance, pitfalls and challenges

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Over the last 15 years, the advent of high-throughput 'omics' techniques has revealed the multiple roles and interactions occurring among hosts, their microbial partners and their environment. This microbiome revolution has radically changed our views of biology, evolution and individuality. Sitting at the interface between a host and its environment, the microbiome is a relevant yet understudied compartment for ecotoxicology research. Various recent works confirm that the microbiome reacts to and interacts with contaminants, with consequences for hosts and ecosystems. In this paper, we thus advocate for the development of a "microbiome-aware ecotoxicology" of organisms. We emphasize its relevance and discuss important conceptual and technical pitfalls associated with study design and interpretation. We identify topics such as functionality, quantification, temporality, resilience, interactions and prediction as major challenges and promising venues for microbiome research applied to ecotoxicology.

17. Gut microbiota and host-related responses of Xenopus laevis tadpoles exposed to nanoparticles

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Engineered nanoparticles (NPs) such as boron nitride nanotubes (BNNT) or graphene family materials like graphene oxide (GO) possess unique properties triggering high expectations for the development of new technological applications in various fields. Boron nitride nanotubes (BNNT) are structurally analogous to carbon nanotubes (CNTs), a carbon-based nanoparticle (CBN), in which boron and nitrogen replace carbon atoms. Such interest for these materials predicts a large scale production in the near future which implies to evaluate the potential consequences of their release in the environment, especially in the aquatic environment in which they are likely to accumulate. However, studies related to the assessment of their ecotoxic potential remain scarce.

Gut microbiota constitutes a compartment of crucial importance in regulation of host homeostasis but most of ecotoxicological studies have neglected its role in toxicity-related responses of host organisms. As CBNs were shown to inhibit larval growth of the amphibian *Xenopus laevis* in a surface-dependant manner [1], this study aims to investigate the consequences of an *in vivo* exposure of *X. laevis* tadpoles to BNNT and GO at 0.1, 1 and 10 mg/L. The monitoring of host physiology and gut bacterial community composition were combined, based on biometric measurements and high-throughput sequencing of 16S rRNA genes, respectively.

Exposure to BNNT led to a significant increase of Xenopus tadpole growth in a dose-dependent manner while growth inhibition was observed in the case of GO exposure. Gut microbiota survey indicated that microbial communities were modified by exposure to both of the tested NPs, leading to a decrease of Shannon index at high BNNT concentration and at low GO concentration. In both cases, the relative abundance of the major phyla was modified after NP exposure leading to a significant change in Firmicutes/Bacteroidetes ratio after exposure to 1 and 10 mg.L-1 of GO. Statistical analysis indicated a strong correlation between host growth-related parameters and the phylum Bacteroidetes.

The obtained results indicate an overall weak toxicity of the tested BNNT compared to GO toward *X*. *laevis* tadpoles. Significant induction of larval growth as well as growth inhibition observed were shown to be correlated with changes in the gut microbial communities of the host. Thus, studying gut microbia composition in response to NPs exposure constitute a good marker of metabolic changes in host.

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Acknowledgement - The authors thank the European Union's Horizon 2020 research and innovation programme under grant agreement No 696656. This research was also supported by the French Ministry of National Education, Higher Education and Research.

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Periphytic biofilms have been used as early-warning signals and as biomarkers of surface water degradation and contamination. Among other environmental stressors, metals and pesticides are known to affect biofilm structure and functions, and their impacts may be exacerbated at higher water temperatures (global warming). In this presentation, results from microcosm exposure experiments conducted at different temperatures will be presented.

The purpose of the first microcosm study was to investigate the influence of temperature on biofilms sensitivity to glyphosate. Periphytic communities were exposed to 100 µg/L of glyphosate at 20°C and 25°C during 22 days. At the start or the experiment and after 7, 14 and 22 days, biofilms were collected from each treatment and both the phototrophic and heterotrophic compartments were characterized with measures of chlorophyll a concentration, microbial ash free dry weight, photosynthetic activity, extracellular enzyme activities of beta-glucosidase, phosphatase and leucine-amino-peptidase. At the end of the experiment, microbial tolerance to glyphosate was evaluated using the PICT approach (Pollution Induced Community Tolerance) in a toxicity test measuring the esterase activity after a 4hexposure to a gradient of glyphosate. Lipids were also extracted from biofilms and fatty acids (FA) were analysed. During the 22 days of exposure, temperature increase had a stronger impact on biological parameters than glyphosate, however glyphosate stimulated microbial activities (respiration and betaglucosidase activity). However, at 20°C, chronic exposure to glyphosate resulted in an increase in community tolerance while at 25°C control biofilms and biofilms chronically exposed to glyphosate had similar tolerance levels. The biofilm response in terms of FA profiles seem to be more sensitive to an increase in temperature than to glyphosate exposure, where the warmer temperature tested resulted in an increase in mono unsaturated FA. All together, the results from this study suggest that an increase in temperature is likely to have a stronger impact on microbial communities than glyphosate exposure, within the range of concentrations and temperatures tested.

The second set of experiments was conducted to evaluate the effects of metal contamination on biofilm taxonomic composition and FA profiles. Experiments were carried out in laboratory microcosms where biofilms were exposed to 5.9 mg/L of nickel, 0.18 μ g/L cadmium or 420 μ g/L of bismuth during independent experiments. The nickel exposure experiment was conducted at 15 and 20°C, while the two other metals were tested only at 20°C. Results showed that both temperature and metal contamination induced a change in biofilm FA profiles.

Both experiments show the importance of environmental factors on biofilm response to contaminants and alert on the potential consequences of climate change on microbial communities already exposed to contamination. It seems also that FA could be used as a supplementary tool to evaluate environmental stressors effects on aquatic ecosystems.

19. Impact of PCBs on the structure of microbial communities

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The commercial production and use of polychlorinated biphenyls (PCBs) began in the 1930s in the context of various applications (electrical insulators, lubricants and fire retardants for electrical equipment, additives in paint, ink, immersion oil, pesticides, etc.). But they were then quickly banned in particular in 1985 by the Member States of the European Union because of their high toxicities. PCBs, recognized as endocrine disruptors and carcinogens, are very persistent aromatic chlorinated chemical compounds currently polluting all types of environment (soil, sediment, water, air). They represent a major ecological problem since approximately 10,000 tons are persistent in environment. In addition, the bio-accumulated PCBs in certain organisms are bio-amplified in the food chain.

Management of these toxic pollutants is still a challenge. Particularly in the face of the very expensive conventional, long and complex elimination techniques (incineration, chemical dechlorination), the more environmentally friendly bioremediation techniques need further study. Since PCBs can be degraded by some soil microorganisms (bacteria and fungi), their biodegradation could generate great interest as an effective and economically cost-reduced means of eliminating these pollutants. In this context, the structure of microbial communities from the 3 life's domains was studied in PCB polluted soil. Heterogeneous PCB contamination levels in the soil made possible to compare the communities present in the polluted areas of the soil *versus* those that are nearly not polluted and allowed the highlighting of microbial taxa potentially PCB adapted and bearing potential functions to possible PCB decontamination that has now to be evaluated.

20. Response of Populus trichocarpa and associated endophytic communities to PAH contamination gradient

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants mainly found in anthropized soils causing environmental and health issues on a global scale. Phytoremediation, using plants and their associated microorganisms, could be a cost-effective and reliable method for soil PAH decontamination. Plants are colonised by diverse microbial communities such as those present in the phyllosphere, the rhizosphere and the endosphere. To date, studies on the dynamic of PAH dissipation in soil-plant system mostly focused on rhizospheric microorganisms. However, it was recently shown that part of the PAH present in contaminated soils could be absorbed by plants and transported in plant tissues. In such context, endophytes could have a major role in the fate of internalized PAH.

As part of the ANR-EndOMiX project, our objective was to study the response of bacterial and fungal endophyte communities and their host (*Populus trichocarpa*) in a gradient of PAH contamination. A multi-contaminated soil, from a former coking plant (experimental site, GISFI) located in Homécourt (54, France) was chosen. It was spiked with various concentrations of phenanthrene, a model 3-cycles PAH. Poplar cuttings were grown for 6 weeks. Metagenomic diversity (16S rDNA and ITS sequencing) of the endophytic communities and plant response at the physiological level (growth, enzymatic activities) were monitored. Moreover, cultivable bacterial and fungal endophytes were isolated and their phenanthrene biodegradation potential as well as their putative PGP properties within the scope of phytoremediation will be explored.

These results will strengthen the understanding of the relationship between plants and their endophytes in the case of PAH soil contamination, how the microbial communities adapt and reveal hints on the function played by the endophytes in this relationship. The identification of endophytes involved in PAH degradation or exerting PGP properties could then provide an interesting tool for microbial assisted plant remediation of PAH.
21. Exposure of Aspergillus flavus NRRL 3357 to Dioxin Results in a Hyper Aflatoxicogenic Phenotype: A Possible Role for Caleosin/Peroxygenase (AfPXG)

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Aflatoxins (AFs) as potent food contaminants are highly detrimental to human and animal health. The production of such biological toxins is influenced by environmental factors including pollutants, such as dioxins. Here, we report the biological feedback of an active AF-producer strain of A. flavus upon in vitro exposure to the most toxic congener of dioxins, the 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (TCDD). The phenotype of TCDD-exposed A. flavus was typified by a severe limitation in vegetative growth, activation of conidia formation and a significant boost in AF production. Furthermore, the level of reactive oxygen species (ROS) in fungal protoplast was increased (3.1- to 3.8-fold) in response to TCDD exposure at 10 and 50 ng mL-1, respectively. In parallel, superoxide dismutase (SOD) and catalase (CAT) activities were, respectively, increased by a factor of 2 and 3. In contrast to controls, transcript, protein and enzymatic activity of caleosin/peroxygenase (AfPXG) was also significantly induced in TCDD-exposed fungi. Subsequently, fungal cells accumulated fivefold more lipid droplets (LDs) than controls. Moreover, the TCDD-exposed fungi exhibited twofold higher levels of AFB1. Interestingly, TCDD-induced hyperaflatoxicogenicity was drastically abolished in the AfPXG-silencing strain of *A. flavus*, suggesting a role for AfPXG in fungal response to TCDD. Finally, TCDD-exposed fungi showed an increased in vitro virulence in terms of sporulation and AF production. The data highlight the possible effects of dioxin on aflatoxicogenicity of A. flavus and suggest therefore that attention should be paid in particular to the potential consequences of climate change on global food safety.

22. Bacterial biotransformation of chlordecone and two degradation products from the In Situ Chemical Reduction process (ISCR)

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Chlordecone (CLD) is a poorly soluble organochloride insecticide intensively applied in the 70s and 80s in soils of the French West Indies to control the black banana weevil. Due to its complex structure (C10Cl10O), this synthetic compound is very persistent in the environment and is presently a public health problem. This has motivated research on (bio)remediation methods to reduce its impact on the environment. In 2016, in laboratory experiments, Mouvet et al. showed that the ISCR (In Situ Chemical Reduction) process leads to the formation of several degradation products and makes it possible to reduce CLD concentrations in Martinique soils by up to 70%. Additional toxicity research has shown that the major metabolites derived from ISCR are neither mutagenic nor genotoxic and have weaker proangiogenic properties than CLD (Legeay et al. 2017). However, to date we have no information on the biodegradability of these metabolites and still little information on the biodegradation of CLD. The objective of this work was first to isolate a bacterial consortia capable of degrading CLD in the absence of oxygen from different environmental sources and then to test the ability of this consortium to degrade two of the major metabolites resulting from ISCR, a mono-hydroCLD (CLD-1Cl) and a tri-hydroCLD (CLD-3Cl).

A microbial consortium, enriched from water treatment plant sludge, was found to be capable of biotransforming 2 mg.L-1 of CLD in a few weeks with a simultaneous production of a 9-carbon compound (C9Cl5H3) with a pentachloroindene structure, identified as the same as that recently described by Chevallier et al. (2018). This consortium also transformed the two CLD metabolites tested. The GC-MS analysis of these three biotransformations shows the unexpected formation of molecules with the same number of C and Cl as the parent molecules but functionalized, which opens up new prospects for degradation. This is the first time that a biotransformation of metabolites from the ISCR process has been studied. The biotransformation kinetics of CLD, CLD-1Cl and CLD-3Cl as well as the molecules newly formed by this new consortium will be presented and discussed in connection with the evolution of its diversity. The identification of the bacterial genera comprising this consortium by metagenomic sequencing (Illumina MySeq) suggests the involvement of bacteria other than the genus *Citrobacter* whose ability to degrade CLD has previously been demonstrated (Chaussonnerie et al., 2016).

The discovery of microorganisms naturally present in the environment capable of degrading CLD opens up interesting perspectives, which could lead, in the long term, to the implementation of bioremediation processes for this pollution.

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23. Impact of natural radioactivity on microorganism communities in different mineral springs in Auvergne (Massif Central, France)

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Radioactivity is a natural phenomenon present on earth since its formation and which could be at the origin of the emergence of life. Several environments are characterized by naturally high levels of radiation. Mineral waters, like groundwater, contain naturally occurring radioactive elements whose concentrations vary according to many parameters, including the geological nature of the land. Over thousands of years, microorganisms have developed various strategies to respond to the constraints induced by this ionizing radiation. Naturally radioactive mineral springs are therefore ecosystems where ionizing radiation could constitute an abiotic driver impacting the diversity and structure of microbial communities.

The TIRAMISU collaboration, within the Zone Atelier Territoires Uranifères (ZATU), is studying a diverse range of radioactive mineral springs in the Massif Central to characterize microbial biodiversity and the adaptations developed by microorganisms in these environments defined by natural, chronic and ancient radioactivity. The Massif Central is a region made up of uranium-rich geological formations (granite massif), well known for its naturally radioactive mineral sources. The objective of this collaboration is thus to determine the impact of radioactivity on different microbial communities (bacteria, diatoms, fungi, archaea), that have likely evolved to these environments for very long periods of time. While diatoms have been observed to undergo deformation due to the radioactivity present in these sources [1], much work is needed to further characterise these interactions. Moreover, no work to date has focused on bacterial, archaeal and fungal communities at these sites and clearly warrants investigation. Through a multi-disciplinary approach of microbial community analysis, physico-chemical, radiological analyses and corresponding simulations of this site will be used to elucidate the impact and mechanisms of radionuclide toxicity and irradiation on the microorganisms at this site.

In this study, water samples from six naturally radioactive mineral springs of varying radioelement concentration gradient (<3.71 ppb U) in the Massif Central were taken in triplicate. A culture on non-selective medium (TBE) was carried out in order to obtain a wide range of bacteria present in our samples. A collection of aerobically cultivable bacterial isolates was thus constituted and characterized via 16S RNA gene Sanger sequencing. Subsequent novel isolates and relatives to radiotolerant strains were subsequently selected for future metabolic, genetic and toxicity experiments. These preliminary results will be discussed in the context of understanding the evolution, functionality and survival of microbial communities and their interactions with radionuclides in naturally radioactive sources.

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24. Array of microbial indicators, a promise for a better monitoring of pesticide effects on stream biological quality

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Freshwater contamination by pesticide residues is a major and growing threat to aquatic communities, ecosystem functioning and ultimately human health worldwide. Typical pesticide contamination in agricultural landscapes is characterized by a cocktail of a large number of active compounds and their main transformation products, each of them found at very low and temporally fluctuating concentrations. This makes the quantification of pesticide residues in streams highly challenging and costly by means of grab chemical sampling. Accordingly, it makes also difficult to characterize the chronic exposure of aquatic communities in pesticide-contaminated streams and the resulting ecological effects on community structure and functions. During the last decade, the development and implementation of pesticide integrative samplers has allowed to improve the monitoring of the chemical quality through the determination of time-weighted average concentrations over an exposure period, leading to a better representativeness of measurements. However, scientists and regulators are still facing the challenge of going beyond the estimation of pesticide concentrations to take into account the ecological effects on exposed aquatic communities in the evaluation of the ecological status in the particular context of pesticide contamination.

To overcome these limitations, microbial communities can be viewed as potential bioindicators that may provide a broad array of structural and functional metrics to diagnose the effects of stream contamination by pesticide residues, and constitute an innovative toolbox to monitor the effect of pesticide residues on stream biological quality. In this study, we evaluated 13 structural and functional metrics on microbial communities from 10 streams that belonged to 3 catchments, and repeated the measurements at 2 seasons in 4 out of the 10 streams. Streams were selected in different agrosystems to be representative of different scenarios of pesticide contamination gradient, as evidenced by pesticide quantification using composite silicone rubber and POCIS integrative chemical samplers (66 molecules targeted). Our metrics were measured on microbial communities associated with sediment (3), periphyton (2) or benthic particulate organic matter (8) and spanned a broad range of microorganism types (bacteria, fungi, algae). They included microbial processes (organic matter decomposition, degradation of targeted pesticides, fungal reproduction rates and enzymatic activities), their resilience to pesticide exposure (Pollution Induced Community Tolerance), as well as community features (fungal, bacterial and algal community structure, fungal biomass, abundance of pesticide degrading micro-organisms). Based on our results and on literature data, we identify and discuss the respective objectives, advantages, disadvantages and knowledge gaps associated with each indicator in the context of estimating the effect of pesticide contamination on the biological quality of streams. Finally, based on a survey of stream and water resource managers, we conclude on their possible applicability and interest for end-users.

25. Deep exploration of the microbial community in sediments from the Seine river basin by combining metagenomics & metaproteomics

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Metagenomics and metaproteomics bring a new dimension to the study of soil microbial communities at a higher level by providing a global outlook on the living organisms in terms of biomass, taxonomic and functional scope. Metaproteomics consist in the analysis of the whole protein content of a sample by using ultra-fast mass spectrometry technology and querying a protein sequence database derived from the metagenomics data. Identifying and quantifying thousands of proteins in an environmental sample give access to the detailed functional analysis of its microbial community. This information can be correlated with the presence of contaminants such as trace metals, persistent organic pollutants, antibiotics, and endocrine disruptors. For example, the *Sphingomonas wittichii* species was identified in a sediment deposit downstream of the Seine basin and may be linked to the presence of dioxins which present-day concentration is not quantifiable, this species having an appropriate enzymatic arsenal to degrade weakly chlorinated dioxins. Combining functional and taxonomical results with geochemical data informs the dynamics of microbial communities in the soil column in terms of enzymatic activities, which reflects the complex anthropogenic impacts. Complex and heterogeneous data such those produced in the course of soil multi-omics and geochemical studies need ambitious analysis development and optimization.

In this work, we optimized the process for interpreting the data for soil samples and analyzed a sediment core from an anthropized floodplain along the Seine River downstream of Paris (France). The sediment core is used as a sedimentary archive with an age-depth model reflecting a posteriori water multi-contamination historical trends, sampled in a site where the sedimentation is homogeneous and undisturbed. Soils are the most difficult samples for meta-omics because most organisms are uncultivated or unsequenced, and consequently protein sequence databases are far from being comprehensive enough. We have shown that innovative bioinformatics cascaded strategies where different types of databases are merged can maximize the interpretation of the omics data. The analysis of the identified microorganisms present in the soil core and their functioning metabolic pathways assessed by the abundances of proteins highlights the microbial complexity and the soil potential in terms of pollutant catabolism. The results interpretation benefits from an impressive corpus of geochemical data acquired on the sediment core such as profiles of concentrations of different pollutants.

Based on this improved methodology and these results, we proposed the alliance of geochemistry and omics as a new environmental diagnostic tool.

26. 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" and has been included in the European Commission's list of priority substances for European freshwater resource (2000/60/EC, 2001) and in the U.S. Contaminant candidate List 4 (Environmental Protection Agency, 2015). At concentration levels of a few µg L-1, diuron is toxic to non-target organisms in the environment and increases the risks for humans by causing endocrine, reproductive and immune disturbances. This plant phytosanitary product, banned in France in 2002, is still used as a biocidal products (in paintings for instance) and released into the environment. In France, data on the use of diuron as a biocide is not documented. However, studies have shown that the tonnages of biocidal uses in 2014 were higher (186 tons) than those of plant phytosanitary products (143 tons) in 2008 (Cerema, 2017). In particular, diuron was detected in 6 of 24 urban WWTPs studied according to the report of the French Ministry for Ecology, Sustainable Development and Energy.

In soil, biodegradation appeared to be the major cause of diuron degradation. The biodegradation pathway for diuron is well established. It processed by successive demethylation steps to form DCPMU (1-3,4-dichlorophenyl) -3-methylurea), DCPU (1-3,4-dichlorophenylurea) and DCA (3,4-dichloroaniline) which exhibit a higher toxicity than diuron. However, in WWTPs, the diuron removal efficiencies are very variable and even negative up to -70%. To date, these large variations in diuron concentration in WWTP effluents remain unexplained. In addition, the negative removal rates of diuron observed even in dry periods suggest that in some cases, the concentrations of this micropollutant in the treated effuents are higher than those in 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.

The present study aims to test the hypothesis that an additional quantity of diuron is produced during wastewater biological treatments from the transformation of compounds other than 3,4-dichloroaniline such as 3-(4-Chlorophenyl)-1-(3,4-dichlorophenyl)-urea and/or from structural analogues of 1,2-Dichloro-4 -nitrobenzene. These compounds should be identified and quantified in wastewater and their fate during wastewater treatment process followed.

The second hypothesis concerns the processes of degradation of diuron by microorganisms. Degradation of diuron to 3,4-dichloroaniline under aerobic conditions has been established by N-demethylation reactions which is catalyzed by cytochrome P450 mono-oxygenase. A selection of bacterial species which are capable of degrading and transforming diuron will be carried out.

Results of this study could help us better understand the factors governing the degradation of diuron in the biological treatments in WWTPs.

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27. Exploring the potential of biobeds to treat the pesticide-contaminated effluents from various agro-food industries: insights into the role of microbiome and plasmidome

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Agro-food industries produce large amounts of pesticide-contaminated effluents, which constitute significant point sources for the contamination of natural water resources. Despite relevant regulation being in place, little is known about the treatment of these effluents. Biodepuration systems like biobeds could be a possible solution. We employed a leaching column study to investigate the potential of biobeds, packed with a biomixture composed of spent mushroom substrate (SMS), straw and soil (50:25:25 by volume), to depurate pesticide-contaminated effluents from (I) seed-coating (carboxin, metalaxyl-M, fluxapyroxad), (II) bulb-disinfestation (thiabendazole, fludioxonil and chlorothalonil) and (III) fruit-packaging (fludioxonil, imazalil) industries. Pesticides were effectively retained by the biomixture with 1.65 - 6.83%, 0.02 - 0.40% and 0.03 - 2.35% of the total amount of pesticides applied detected in the leachates of the columns receiving effluents from seed-coating, bulb-dipping and fruit-packaging industries, respectively. Fluxapyroxad and imazalil were mostly retained by the biomixture (88.7 and 78.4 % respectively). Fludioxonil showed a similar pattern and it was retained to levels of 82.8 and 44.1% when applied as seed-coating effluents and fruit-packaging effluents, respectively. All pesticides were mainly retained at the upper layer of the columns (0 - 20)cm) except of carboxin and metalaxyl-M which diffused to deeper layers (20-80 cm). Carboxin, metalaxyl-M, thiabendazole and chlorothalonil were efficiently dissipated by the biobeds (86.5, 92.6, 73.9 and 88.7% respectively). Triplicate columns bioaugmented with tailored made bacterial (Ochrobactrum sp.,) and fungal (Mycosphaerella tassiana) inocula able to rapidly degrade two of the most persistent pesticides, fludioxonil and imazalil, respectively showed enhanced pesticide dissipation. Samples from all treated columns (at three different layers), including triplicate columns fed with tap water without pesticides, were collected along the study to study the response of the soil microbial community to this heavy pesticide exposure using amplicon sequencing and the role of plasmidome on microbial response via q-PCR. Overall, our observations suggest that biobeds could efficiently depurate effluent from different agro- food processing industries and the dissipation processes prevailing vary based on the environmental fate properties of the pesticides contained in the effluents.

Acknowledgments:

PK is supported by the project MINOTAUR (MIS 5030360, project code: T1EDK-02566) co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH – CREATE – INNOVATE

28. Variability in microbial diversity during harmful algal blooms in coastal Tunisian waters

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Harmful Algal Blooms (HABs) can negatively impact aquatic ecosystems and associated economical activities, as well as human health. Most toxic HAB taxa belong to the dinoflagellates, including species of the genera *Alexandrium* and *Gymnodinium*, which proliferate recurrently in various marine areas around the world by forming large blooms in coastal waters. A major scientific effort has been deployed to identify the factors explaining these proliferations, however few studies have evaluated the microbial community diversity associated with HABs, despite their major role in the functioning of marine ecosystems particularly in the southern Mediterranean Sea and their presumed role in the HABs bloom control.

The aim of the study was to investigate the diversity of marine prokaryotes during dinoflagellate bloom events caused by *Gymnodinium impudicum* and *Alexandrium minutum* at northern (Carthage, Gulf of Tunis) and southern (Sfax-Sidi Mansour, Gulf of Gabès) Tunisia, during the summer 2019. Surface coastal waters were sampled consecutively for 4 days during the bloom peak and decline phases.

By combining microscopic observations and 16S rRNA gene sequencing (Illumina MiSeq), the variability of prokaryotic community composition was investigated in relation to bloom-forming species and environmental variables (temperature and nutrients).

The results showed that the abundance of microphytoplankton (including dinoflagellates) in Carthage (impacted by *Gymnodinium impudicum*) was higher than that observed at Sfax (impacted by *Alexandrium* minutum). During the dinoflagellate bloom monitoring, the prokaryotic communities were mainly dominated by archaeal MGII group, Pelagibacterales, Rhodobacterales and Flavobacteriales at Carthage, while Archaea and Pelagibacterales were found in low proportion at Sfax, with relatively stable contents of Rhodobacterales, and Flavobacteriales. At Sfax, the prokaryotic communities were largely dominated by Chromatiales at the *Alexandrium* peak, then reached a maximum (representing the half of prokaryotes) the next day, before decreasing to represent only 2-3% of the community at the end of the survey. This decrease was accompanied by an increase in Actinomycetales. These results indicated a shift in prokaryotic community diversity during dinoflagellate blooms, suggesting different ability of bacterial taxa to utilize organic matter from dinoflagellates.

29. Effect of glyphosate application on microbial community dynamics of four German agricultural soils

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Glyphosate is the world's most important herbicide. Despite its widespread and heavy use in agriculture and forestry, the fate of glyphosate in the environment, its microbial degradation processes and the impact upon soil ecosystems are still not fully understood.

In this study, a controlled microcosm experiment was carried out to determine the effect of glyphosate application on the microbial community structure and size of four different soils from the Ammer valley in Baden-Württemberg, Germany. A single glyphosate application (15 mg/kg) was added to soil microcosms from four different field sites which were incubated in the dark at 60% water-filled pore space and 20°C for 56 days. Microcosms without glyphosate application were also set up as controls. Soil samples were taken on the day of glyphosate addition (Day 0) and after 7, 28 and 56 days. Capillary electrophoresis-mass spectrometry was used to quantify glyphosate and its major transformation product aminomethylphosphonic acid (AMPA). Changes in the microbial community structure and size were evaluated using 16S rRNA gene amplicon sequencing and qPCR.

Results show that even though glyphosate was rapidly degraded in the four soils, the impact of glyphosate application on the microbial community structure and size (gene copy number) was small. Only minor changes in composition and diversity could be detected with our approach, suggesting glyphosate degradation in soil might be a concerted effort by a wide bacterial network or could be occurring co-metabolically without microbial growth. Additionally, using batch experiments, glyphosate adsorption isotherms were determined for two of these soils, suggesting low concentrations in the aqueous solution throughout the experiment. The sorption/desorption dynamics of glyphosate with the soil matrix heavily influences its bioavailability through time, which may explain the minor effects observed on the microbial community. Future efforts will focus on detecting the activity of glyphosate degradation genes and pathways through the use of RNA-based omics techniques in similar microcosm setups.

30. Microbial diversity and pathways mediating mercury transformations in Antarctic aquatic ecosystems

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Neurotoxin methylmercury has the feature, in addition to its high toxicity for living organisms, to be easily incorporated, bioaccumulated and biomagnified through the aquatic food web. Antarctic regions receive atmospheric mercury mainly through long-range transport from other continents. Microorganisms (Archaea and Bacteria) are implicated in the transformation of mercury to methylmercury and their activity deeply impact the methylmercury availability for the whole food web. In a context of increasing releases of heavy metals to aquatic environments and the atmosphere, it is crucial to elucidate the fate of mercury in Antarctic aquatic ecosystems from microbial point of view. We investigated the microbial diversity in pristine Antarctic lakes (South Shetland Islands, Antarctic, Chile) where benthic total mercury concentration was around 14 ppb. Up to 6.3% of the active community was constituted by putative methylators and a positive significant correlation was found between total mercury concentration and putative methylating microorganism relative abundance. Putative methylating Archaea Methanoregula and Methanosphaerula have been detected but did not seem active in the studied ecosystems (RNA metabarcoding VS DNA metabarcoding). By combining molecular data and a cutting-edge mercury-isotope based approach, mercury methylation was found to outweigh methylmercury demethylation rates. Metagenomic data will allow to better decipher the mercury-related pathways in Antarctic lakes. This investigation represents the first attempt to disclose the implication of microorganisms in the cycle and bioavailability of mercury through both activity and molecular approaches in Antarctic aquatic systems.

31. Chronic-exposure of river sediment microbial communities to environmental concentrations of copper induces diversity changes and tolerance acquisition without increasing sensitivity to arsenic

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Many metals accumulate in the benthic compartment of aquatic ecosystems but, to date, little is known about the response of natural microbial sediment communities following metal exposure. In sediments, microorganisms are highly abundant and contribute to many ecological functions. Using copper (Cu) as a model for metal contamination, a microcosm experiment was carried out using natural surface sediment from the Ain River (France). It aimed to characterize the structural and functional response and adaptation mechanisms of microbial communities exposed for 21 days to a realistic Cu concentration (~50 mg Cu kg-1 dry sediment). The resulting effects on microbial sensitivity to acute Cu and Arsenic (As) toxicity was characterized to gain knowledge on potential positive or negative cross-tolerance processes.

Chronic Cu exposure of the bacterial and archaeal communities led to rapid and significant effects that persisted from Day 0 to Day 21 and resulted in both diversity (richness and equitability) decrease and community restructuring. A statistical analysis at the taxa level identified the main microbial groups responsible for the observed community structure changes, including taxa that were negatively affected (potentially sensitive) and favoured by Cu exposure. To understand the consequences of these changes on the Cu tolerance capacity at the community level, a PICT (Pollution Induced Community Tolerance) approach was implemented using acute toxicity tests on β -glucosidase and phosphatase enzymatic activities. The PICT concept predicts that loss of the most sensitive taxa and gain of more tolerant ones should increase the capacity of Cu-exposed communities to tolerate acute Cu toxicity. The observed increase of EC50 values for Cu-exposed communities confirmed that changes in the structure and composition increased community tolerance to the acute toxicity of this metal. The absolute quantification of cusA, which encodes a Cu membrane transporter efflux pump belonging to the resistance-nodulation-division (RND) protein family, did not reveal any abundance difference. However, when considering the ratio of the number of *cusA* copies to the number of 16S rRNA gene copies, an increasing trend was observed from Day 7 to Day 21 in chronically Cu-exposed communities. It suggests that some of the microorganisms that were more abundant under Cu exposure harbored this gene, lending them a greater Cu tolerance ability. Although diversity loss and functional costs of adaptation could have increased the community sensitivity to subsequent toxic stress, no increased sensitivity to As acute toxicity was observed following Cu-exposure. This suggests that induced tolerance to Cu and As was supported by different species with different metabolic capacities. These results, together with previous ones demonstrating that Cu-exposure significantly impacted the functional potential of benthic microbial communities, underline that metal exposure could significantly impact the overall functioning of aquatic ecosystems. Our findings also confirm that laboratory tools such as the PICT approach can serve to assess ecotoxicological impacts in sediment communities and reveal underlying negative effects such as loss of biodiversity and functional impairment.

32. Response of river biofilms to herbicide contamination: taxonomic and functional changes and their implications for nutritional quality

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Agricultural practices can lead to pesticide contamination of surface waters. In the Province of Quebec (Canada), corn and soybean crops contribute to the massive use of herbicides, including atrazine, which has been banned in Europe since 2003 but is still used in North America. Several herbicides, such as glyphosate, atrazine and S-metolachlor, are frequently detected in Quebec watercourses. These organic compounds have the potential to affect living organisms by altering various metabolic functions. In particular, they can affect the functioning of periphytic biofilms that are at the base of aquatic food webs, possibly disturbing the entire food chain.

The aim of this PhD project is to study the effects of herbicides, alone and in mixtures, on the taxonomic and functional composition of the microorganisms composing fluvial biofilms as well on their nutritional quality for primary consumers feeding on them. Experiments under controlled conditions will be carried out where changes in the microorganism assemblages of the biofilms will be monitored (proportions of the major algal groups, diatom assemblages, micromeiofauna, bacterial communities) and fatty acids (FAs) will be measured. FAs are key nutritional components and will be used as biomarkers of stress and as proxies of biofilm nutritional quality. Changes in FA profiles may reflect (1) a change in the microorganisms composing the biofilms (eg., replacement of species rich in essential polyunsaturated fatty acids (PUFAs) by others that are PUFA-poor), (2) a direct alteration in FA metabolism or (3) a combination of these two responses. Oxidative stress-related enzymes will also be monitored under the different exposure conditions. Given their fundamental role in carbon fluxes and energy in aquatic systems, the composition of bacterial communities will be investigated using 16S rRNA gene sequencing, while functions will be examined based on carbon source utilization abilities (BIOLOG). Biofilms collected from the experiments under herbicide contamination will also be used to feed *Gammarus sp* (scuds) with the purpose of studying the response of primary consumers (growth, FA profiles, bioaccumulated herbicides) to herbicides in the water and in their diet.

As a complement to exposure experiments under controlled conditions, a field study will be conducted in agricultural regions of southern Quebec. Biofilms will be collected in several watercourses known for their elevated concentrations in herbicides and will be compared with biofilms collected in other agricultural areas characterized by lower herbicide contamination. Several biological descriptors will be investigated such as biofilm FA profiles, and both algal and bacterial taxonomic composition.

This poster is mainly intended at presenting the general objectives of the PhD project. Preliminary results will also be presented.

<mark>33.</mark> The unexpected role of bioaerosols in the Oxidative Potential of atmospheric Particulate Matter (PM)

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Atmospheric particulate-matter (PM) is defined as a complex and dynamic mixture of particles from both chemical and biological origins. Exposure to PM has been linked with a wide range of deleterious health effects in both humans and animals. These include among others, cardiopulmonary disease, lung cancer, or asthma. One underlying mechanism explaining many health effects of PM is its capacity to carry out or catalyze the formation of reactive-oxygen-species (ROS) within lung cells, responsible for further oxidative stress and airway inflammation. This intrinsic property of PM called oxidative potential (OP) is easily revealed in biochemical and acellular assays by monitoring anti-oxidants depletion when in contact with PM. Bioaerosols represent up to 15-25% of PM by mass, but there is currently no assessment of their impact on PM Oxidative Potential (OP), or capacity of particulate matter (PM) to produce damaging oxidative reactions in the human lungs. Here, the OP of selected bioaerosols (bacteria cells *vs* fungal spores) was assessed through the cell-free DTT assay.

Selection of model bioaerosols: Some common atmospheric bacterial and fungal species have been selected. For bacteria, two Gram-positive cocci (*Staphylococcus epidermidis* and *Micrococcus sp.*) and one Gram-negative rod (*Pseudomonas fluorescens*) were selected. Concerning fungi, spores of *Stachybotrys chartarum* and *Penicillium sp.* and of *Aspergillus brasiliensis* and *Aspergillus fumigatus* were used. These fungal species are commonly used in exposure models because of their frequent occurrence in indoor air contamination. Commercial reference strains (CIP69.13T, ATCC14990 and ATCC16404, respectively) were used for *P. fluorescens, S. epidermidis* and *A. brasiliensis.* The other species were isolated from urban and natural environments: indoor for *Micrococcus sp., S. chartarum* and *Penicillium sp.*, and outdoor for *A. fumigatus*.

The DTT assay for OP measurement: The dithiothreitol (DTT) assay is a cell-free method commonly used to assess the oxidative potential of airborne redox-active chemical compounds. Briefly, DTT consumption is continuously monitored when in contact with PM, and the depletion of DTT (in excess) is proportional to the concentration of redox-active species present in PM. The protocol established by Charrier and Anastasio, (2012) was adapted to fit our constraints, and significant changes were brought in the sample pretreatment step.

Results showed that bioaerosols induce Reactive-Oxygen-Species (ROS) production, varying along with the microorganism type, species, and concentration. Fungal spores show up to 10 times more ROS generation than bacterial cells. At the highest concentrations, fungal spores present as much oxidative reactivity as the most redox-active airborne chemicals (Copper, Naphtoquinone). Moreover, bioaerosols substantially influence the intrinsic OP of ambient PM and that of its chemical constituents: in presence of *A. fumigatus* spores, the OP of Cu/NQ is increased by a factor of 2 to 5, whereas, 104 and 105 *S. epidermidis* bacterial cells.mL-1 halve the OP of Cu/NQ. Finally, viable and gamma-rays-killed model bioaerosols present similar oxidative reactivity, suggesting a metabolism-independent cellular mechanism. These results reveal the importance of bioaerosols for PM reactivity. PM toxicity can be modified due to bioaerosols contribution or by their ability to modulate the OP of toxic chemicals present in PM.

34. Preservation of microbial consortia: impact of storage at 4°C and -80°C on an As(III)oxidizing community

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The use of mixed microbial communities in biotechnologies offers many advantages compared to single/pure strains (Brenner et al., 2008):

- They can face environmental changes in an easier manner than monocultures can do.

- They can perform complicated tasks/functions such as that requiring multiple steps, which can be difficult or even impossible for many individual strains.

- Microbial consortia represent a source of genes and functions, as well as resources for future and novel biotechnological applications as they involve non-cultivable and/or uncharacterized microorganisms.

- Microbial communities are naturally mixed in the environment and consortia are thus more representative of the "real" life.

However, the preservation, especially over the long-term, of stable microbial consortia is still underlined as a challenge. It has become a necessity to safeguard the accessibility to microorganisms already recognized to have interesting features and to be a pool of microbial resources. Several factors are known to be critical for a good storage, such as cell size and type, temperature, amount of cells, physiological state, the use of a cryoprotectant for cryoconservation and lyoprotectants for lyophilisation, the period of time of cryoprotectant/cells contact before cooling, the rates of cooling and thawing... These parameters have been identified as critical for the storage of pure microbial strains but little is known on how to preserve microbial consortia.

Here, the impact of two ways of storage widely used in laboratory, cryoconservation at -80°C in 20% glycerol and 4°C storage, was studied on a microbial community selected for its ability to oxidize As(III) into As(V), an important function in the frame of As bioremediation purposes. The community was characterized before and after storage, over a period of up to 12 months, focusing on the following parameters: viability (Live and Dead labelling approach), cell growth (microscopic counts), activity (As(III) oxidation and carbon sources utilization Biolog profile), and diversity fingerprints. Results showed no impact of both ways of storage on As(III) oxidation ability and cell growth, but storage led to an impact on cells viability. An increase of biodiversity after storage at -80°C was observed, probably due to the presence of glycerol that may have allowed the growth of strains that were in minority before storage. On the opposite, storage at -4°C tended to decrease biodiversity. Results also showed an evolution in carbon source utilization that varied according to the temperature and the time of storage. In conclusion, the function of interest, As(III) oxidation, was well preserved whatever storage temperature and duration, but microbial diversity and viability were impacted, even during short term storage.

Brenner K, You L, Arnold F (2008) Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol 26: 483-489.

35. Nematodes trophic groups changing via reducing of bacterial population density after sediment enrichment to ciprofloxacin antibiotic: Case study of Marine Mediterranean community

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diversity.

An experiment was carried out using microcosms to evaluate the impact of the fluoroquinolone antibiotic on nematode trophic groups structure and bacterial abundance. Sediment samples were experimentally enriched with four increasing doses of ciprofloxacin [D1 (50 ppm), D2 (100 ppm), D3 (200 ppm) and D4 (500 ppm)] and compared to non-enriched sediments (used as control). Ciprofloxacin changed the trophic composition of nematodes taxa where the relative abundance of microvores (M), epigrowth feeders (EF) and ciliate consumers (CF), raised in a control microcosm, was highly affected and significantly decreased in response to the increasing doses. Nevertheless, the abundance of deposit feeders (DF), optional predators (FP) and exclusive predators (Pr) showed a significant increase. Results from the multivariate analysis showed a clear impact of this antibiotic on nematode trophic assemblages. Microcosms treated with the three highest doses [D2, D3 and D4] were different from the control. The exceptions were those treated with the lowest dose, D1, and which were grouped with the control. The SIMPER analysis results showed that the average dissimilarity continuously increased in the treated microcosms compared to the control. Furthermore, our results have shown that ciprofloxacin also leads to a significant decrease in bacterial density with the highest dose, which could explain the results obtained for nematode trophic groups distribution. Thus, the bacteriophages nematodes only use bacteria as a nutrition source and the lack or presence in small quantity of this food could induce a decrease in their abundance as well as changing of nematodes repartition. groups Our work demonstrates that the nematode responses were dependent on sediment enrichment with ciprofloxacin and opens new perspectives on the potential impact of antibiotics on functional traits

36. Archaea and Bacteria evolve differently in response to a Mediterranean extreme storm event

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Urban rivers are mainly influenced by the terrestrial environment including land-use activities. Pollution in surface waters occurs regularly via urban sewage and runoff, which are more intense during storm events. Rain events are particularly intense and frequent in Mediterranean climate regions and will become more pronounced as a result of global changes. Floods and Combined Sewer Overflows (CSOs) occur very often under this climate regime and lead to large loads of pollutant mixtures reaching surface waters through river sediments and in-sewer solids resuspension, which indicates the major role of suspended particles in freshwater quality. We have previously demonstrated that the particle-attached bacteriome responded significantly to multipollution phenomena derived from CSOs and floods. Resident riverine communities shifted in an urban-associated community whose key players were pollutant-resistant and/or pathogenic bacteria. Contrarily to Bacteria, the diversity and importance of Archaea remain poorly understood and no environmental studies deal with xenobiotics impact on this domain.

We aimed to provide a first insight on riverine particle-attached archaeal communities shifts following an extreme storm that lead to multipollution events into a typical coastal Mediterranean river.

We have been able to identify a significant relationship between the *in situ* fluviatile archaeome, the river hydrodynamics and the major changes of physicochemical parameters, including several families of contaminants (faecal indicators, trace metals, pesticides and pharmaceuticals) and nutrients, that occurred during CSOs and the flood. A comparison with the bacteriome demonstrated a different response between both domains. The fluviatile archaeome showed a specific community in summer as well as a later response to multipollution events. Bacterial communities were more diverse and structured stronger in response to multipollution events during the storm than Archaea as well as than to environmental changes according to seasons. As for Bacteria, we found several urban-specific archaeal taxa significantly related to multipollution events at the beginning of the event, during first flushes. These taxa were derived from in-sewer solids and soil/sediments resuspension via CSOs and runoff, respectively. Other Archaea never identified as urban-taxa appeared at this moment as well. Archaeal dynamics were significantly correlated to highly pollutant-resistant and/or pathogenic bacteria that emerged at this moment. At the highest water discharge, however, Archaea related to the nitrogen cycle were identified while Bacteria originated from terrestrial environments but also from wastewater still appeared at this moment.

To conclude, Archaea as well as Bacteria taxa linked to pollutant mixtures could provide excellent means of assessing water quality in view of developing new bioindicators for rapid assessment of environmental risk in aquatic ecosystems. We further discuss risks to public health and ecosystems in this scenario of continuous disturbance of river resident microbial communities subjected to multipollution phenomena associated to CSOs and floods and highlight why urges improving certain urban management practices.

37. Removal of microbial communities (Archaea and Bacteria) from wastewaters by photooxidation on TiO2 under controlled radiation

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State of the art

Given the current context of water scarcity, wastewater treatment is a priority, especially for public health. Used waters are only partially treated in wastewater treatment plants (WWTPs) which reach 95% purification rates. Consequently, when wastewaters are released in the environment they contain microorganisms and abiotic contaminants that are not degradable by biological processes. New technologies, known as tertiary or quaternary treatments in WWTPs, have emerged. They are based on the production of radicals capable of non-selectively degrading carbonaceous contaminants. The scientific community is mainly focusing on one of these advanced oxidation processes, solar heterogeneous photo oxidation. This process is based on the photoexcitation of solid photocatalysts, thus capable of producing radicals. This sustainable technology, which has been the subject of numerous scientific studies, ensures the degradation of abiotic but also microbiological contaminants. Studies show its ability to disinfect the many faecal indicators in wastewater.

Aims and Scope

We aimed at defining the operating conditions to ensure the best microbial degradation by photo oxidation with a solid photocatalyst in powder form: titanium dioxide (TiO2). Several levels of treatment were applied to wastewater collected from WWTPs in order to compare their impact on the microbial communities. The first variable was the proton flux density: 10 and 50 w.m-2. The second was the presence or absence of TiO2. The originality was to analyse the evolution of two microbial compartments (Bacteria and Archaea) during the oxidative treatment using metabarcoding sequencing of the 16S rRNA gene.

Results and Discussion

A significative reduction in the number of bacterial reads and therefore on the alpha diversity was obtained at the end of photocatalysis treatment, particularly with the strongest method (50w.m-2 and TiO2), but not in the absence of TiO2. Regarding Archaea, all treatments had low impact on the number of reads at the end of all treatments, with no significant alpha diversity measurements between the beginning and the end of the experiments except Chao1 estimator and for photocatalysis treatments only. Beta diversity analysis demonstrated significantly different community structures at the end of all treatments indicating a shift in a different assemblage throughout the experiments. Changes in taxonomic composition were observed at the end of all treatments for both Bacteria and Archaea, but changes were more drastic under the strongest treatment. Bacterial communities were affected along treatments in a random way, with some phyla increasing with one treatment and decreasing with another treatment. Only Firmicutes and Planctomycetes phyla augmented while Patescibacteria decreased with the three treatments. Concerning archaeal domain, only two major phyla were represented from the beginning of the experiment and the response was a decrease of the Nanoarchaeota at the expense of the Euryarchaeota for all treatments. In all cases, highly resistant microorganisms remained at the end of all treatments, such as Arcobacter sp. or Microthrix sp. for Bacteria or Methanobrevibacter sp. for Archaea. In perspectives, it would be interesting to directly study the evolution of resistance genes along the different treatments.

38. Comparison of the in vitro activity of novel and established nitrification inhibitors used in agriculture on soil ammonia- and nitrite-oxidizers: working out more effective nitrification inhibition strategies

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Nitrification inhibitors (NIs) applied in soil reduce nitrogen fertilizer losses from agricultural ecosystems. Currently available NIs appear to selectively inhibit ammonia-oxidizing bacteria (AOB), while their impact on other groups of nitrifiers is limited or unknown. Previous soil microcosm studies showed that ethoxyquin (EQ), an antioxidant used as a preservative in fruit packaging plants, is rapidly transformed to quinone imine (QI) and 2,4-dimethyl-6-ethoxyquinoline, and suggested a strong inhibitory effect on ammonia-oxidizers. We aimed to define *in vitro* the spectrum of activity and the inhibition thresholds of EQ and its derivatives, compared with other NIs (dicyandiamide (DCD), nitrapyrin (NP), 3,4-dimethylpyrazole-phosphate (DMPP)) that are widely applied in an agricultural setting. The activity and growth of five soil-derived isolates (two AOB (Nitrosomonas europaea, Nitrosospira multiformis), two ammonia-oxidizing archaea (AOA) ("Candidatus Nitrosocosmicus franklandus", "Candidatus Nitrosotalea sinensis"), and one nitrite-oxidizing bacterium (NOB) (*Nitrobacter* sp. NHB1)) were monitored in liquid cultures, via nitrite production or consumption and qPCR of phylogenetic markers, respectively, for a broad range of NIs concentrations. To establish possible correlations between NIs presence and inhibition, their degradation/transformation in the liquid cultures was also determined via HPLC. With the exception of NP, a differential sensitivity of the key nitrification contributors to NIs, was shown. DCD and DMPP, the only NIs currently registered for use in Europe, selectively inhibited AOB, suggesting a suboptimal efficiency of these compounds. In contrast, EQ, and primarily its major transformation product QI, showed high potency against AOA. NP, EQ, and its derivatives were also active against NOB, in contrast to DCD and DMPP. Our findings provide benchmarking knowledge on the activity range of known and novel NIs with practical implications for their use and the development of novel NIs with broad or complementary activity against all ammonia-oxidizers.

Acknowledgments

This work is part of the project "NITRIC – Looking up for **N**ovel n**ITR**ification **I**nhibitors: New stories with old **C**ompounds" which has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No. 1229.

39. In soil assessment of the efficacy and off-target microbial toxicity of quinone imine and other established nitrification inhibitors used in agriculture

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Nitrification inhibitors (NIs) minimize nitrogen fertilizer losses from agricultural ecosystems by reducing the activities of soil nitrifying prokaryotes. Previous in vitro studies suggested a suboptimal efficiency of dicyandiamide (DCD) and 3,4-dimethylpyrazole-phosphate (DMPP), the only NIs currently registered for use in Europe, due to their selective inhibitory activity towards ammonia-oxidizing bacteria (AOB), and indicated nitrapyrin as the sole NI effectively inhibiting both AOB and ammoniaoxidizing archaea (AOA). In contrast, ethoxyquin, a potential novel NI, showed high activity against AOA, attributed to its major transformation product quinone imine (QI). To eliminate potential limitations in predicting the behavior of these NIs in-soil, we aimed to assess their efficacy under a range of conditions known to affect the activity of ammonia-oxidizers (soil pH, NH4+-amendment) or the performance of NIs (application rate, persistence) in soil by evaluating their impact on the size, activity and structure of soil ammonia-oxidizing populations. DCD and QI were more potent than nitrapyrin and DMPP in inhibiting nitrification in the acidic soil, while in the alkaline soil all NIs were equally effective. At maximum concentrations, nitrapyrin and DCD were more efficient on AOB in the alkaline soil, as opposed to QI and DCD, that were more effective against AOA in the acidic soil. NIs persistence in soils was not correlated with their impact on ammonia-oxidizers. Results on the effects of NIs on the community composition of ammonia-oxidizers and off-target microbial groups (i.e. bacteria, archaea, fungi) are pending. Our findings could be exploited for the development of more efficient strategies for N conservation in agricultural soils.

Acknowledgments

This work is part of the project "NITRIC – Looking up for **N**ovel n**ITR**ification **I**nhibitors: New stories with old **C**ompounds" which has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No. 1229.

40. Isolation and characterization of a Mycosphaerella tassiana isolate able to rapidly degrade the recalcitrant fungicide Imazalil

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Imazalil (IMZ) is a systemic imidazole fungicide widely used by fruit-packaging plants to control fungal infestations during storage. Its application in dense solutions leads to the production of large pesticidecontaminated wastewater volumes, and according to the European legislation those effluents need to be treated on site, in order to get the pesticide approval of use. Considering the lack of efficient treatment methods, the development of tailored-made inocula to be used in biodepuration systems appears as an appropriate and low-cost solution. However, nothing is known about the biodegradation of IMZ. We report for the first time, the isolation of a microorganism able to rapidly degrade IMZ. Enrichment cultures from a soil receiving regular discharges of effluents from a fruit-packaging plant led to the isolation of a fungal strain which identified via ITS sequencing as Mycosphaerella tassiana. Growth in various selective and broad range media, in the presence of antibacterial and antifungal agents, confirmed the direct involvement of this fungal strain in the degradation of IMZ and negated the role of potential bacterial contaminants. The degrading capacity and the growth of the fungal isolate was evaluated at increasing IMZ concentrations and pH values (4, 6, 8), in both selective (MSMN) and nutrient-rich (PDB) growth media. The fungus showed maximum degradation capacity at pH 4 where it was able to degrade up to 200 mg/L of IMZ, although its degradation capacity was reduced at increasing IMZ concentrations. Measurements of fungal growth in the presence or absence of IMZ suggested a slower proliferation of the fungus in the presence of the fungicide, an effect which magnified at the highest dose rates. This response suggests that the degradation of IMZ by the fungus is most probably a detoxification mechanism rather than an energy-gain process. On-going genomic and transcriptomic analysis will shed light on the biodegradation mechanism, while shotgun metabolomic analysis will explore the transformation pathway of the fungicide. Our study provides first evidence for the biodegradation of IMZ, a highly recalcitrant and relevant environmental contaminant, by a microbial isolate.

Acknowledgement: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)

41. Benthic communities as indicators of large lake surface sediment contamination: A casestudy in Lake Geneva.

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In lake ecosystems, surface sediments contain large amounts of biomass comprising a wide range of specific and functional biodiversity. Microorganisms and invertebrates composing lake benthic communities play a crucial role in biogeochemical cycling and food-webs. Studying the impacts of contaminants on these sediment-associated communities would take into consideration specific and functional biodiversity in effect-based studies and monitoring programs. However, despite a number of ecotoxicological approaches available for evaluating sediment quality, there is a limited number of tools available at the community level to assess the *in situ* effects of contaminants in lake surface sediments.

To pave the way for implementing relevant ecotoxicological approaches at community level in lake sediments, we initiated an international collaborative study combining approaches from ecology, ecotoxicology, chemistry and geochemistry. We examined the spatio-temporal variations in the structure and functions of benthic communities collected in surface sediments from eight sampling stations in Lake Geneva covering a large range of contamination levels by metals and organic compounds.

Structural and functional characteristics of the bacterial communities varied according to sampling dates and locations. Based on the pollution-induced community tolerance (PICT) concept, the microbial tolerance and resistance potential to copper was positively correlated with copper concentrations in sediments. Microbial adaptation in response to chronic contaminant exposure was also evidenced locally by higher abundances of several genes involved in resistance processes to several substances including antibiotics.

In invertebrate communities, the relative distribution of sensitive and tolerant oligochaete species was also related to the contamination conditions. Interestingly, toxicity tests carried out in the laboratory on model populations of ostracodes and nematodes revealed, in some cases, sediment toxicity levels that were not suspected by the study of the native oligochaete community.

Overall, our results confirm the relevance of using benthic communities to develop *in situ* diagnostic tools for the evaluation of the ecotoxicological quality of lake sediments. They also confirmed the need for considering different kinds of organisms (in our case microorganisms and invertebrates) and combining complementarity ecotoxicological approaches to improve current procedures for environmental risk assessment of contaminants in sediments.

42. Exploring climate change feedbacks of the extreme weather events on the interaction between rhizosphere microbiota and plants

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Climate change is one of the biggest challenges of our times, with agricultural sector predicted to be among those mostly affected. It is projected that climate variability will cause a substantial effect on agroecosystem functioning with measurable consequences on agricultural production and food security. Project "Exploring adaptation potential of rhizosphere microbiota to climate change: towards sustainable agriculture in the future (PERSPIRE)", funded by the European Regional Development Fund (ERDF), aims to study the effects of the extreme weather events, already being experienced as a consequence of climate variability, on the complex interaction between rhizosphere microbiota and plant holobiont. Rhizosphere microbiota accomplish various functions that contribute a wide range of essential services by acting as the primary driving agents of nutrient cycles, regulating dynamics of soil organic matter, soil carbon sequestration and greenhouse gas emission, degrading pollutants, modifying soil physical properties and water regimes therefore indirectly being responsible for the health, growth, development and productivity of the plants. In the same time, due to unpredictable nature of the extreme weather events, studies of their impacts on the climate-ecosystem feedbacks are limited, with almost nothing known of the way in which extreme weather affects the rhizosphere microbiota. In view of a changing climate, not only crop yields, but also agricultural practices, including crop protection, is expected to be deeply affected. Combination of increased volatilization and accelerated degradation under climate change scenario, both strongly affected by a high moisture content, elevated temperatures and direct exposure to sunlight, is expected to reduce environmental concentrations of both synthetic and natural active ingredients. In such "new" condition pesticides are presumed to be used in higher amounts, doses, frequencies and different varieties or types of products applied. Climate change will therefore surely change what we currently know about the behaviour of pesticides in soils as well as their effect on microbiota. As intense rainfall events will in many cases result in soil saturation/hypoxic or anoxic conditions both microbiota, plant as well as pesticide behaviour is expected to be greatly affected. To tackle the uncertainty of the climate change effects on the agroecosystems, within the project PERSPIRE, we will settle a microcosm experiment in which selected model plant Brassica oleracea var. viridis (collards) will be grown in conditions mimicking expected climate change scenario: (1) exposing soil and holobiont to repetitive short-term flooding events (48h) and (2) additions of high amounts of herbicide pendimethaline [N-(1-ethylpropyl)-3,4dimethyl-2, 6-dinitrobenzenamine] to soil. Complex interactions between rhizosphere microbiota and plant holobiont in these "new" climate-change driven conditions will be studied on different levels: (i) within the rhizosphere microbiota (changes in the structure, function and activity), (ii) within the plant (changes in physiology, productivity and growth), (iii) within soil compartment (changes in plantnutrient capacity and pesticide behaviour). Studying the responses of the rhizosphere microbiota and plants in experiments mimicking expected climate change scenarios is necessary not only to better understand effects of climate change on the agricultural sector but also to explore resilience and recovery capacity of the plants under expected climate change.

43. To shift or not to shift: Agricultural run-off induced regime shifts in phototrophic communities

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Anthropogenic stressors such as nutrients or pesticides are widely abundant in our agricultural landscape. Yet, they not only occur alone but are mostly abundant in combination. Individual stressors can have contrasting effects, e.g. nutrients enhance growth of phototrophs while herbicides inhibit their photosynthesis and lead to a decline of phototrophic biomass. However, applied in combination stress responses may result in additive, synergistic or antagonistic interactions. Stressor interactions may be especially relevant in complex ecosystems like small ponds, which are characterized by alternative stable states: nutrients lead to eutrophication including regime shifts from macrophyte- to phytoplankton-dominated aquatic systems while herbicides decrease primary production. Still it is unknown whether pesticides, in particular herbicides, can lead to this regime shift, too, or if they prevent shifts from happening. In our study we use microcosm experiments with photoautotroph organisms (microalgae, periphyton and macrophytes) to test the effect of stressors representing an agricultural run-off (ARO): nitrate, pesticides (Terbuthylazine, Pirimicarb, Tebuconazole) and a trace metal used in organic farming (copper). Additionally, we tested if elevated temperatures as occurring during heatwaves, influences the effects of the ARO-mixture. Using a multifactorial dose-response design, we tested stressors individually and in mixture using a gradient of their concentration. The overall aim was to calculate the stressor interaction based on their ability to induce a regime shift in our system. Our results show that nitrate and pesticides partly show effects on the phototrophic biomass in opposite direction when applied separately. Most significantly, their presumed and partly proven contrasting effect diminished when present in mixture; instead the stressors act synergistically and induce a strong regime shift which is indicated by a decrease in macrophyte biomass and an increase in phytoplankton biomass. Furthermore, during elevated temperature, which might enhance both stressors in their antagonistic way, the regime shift is intensified. Thus, if all three stressors are present, the synergistic effect is the strongest. We hypothesize that the leading mechanism behind this finding is not the photosynthesis inhibiting effect of the pesticides, but indirect shading through dense phytoplankton communities. This scenario is expected to occur more often in future as more heatwaves occur in the fate of climate change. More generally, our experiment shows the complexity of stressor interaction and that antagonistic stressors can lead to indirect (in our case trough shading) synergistic effects.

44. Natural freshwater biofilms: a potential nature-based solution for sewage waters treatment

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The treatment of sewage waters has improved significantly in last decades achieving a general reduction of the impacts generated by wastewater release to the environment. However, treated sewage waters are still one of the main sources of nutrients, pollutants and fecal bacteria to the receiving water bodies. Nature-based solutions are defined as "solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience". The project INNOQUA has developed and implemented an innovative wastewater treatment based on the purifying capacity of biological organisms and biofilms with the help of physicochemical factors such as sunlight and sedimentation. Briefly, three reactors have been implemented based on the biological activity of earthworms, zooplankton and microalgae/microbial biofilms. The efficiency of a bioreactor based on river biofilms activity (BioSolar) has been tested at a pilot-plant fed with the influent of a wastewater treatment plant (WWTP) located in Catalonia (NE Spain). Biofilms are ubiquitous microbial communities, made up of bacteria, algae, fungi, and microfauna, embedded in a mucopolysaccharide matrix, that grow adhered to any solid surface in aquatic environments. Moreover, biofilms have been demonstrated to be relevant actors in the self-depuration processes occurring in rivers which is considered one of the most relevant services provided by these ecosystems. The idea behind this research is that those biological processes naturally occurring in freshwater ecosystems can be used, and maximized under controlled conditions, to improve the efficiency of sewage water treatment facilities improving the quality of the water discharged to the receiving bodies. Through this presentation the results of two experiments will be presented and compared. In the first experiment, the BioSolar received the treated water from the conventional secondary treatment of the WWTP whereas in the second one the incoming water come from the earthworm's reactor (Lumbrifilter) which received raw untreated water from the WWTP inlet. Different parameters (nutrients concentrations, chemical oxygen demand, total suspended solids, turbidity, fecal bacteria abundances, biological oxygen demand) have been measured once per week during 4-6 weeks in the influent and effluent of the BioSolar reactor to evaluate its efficiency. At the same time, biofilms have been characterized measuring the abundances of the main phototrophic groups (green algae, diatoms and cyanobacteria) and the biomass (chlorophyll-a and ash free dry mass). The preliminary results obtained indicate that: a) overall the quality of water released by lumbrifilter is significantly better than from the secondary treatment of the WWTP; b) the overall BioSolar efficiency is greater when the incoming water arrives from Lumbrifilter compared to secondary treatment (average reduction of all parameters considered of 64% and 47%, respectively); c) the BioSolar showed the best efficiency of reduction (>50%) for total suspended solids, turbidity, biological oxygen demand and fecal bacteria indicators in both experiments; d) the greater efficiency of the BioSolar could be related with the higher abundances of green algae within biofilm communities. This study demonstrated the great potential of nature-based solutions grounded on the purifying capacity of biological organisms for wastewater treatment.

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45. Diat.barcode: a DNA tool to decipher diatom communities for the evaluation environmental pressures

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Diatoms (Bacillariophyta) are ubiquitous microalgae used worldwide for water quality assessment. Their huge taxonomic diversity and differential sensitivity to contaminants make them excellent indicators of freshwater pollution and turns them into organisms of interest for ecotoxicological studies.

Current standardized methodologies for diatoms are based on microscopic determinations, which are time consuming and prone to identification uncertainties. DNA metabarcoding has been proposed as a way to avoid these flaws, enabling the sequencing of a large quantity of barcodes from natural samples. A taxonomic identity is given to these barcodes by comparing their sequences to a barcoding reference library, which should contain a representative collection of reference sequences with a good coverage of diatom diversity. Moreover, the reference database needs to be carefully curated by experts, as its content has an obvious impact on species detection and on the final ecological assessment.

Diat.barcode is an open-access library for diatoms linking diatom taxonomic identities to *rbcL* barcode sequences (a chloroplast marker suitable for species-level identification of diatoms), which has been maintained since 2012. Data are collected from three sources: (1) the NCBI nucleotide database, (2) unpublished sequence data from culture collections, and more recently (3) environmental sequences. Since 2017, an international network of experts in diatom taxonomy has been curating this library. The last version of the database (version 8.1), includes 7805 entries corresponding to 298 different genera and 1472 different species. In addition to the taxonomic information, morphological features (e.g. biovolumes, chloroplasts, etc.), life-forms (mobility, colony-type) and ecological features (taxa preferences to pollution) are given.

The database can be downloaded from the website (www6.inrae.fr/carrtel-collection/Barcodingdatabase/) or directly through the R package diatbarcode. Ready-to-use files for popular metabarcoding pipelines (mothur and DADA2) are also available.

The reference database *Diat.barcode* is a valuable tool to decipher diatom communities with DNA metabarcoding, which enables (1) the bioassessment of water quality and (2) the evaluation of specific impacts of pollutants (e.g. herbicides, metals, pharmaceutical compounds). Furthermore, by linking barcode sequences to ecological guilds, *Diat.barcode* makes it easy to study the link between diatom functional traits and environmental pressures.

46. Bioaugmentation involving a bacterial consortium isolated from a foaming agent conditioned soil from tunnelling

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Bioaugmentation is a green technology used for removing contaminants from soil and water. The addition of selected microbial strains promotes the catabolic potential of soil microbial communities for the degradation of pollutants. The anionic surfactant sodium lauryl ether sulphate (SLES) is the principal component of several commercial foaming agents (FA) used for soil conditioning in the tunnelling industry. Huge amounts of soil debris are produced during the excavation process and the presence of SLES can affect the re-use of the spoil material as a by-product in green areas close to water bodies. A prompt SLES biodegradation is a key point for the re-use of the excavated soil because it can be toxic for aquatic organisms. A bacterial consortium capable to degrade completely this anionic surfactant (at the concentration of 250 mg/L) in only 24 hours was identified and characterized by NGS and FISH analysis from a foaming agent conditioned soil, using enriched cultures. Most bacteria identified in the bacterial consortium belonged to Gamma-Proteobacteria (99%) and the Pseudomonas genus (ca 90%) was the predominant one. First of all, it was tested the capacity of the bacterial consortium to grow on 14 different SLES concentrations in a commercial foaming agent (from 0.5 mg/L to 4g/L). Subsequently, biodegradation experiments on the same FA-conditioned soils were performed. For this purpose, microcosms containing uncontaminated soil and soil treated with a foaming agent containing SLES, both derived from a tunnelling construction site, were set-up. Half of the soils were inoculated with the SLES degrading bacterial consortium. Soil sub-samples were collected at selected times to determine the SLES degradation (Blue Active Substances method) and microbial abundance (DAPI counts) and cell activity (dehydrogenase activity) increase. The bacterial consortium isolated can be used for enhancing SLES biodegradation in real tunnelling construction sites for a prompt re-use of the soil debris.

47. Bacterial hppd: a biomarker of exposure of soils to beta-triketone herbicides?

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 β -triketone herbicides are among the most used herbicides in corn crop to control broadleaf weeds. These herbicides inhibit the 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) and lead to bleaching and death of weeds. This enzyme is not only found in plants but in all living organisms, including microorganisms where it takes part to the tyrosine degradation pathway. Thus, microorganisms classified as "non-target organisms" by current EU regulation for pesticide authorization, might be impacted by β -triketones, with possible domino effect on microbial functions supporting soil ecosystem services (Thiour-Mauprivez *et al.* 2019). Since microorganisms have been proposed by EFSA as key-drivers to be monitored to better protect soil ecosystem services, we tested the hypothesis that *hppd* bacterial community can constitute a biomarker of exposure to β -triketones. Within this context, we developed a toolbox to monitor the abundance, the diversity and the activity of the *hppd* bacterial community.

Abundance and diversity of *hppd* bacterial community were tested in a lab-to-field experimental design following the tiered-approach recommended by EFSA to conduct pesticide environmental risk assessment (ERA). Under lab conditions, soil microcosms not exposed (control) or exposed to x1 or x10 times the agronomical dose of sulcotrione (active ingredient) or Decano[®] (one of the commercial formulation of sulcotrione) were studied. Under field conditions, samples were collected in corn crop exposed to β -triketones or not (control). Analytical chemistry was applied to all samples to study the dissipation of β -triketone, search for residues and estimate the scenario of exposure of soil microorganisms. Nucleic acids (DNA/RNA) were extracted from soil samples. Home-made degenerated primers, specific to the *hppd* gene of soil bacteria, allowed us to measure the abundance (quantitative PCR), the composition (α -diversity) and the diversity (β -diversity) (NGS) of the *hppd* bacterial community (Thiour-Mauprivez *et al.* 2020). Finally, the inhibition of the activity of the 4-HPPD was assessed on pure bacterial strains under wet lab conditions to measure EC50 of β -triketones.

Our poster will be presented to the audience with the aim to identify the better proxy of the *hppd* bacterial community that could be used as a biomarker to reflect the exposure of soil microbial community to β -triketone residues. As a perspective my work might be extended to other pesticides targeting other enzymes that are also present in so call non-target organisms such as sulfonylureas inhibiting acetohydroxy acid synthase (AHAS).

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48. Biodegradation rates of pharmaceuticals in activated sludge are dependent on concentration

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A large number of pharmaceuticals is entering our environment through wastewater treatment plants (WWTP) effluents, which can ultimately cause human and environmental risks. Biodegradation is the main removal mechanism in the WWTP and is often modelled with statistical quantitative structure activity relationships, depending on solely chemical properties. These models often explain approximately half of the variation in biodegradation. In order to improve fate (and thus risk) predictions of pharmaceuticals, especially more knowledge is needed on the influence of the microbial community on biodegradation in the WWTP.

Activated sludge was sampled from a Dutch WWTP (Groesbeek, Gelderland) in June and December 2019 and was spiked with nine pharmaceuticals with varying biodegradation rates: acetaminophen, carbamazepine, diatrizoate, diclofenac, fluoxetine, metoprolol, metformin, terbutaline and phenazone. The activated sludge was spiked with two environmentally relevant concentrations: either 3 nM or 30 nM of pharmaceuticals mix, incubated in 120 mL glass bottles and the concentration was measured over time (t = 0, 4, 8, 24, 48 and 96 hours) by means of LC-MS/MS. Concentrations over time were used to calculate pseudo first-order biodegradation rate constants (kb). Microbial community was analysed by bacterial 16S rRNA gene amplicon sequencing.

In both experiments, acetaminophen and metformin biodegraded within 8 hours of the experiment, with kb in the range of 0.39-0.55 1/h and 0.02-0.32 1/h, respectively. These pharmaceuticals were followed by metoprolol and terbutaline, with kb in the range of 0.007-0.02 1/h and 0.009-0.021 1/h, respectively. The pharmaceuticals fluoxetine and phenazone showed either no or a low biodegradation rate of 0.015 and 0.002 1/h, respectively, only in case activated sludge was spiked the highest concentration. For diclofenac, diatrizoic acid and carbamazepine either no biodegradation or a very low production rate was observed (< 0.002 1/h). The kb values of pharmaceuticals that were highly to intermediately biodegradable (acetaminophen, metformin and metoprolol) were influenced by the initial pharmaceutical concentration: kb increased at higher concentrations. No significant differences in microbial community composition were observed between the start and end of the experiment, and between the two experiments inoculums (in June and December). This indicates that (i) the kb values we found are likely similar in the WWTP as we found no effect of incubation on microbial community composition, and (ii) the effect of concentration on kb was not caused by community changes. Instead we expect that specific (groups of) microbes have higher transcription rates in case of higher concentrations, resulting in a fast response and increased biodegradation.

From our results, we can conclude that different biodegradation rates were obtained from a short-term biodegradation assay (< 5 days), driven by pharmaceutical concentration. Modelling biodegradation as pseudo-first order reaction does not include the short-term microbial responses to concentration. In order to improve fate assessments of pharmaceuticals in WWTPs, more knowledge is needed on microbial mechanisms influencing biodegradation. Methods such as transcriptomics may help in future studies to model biodegradation more accurately.

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