# EcotoxicoMic 2017

## 1st International Conference on Microbial Ecotoxicology

21st-24th November, 2017
Lyon, FRANCE

ecotoxicomic

The emerging Network of
Microbial Ecotoxicology

Book of Abstracts

https://ecotoxicomic.sciencesconf.org
ecotoxicomic@sciencesconf.org

FONDATION ROVALTAIN
RECHERCHE EN ECO-TOXICOLOGIE
Pour un monde sans pollution

# Welcome to EcotoxicoMic 2017!

We are very proud to welcome you to Lyon for the first International Conference on Microbial Ecotoxicology.

Organized by the emerging Network of Microbial Ecotoxicology (EcotoxicoMic) & the Rovaltain Foundation, the conference will focus on the main research advances on Microbial Ecotoxicology, which is an emerging branch of Science at the crossroad between microbial ecology, microbial toxicology, ecotoxicology, physics and chemistry.

This event will gather for the first time more than 160 microbial ecotoxicologists representing 23 countries from around the world and belonging for both the private and academics sectors. It will address important issues related to increasing demands from worldwide politics and society rising with the threats on environmental and human health caused by intense anthropogenic activities.

Thanks to the support of our many partners, the quality of the presentations and the presence of world-renowned speakers we hope that this event will be a success and will start a long series of passionate conferences dedicated to Microbial Ecotoxicology!

We wish you a very fruitful conference with numerous exciting communications, discussions and prospects of collaboration and a very pleasant stay in Lyon.

Stéphane Pesce, Irstea
Co-Funder of the EcotoxicoMic Network

Philippe Garrigues
President of the Rovaltain Foundation

# Who we are.

ecotoxicomic

### The Emerging Interdisciplinary Network of Microbial Ecotoxicology

The EcotoxicoMic Network aims at enhancing visibility and structuring microbial ecotoxicology community by developing transverse actions between studied ecosystems and researchers belonging to various research organizations.

### Rovaltain Foundation

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

# Programme

| | Tuesday November 21 | Wednesday November 22 | Thursday November 23 | Friday November 24 |
|---|---|---|---|---|
| | OPENING SESSION | SESSION FUNCTION | SESSION MULTISTRESS | SESSION TROPHIC INTERACTIONS |
| 08:40 - 09:00 | | **Keynote Conference**<br>**How do aquatic microbes respond to emergent contaminants?**<br>**F.CASSIO** | **Keynote conference**<br>**Combined chemical and non-chemical stress: towards a mechanistic understanding of stressor interactions,**<br>**Mechthild SCHMITT-JANSEN** | **Keynote conference**<br>**The complexities of pollutants and ecological interactions: Lessons from freshwater ecosystems,**<br>**Rick RELYEA** |
| 09:00 - 09:20 | | | | |
| 09:20 - 09:40 | | *Gallois (Gallois et al. Insights into uranium tolerance of Microbacterium oleivorans A9 by proteogenomic analyses)* | *Rocca (Rocca et al. The Microbiome Stress Project: A database for identifying stressor impacts on microbial community structure across multiple environments)* | *Vincent (Vincent et al. Abiotic, biotic (microflora and fauna) and functional parameters of derelict soils - towards a potential use of derelict soils as a ressource)* |
| 09:40 - 10:00 | | *Meistertzheim (Dussud et al. Influence of plastic litters on marine microbial life)* | *Reoyo-Prats (Reoyo-Prats et al. Changes in bacterial coommunities in response to multicontamination phenomena and other anthropogenic forcing during different hydrological regimes in mediterranean watercourses)* | *Zubrod (Zubrod et al. Are fungicides a threat for heterotrophic aquatic food webs?)* |
| 10:00 - 10:20 | | **Coffee Break** | **Coffee Break** | *Spina (Spina et al. Evaluation of the ecotoxicologycal impact of municipal wastewaters on wildlife: a case study in Tuscany)* |
| 10:20 - 10:40 | | | | **Coffee Break** |
| 10:40 - 11:00 | | *Chaumet (Chaumet et al. Transfer and distribution of diuron in biofilms and joint toxic effects)* | *Rossi (Rossi et al. Interactive effects of pesticides and nutrients on microbial communities responsible of litter decomposition in streams)* | |
| 11:00 - 11:20 | | *Cheloni (Cheloni et al. Morphological plasticity in Chlamydomonas reinhardtii: an adaptive response to micropollutant stress)* | *Crouzet (Nazaret) (Crouzet et al. Monitoring the impact of multi-stress contamination on an environmental opportunistic pathogen, Stenotrophomonas maltophilia: application to antibiotics and metals)* | *Neury-Ormanni (Neury-Ormanni et al. Changes in the interactions between periphytic microalgae and micromeiofauna induced by environmental exposure to diuron and/or imidacloprid)* |
| 11:20 - 11:40 | | *Grosjean (Grosjean et al. Genome-wide analyses of rare earth elements responsive genes in microbial model organisms)* | *Proia (Proia et al. Biofilm phosphorus uptake capacity responds to chemical and environmental stressors in river ecosystems)* | *Pascoal (Pascoal et al. Impacts of silver nanoparticles on aquatic detritus-based food webs)* |
| 11:40 - 12:00 | | **Poster Corner session (N cycle, bioremediation, genomic)** | **Poster Corner session (Omics, biomarkers/biassays)** | *Arslan (Arslan et al. Dysbiosis in plant-endophyte partnership: repeated short exposures of sulfamethoxazole and trimethoprim at micro-concentrations can disturb the microbial community in soft rush, Juncus effusus)* |
| 12:00 - 12:20 | | | | **CLOSING** |
| 12:20 - 12:40 | **Welcome** | **Poster session** | **Poster session** | |
| 12:40 - 13:00 | | **Lunch** | **Lunch** | |
| 13:00 - 13:15 | | | | |
| 13:15 - 13:40 | **INTRODUCTION** | | | |
| 13:40 - 14:00 | **Opening Conference**<br>**Microbial ecotoxicology : Past, present, and future,**<br>**E. TOPP** | | | |
| 14:00 - 14:20 | | *Simonin (Simonin et al. Novel nanopesticides introduce new challenges to microbes in agro-ecosystems and downstream wetlands)* | *Bourgeois (Bourgeois et al. Spatio-temporal dynamics of TPMT-harboring bacteria among urban deposits reveal significant shifts in Pseudomonas and Aeromonas populations matching pollutant concentrations)* | |
| 14:20 - 14:40 | *Brandt (Brandt- Improving ecotoxicological assessment of chemicals using soil microbial communities)* | *Lemmel (Lemmel et al. Diversity and functions of microbial communities in historically multi-contaminated soils)* | *Freixa (Freixa et al. Ecotoxicological effects on the interactions of fullerenes and organic micro-contaminants in stream biofilms)* | |

| | SESSION BIODEGRADATION | | SESSION RISK ASSESSEMENT |
|---|---|---|---|
| 14:40 - 15:00 | **Keynote conference**<br>**The role of microbial communities in pollutant fate, distribution and degradation,**<br>**E. EDWARDS** | *Eriksson (Eriksson et al. Long-term effects of triclosan on community function, community tolerance and metagenomic composition in marine periphyton communities)* | *Stachowski (Stachowski et al. Influence of natural dissolved organic matter on herbicide toxicity to two marine microalgae)* |
| 15:00 - 15:20 | | **Keynote conference**<br>**Ecotoxicomic: A team player part of the global game in Ecotoxicology**<br>**P. GARRIGUES** | *Bonet (Bonet et al. How climate change enhances nanoparticle toxicity towards freshwater biofilms - NanoTox project)* |
| 15:20 - 15:40 | *Vuilleumier (Torabi et al. Dissipation of chloroacetanilides in agricultural soil microcosms: insights from stable isotope fractionation and bacterial community response)* | **Towards an international EcotoxicoMic network : for an emerging discipline facing contemporary environmental threats worldwide**<br>**J.F. GHIGLIONE**<br>**Followed by a Round Table** | **Keynote conference**<br>**Assessing the soil microbial ecotoxicity of pesticides: Advances, limitations and new risk assessment schemes,**<br>**Dimitrios KARPOUZAS** |
| 15:40 - 16:00 | *Petric (Petric et al. Insights into flexibility of Rhodococci in transformation of PCBs)* | | |
| 16:00 - 16:20 | *Carles (Carles et al. Capacity of leaf-associated microorganisms to biotransform pesticides in rivers)* | **Coffee Break** | *Monier (Monier et al. Real-time and in situ monitoring of aquatic environments using indigenous microbial community-based biosensors)* |
| 16:20 - 16:40 | **Coffee Break** | | *Tlili (Tlili et al. Tolerance of stream biofilms to micropollutants indicates causality and reflects community recovery)* |
| 16:40 - 17:00 | | *Mougin (Mougin. RECOTOX: a network of research sites to implement multiple and multi-scale approaches for a posteriori environmental risk assessment in agricultural systems)* | **Coffee Break** |
| 17:00 - 17:20 | *Desiante (Desiante et al. Micropollutant biotransformation potential of natural river biofilms)* | *Capdeville (Capdeville et al. Responses of microbial communities in mangrove ecosystem under the influence of pretreated wastewater discharges)* | |
| 17:20 - 17:40 | *Duran (Duran et al. Hydrocarbon impact on microbial communities in marine coastal sediments)* | *Baudy (Baudy et al. Do anthropogenic stressors impact the functional performance of leaf-degrading aquatic fungi? A case study using microcosms, fungicides, and modern biochemical tools)* | *Crouzet (Crouzet et al. Unrecognized soil algal and cyanobacterial communities as a model, for herbicide risk assessment in agricultural soils)* |
| 17:40 - 18:00 | *Puglisi (Puglisi et al. Selective colonization processes of plastic-loving bacteria in an abandoned landfill site)* | *Barros (Barros et al. Proteomic responses to nanoparticulate and ionic silver in fungi from metal-polluted and non-polluted streams)* | *Pesce (Pesce et al. Towards the implementation of Pollution Induced Community Tolerance (PICT) approaches in water regulatory frameworks: Developing standard procedures for the sediment compartment)* |
| 18:00 - 18:20 | *Laroche (Laroche et al. Dynamics of bacterial communities in a field-scale pilot treating As-rich acid mine drainage)* | | Best Student Poster Award |
| 18:20 - 18:40 | **Poster session** | | |
| 18:40 - 19:00 | | | |
| 19:00 - 19:20 | **Welcome Cocktail** | | |
| 19:20 - 19:40 | | | |
| 19:40 - 20:00 | | | |
| | | | **Conference Dinner** |

# Detailed poster corners programme

## Wednesday, November 22, 2017 @ 11:40-12:20

### Bioremediation

- Bioremediation of an aged dioxins and furans contaminated soil by saprotrophic telluric fungi and a mild chemical oxidation, Isabelle Delsarte
- On-farm bioremediation of leftover pesticide solutions using biobed, Talaat Elsebai
- Dichloromethane degradation in multi-contaminated groundwater: coupling biomolecular and compound-specific isotope analyses, Louis Hermon
- Microbiology of Acid Mine Drainage: from natural attenuation to bioremediation, Marina Héry
- Microbial resources management for bioremediation, Jean-Michel Monier

### Ecotoxicological effects on N cycle

- Metal pollution effects on ammonia oxidation, Maria Argudo
- Do plant traits matter in the microbial ecotoxicity of metal nanoparticles in soil?, Agnès Richaume
- Mixtures effect of antibiotics on soil nitrification and denitrification - an experimental and modelling approach, Olivier Crouzet
- Impact of pesticides on denitrification in groundwaters, Caroline Michel
- Long Term Exposure of Agricultural Soil to Veterinary Antibiotics Changes the Population Structure of Symbiotic Nitrogen-Fixing Bacteria Occupying Nodules of Soybeans (Glycine max), Alain Hartmann

### Genomic approaches in microbial ecotoxicology

- SYNAQUA: A French-Swiss program to modernize the environmental biomonitoring of Lake Geneva and transboundary rivers, Agnès Bouchez
- Environmental persistence of pharmaceutical residues from hospital and urban effluents and their impacts on environmental aquatic microbial communities, Agnès Bouchez
- Shifts in planktonic and biofilm prokaryotic community composition along a chemical multicontamination gradient in a north-western coastal mediterranean environment (Toulon bay, France), Clément Coclet
- Combination of high-throughput sequencing and geochemistry methods for evaluating the impact of single and multi-metal pollutions in soil at the aggregate scale, Jean Martins

## Thursday, November 23, 2017 @ 11:40-12:20

### Omics approaches in microbial ecotoxicology

- Quick metaproteomics for environmental risk assessment, Jean Armengaud
- From the screening of soil eukaryotic metatransciptomes to the functional characterization of new metallothioneins, Laurence Fraissinet-Tachet
- Volatolomics in microbial ecotoxicology, a pathway for detecting metabolic signatures of exposure to xenobiotics, Kevin HIDALGO

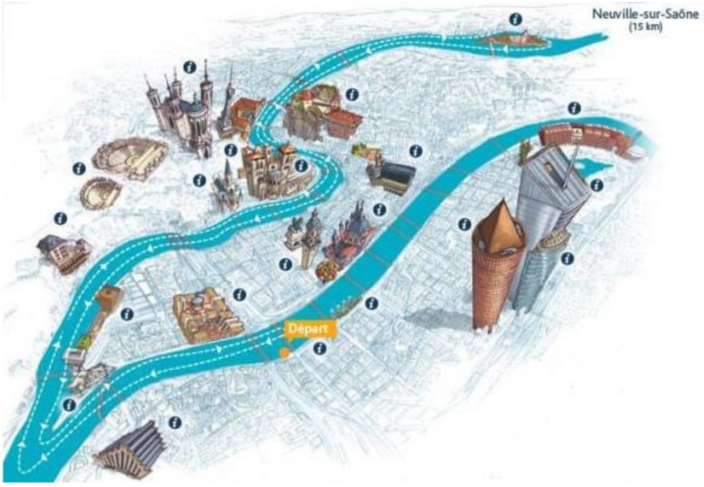
### Innovative microbial biomarkers and bioassays for environmental risk assessment

- Development of a standardized aquatic hyphomycete laboratory assay for fungicide risk assessment, Patrick Baudy
- Integrated biomarker and ecological response (IBER) index, Helena Guasch
- Microbial molecular and isotopic biomarkers for evaluating natural attenuation of chlorinated hydrocarbons in multi-contaminated sites, Jennifer Hellal
- High throughput microbial array for complex environmental sample assessment, Anna Hua

# Conference Dinner

The conference dinner will be held on Thursday evening, 23rd November. The organizing committee invites you to admire the most beautiful urban landscapes aboard the Hermès restaurant boat of the Lyon City Boat company!

## PROGRAM



### DEPARTURE & RETURN

**Boarding time at 8:00 pm**
**In front of the 16 dock Claude Bernard, Lyon**

⚠ **DON’T BE LATE!!!**

Left bank-side of the Rhône at the foot of the University bridge

Departure at 8:30 pm – Dinner served on board

Return around 11:00 pm

During the ILE BARBE TOUR, we will sail along the Rhône docks to the confluence neighborhood where the Saône & the Rhône meet. The travel will stretch through the historic city & up to the Barbe island.

# Partners

## Platinium Partners

**Irstea**

The National Research Institute of Science and Technology for Environment and Agriculture is a research organization which, since more than 30 years, has been working on major issues of a responsible agriculture and territory sustainable planning, water management and related risks, drought, floods, inundations, the biodiversity and complex ecosystem study in their interrelation with human activities.

**Auvergne Rhone Alpes Region**

With major national research centres based in the region, 14 competitivity hubs and 18 clusters, Auvergne-Rhône-Alpes files the second largest number of patents in France, and has huge potential for innovation. Very much open to forging international ties, the Auvergne-Rhône-Alpes region has over the years made connections with other areas, both in Europe and across all continents – thus supporting international projects, ensuring its citizens – In particular young people – are free to move around, and encouraging regional businesses to spread their knowledge and experience.

## Gold Partners

**AFB**

The French Agency for Biodiversity is a public institution of the Ministry of the Environment, Energy and Sea. It supports the implementation of public policies in the fields of knowledge, preservation, management and restoration of biodiversity of terrestrial, aquatic and marine environments. It supports public actors but also works in close partnership with socio-economic actors. It also aims to reach out to the public to mobilize citizens in favor of biodiversity.

**IRD**

A crucial player in providing scientific advice on the major challenges of development. The Institut de recherche pour le développement, an internationally recognised multidisciplinary organisation, is a French public research establishment operating under the joint authority of the French Ministry for Higher Education, Research and Innovation and the French Ministry for Europe and Foreign Affairs. 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.

**INRA**

The French National Institute for Agricultural Research is Europe’s top agricultural research institute and the world’s number two centre for the agricultural sciences. Its scientists are working towards solutions for society’s major challenges for food, nutrition, agriculture and the Environment.
The Department Environment and Agronomy of INRA is supporting the 1st international congress in Microbial Ecotoxicology as one of his actions to encourage new and innovative researches to monitor the impact of agricultural practices on natural resources.

**ECOTOX, the INRA's network of ecotoxicologists**

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

**BRGM, the French geological survey**

The Bureau de recherches géologiques et minières is France’s reference public institution for Earth Science applications in the management of surface and subsurface resources and risks.

**Ifremer**

The Institut français de recherche pour l'exploitation de la mer is an oceanographic institution in France. Ifremer contributes, through studies and expert assessments, to the knowledge of the oceans and their resources, the monitoring of marine environment and the coastline and sustainable development of maritime activities.

**Frontiers**

Frontiers is a community-rooted, open-access academic publisher. Our 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 is the #2 most cited open-access journal in the category of Microbiology, and overall the #8 most cited journal in Microbiology.**

## Bronze Partners

**CNRS**

The National Center for Scientific Research, or CNRS, is a public organization under the responsibility of the French Ministry of Education and Research. As the largest fundamental research organization in Europe, the CNRS carries out research in all fields of knowledge, through its ten institutes.

### Genoscreen

GenoScreen is a French biotech company. Thanks to its 30 highly experienced staff GenoScreen offers innovative services to public and private sector research groups and develops dedicated solutions based on DNA/RNA information. Our innovation strategy has generated a stream of successful projects, including top-level expertise in the characterization of microorganisms. GenoScreen supports companies of the environmental sector in various fields of application: water and air treatment, diagnostic and decontamination of polluted soils, monitoring of microbial biodiversity and ecosystem and biosafety.

### FST Lyon 1

La Faculté des Sciences et Technologies est une composante de l'Université Claude Bernard Lyon 1. Elle regroupe l'ensemble des acteurs (enseignants-chercheurs, chercheurs, personnels administratifs et techniques, étudiants) autour d'un projet de formation et de recherche en sciences et technologies à l'Université ; son activité se concentre principalement sur le campus Lyon Tech La Doua ouvert sur la ville de Villeurbanne et la métropole de Lyon.

### BioEnviS Research Federation

The FR BioEnviS is a research federation regrouping several research units from the Lyon scientific community, operating in the field of environmental biology and its implications in the field of global health (human as well as animal or plant). It includes the agronomic, veterinary or health concerns of the non-anthropized environments.

### Microbial Ecology Laboratory

The Research Unit UMR 5557 has title and theme for Microbial Ecology, discipline at the crossroads of two major research fields: ecology and microbiology. Microbial ecology aims to know the physiology of microorganisms and the influence of their activities on other organisms and the environment in general, and, symmetrically, to clarify the impact of biotic and abiotic factors of the environment of these organisms, from the individual to the community in an ecosystem.

**Springer** is a leading global scientific, technical and medical portfolio, providing researchers in academia, scientific institutions and corporate R&D departments with quality content through innovative information, products and services.

# Committees

## Scientific committee


Jean-François Ghiglione
(CNRS)


Cécile Bernard
(MNHN)


Christophe Leboulanger
(IRD)


Chloé Bonnineau
(Irstea)


Fabrice Martin-Laurent
(INRA)


Virginie Chapon
(CEA)


Jean-Michel Monier
(ENOVEO)


Jennifer Hellal
(BRGM)


Stéphane Pesce
(Irstea)


Corinne Leyval
(CNRS)


Thomas Pollet
(INRA)


Sylvie Nazaret
(CNRS)


Wilfried Sanchez
(FCS Rovaltain)


Agnès Richaume
(Univ. Lyon)


Stéphane Vuilleumier
(Univ. Strasbourg)


Sabine Stachowski-Haberkorn
(IFREMER)

## Local organizers

Chloé Bonnineau, Irstea
Emilie Egea, FCS Rovaltain
Jean-Michel Monier, ENOVEO
Sylvie Nazaret, CNRS
Stéphane Pesce, Irstea
Agnès Richaume, University of Lyon
Wilfried Sanchez, FCS Rovaltain

# Frontiers Research Topic Publication

Frontiers, which is Gold Sponsor of ECOTOXICOMIC 2017, will edit **a Research Topic dedicated to Microbial Ecotoxicology** (Eds S. Pesce, F. Martin-Laurent, E. Topp, & JF. Ghiglione).

An exclusive special publishing discount will be offered to Conference delegates choosing to publish in the participating “Frontiers in” journals.

## Reduced Publishing Fees

Frontiers journals are Open Access and authors pay a publishing fee. For the general fee table, please visit the Frontiers website: http://www.frontiersin.org/about/PublishingFees.

Exclusively, for attendees of EcotoxicoMic 2017 Frontiers will offer a **50% discount** on the full publishing price for submissions to ***Frontiers in Microbiology*** and a **75% discount** to authors publishing in ***Frontiers in Environmental Science.***

Authors who wish to contribute, but have not attended the conference will receive a 15% discount

## Abstract submission

Manuscript submission to the Research Topic is now opened.

Deadlines for the Research Topic are as follow:

- Abstract: 15 December 2017
- Manuscript: 15 April 2018

**The articles will be published as soon as they are accepted**.

Articles will be compiled into a free-of-charge and free-to-access ***eBook***, which we can be distributed electronically.

Please do not hesitate to share this information within your networks.

More information on:

https://www.frontiersin.org/research-topics/6045/microbial-ecotoxicology

# Sessions

# OPENING Session

**Chair: Stéphane PESCE**

## Keynote conference: ***Microbial ecotoxicology : Past, present, and future***, Dr. Edward TOPP, Agriculture and Agri-Food Canada, London Ontario, Canada.

Dr. Ed Topp is a native of Montréal. He studied microbiology at McGill University, and he received his PhD in 1988 from the Department of Microbiology at the University of Minnesota. He is a Principal Research Scientist with Agriculture and Agri-Food Canada and also has adjunct appointments with the Department of Biology at Western University in London Ontario, and the Department of Soil and Water Sciences at the University of Florida in Gainesville. Dr. Topp has led several national studies that seek to better understand and to better manage the risks that food production practices pose for environmental quality and human health. This work has included terrestrial and aquatic exposure and fate assessments for numerous pharmaceuticals including various antibiotics, impacts of antibiotics on soil microorganisms, and fate of antibiotic resistance genes in soils receiving animal manures or sewage sludge. Dr. Topp is the national coordinator for the Genomics Research and Development Initiative project on antimicrobial resistance, a key component of the innovation pillar of the Canadian National AMR Action Plan. He is a former president (2011) of the Canadian Society of Microbiologists..

# Session BIODEGRADATION

## The role of microbial communities in pollutant fate, distribution and degradation

**Chairs: Elizabeth A. EDWARDS and Corinne LEYVAL**

A major challenge in microbial ecotoxicology concerns our ability to evaluate the role of microorganisms in the fate of the pollutants in the ecosystems (ecodynamic concept). Individual microorganisms were shown to degrade a large spectrum of pollutants but in the environment, the biodegradation is realized by a succession of microbial organisms. The relation between chemical evidence of biodegradation and the microbial 'degraders' is generally indirect and under-studied, which makes the prediction of the fate of pollutants in complex ecosystems difficult. Biotechnological approaches that are designed to carry out pollution remediation have received a great deal of attention in recent years, where the growing knowledge of bioremediation strategies proposed by microbial ecotoxicologists is of great interest. Biodisponibility concept is one of the challenge in biodegradation, exploring the accessibility of pollutants to biological barriers which separate microorganisms from their surrounding environment and limit their metabolization. In this session, original results facing this challenging field are welcome, and especially those at the interface between chemistry/microbiology disciplines.

Keywords: Bioremediation, Biodegradation, Biotransformation, Bioavailability, Metabolisation, Retroactive action, Ecological engineering

Keynote conference: ***The role of microbial communities in pollutant fate, distribution and degradation***, Prof. Elizabeth A. EDWARDS, Toronto University, Canada.

Dr. Elizabeth Edwards holds Bachelor's and Master's degrees in Chemical Engineering from McGill University, Montreal, and a PhD degree in Civil and Environmental Engineering from Stanford University. She is internationally known for her work on anaerobic bioremediation, the application of molecular biology and metagenomics to uncover novel microbial processes, and the transition of laboratory research into commercial practice to develop bioremediation and bioaugmentation strategies for groundwater pollutants. She is also the founding director of BioZone, a Centre for Applied Bioscience and Bioengineering Research at the University of Toronto and a Tier 1 Canada Research Chair in Anaerobic Biotechnology. In 2016, she was awarded the Canada Council of the Arts Killam Prize in recognition of outstanding career achievements.

# Session FUNCTION

## Impact of pollutants on microbial functions

**Chairs: Fernanda CASSIO and Ed TOPP**

Microbial communities support a wide range of functions in ecosystems (e.g. primary production in waters, denitrification in soils and sediments…) and thus play a key role in biogeochemical cycles. Therefore the effects of pollutants observed at microbial level can have consequences at higher level of biological organization and alter ecosystem functions. The plasticity of microbial communities supports their adaptative response to the selective pressure exerted by pollutants. Functional indicators of adaptation (e.g. tolerance acquisition) have been used as indicators of contaminant effects and linked to changes in microbial structures. During the last decade, omics techniques (metatranscriptomics, metabolomics, metaproteomics) have been widely applied to complete the information given by classical indicators.

This session will then focus on the impact of contaminants on microbial functions and the consequences at different scales: from cellular biochemical pathway to biogeochemical cycles and ecosystem functions. To cover these different scales, we invite presentations of lab studies performed at different levels of biological organization (from single-species to communities) as well as field studies. Studies at community level, exploring the impact of contaminants on community and ecosystem functions are welcome as well as studies linking functional changes with structural ones (e.g. taxonomic diversity). In this session we also intend to show the wide range of techniques developed and validated to assess microbial functions in various ecosystems, in particular this session will explore the added value of omics tools and clarify how (meta)omics tools can be combined with more classical parameters to unravel the influence of contaminants on microbial functions, especially at community level.

Keywords: Ecosystem functions, Biogeochemical cycles, Functional diversity, Microbial plasticity / adaptation, Link between functions and taxonomic diversity, Metatranscriptomics, metabolomics, metaproteomics

### Keynote conference: ***How do aquatic microbes respond to emergent contaminants?*** Dr. Fernanda CASSIO, Center of Molecular and Environmental Biology, Braga, Portugal.

Dr. Fernanda Cássio is an Associate Professor with habilitation at the University of Minho, in Portugal. She got her PhD in Microbiology. She is the head of the Centre of Molecular and Environmental Biology, rated as Excellent by the Portuguese Foundation for Science and Technology. She has been studying the role of decomposers of organic matter in aquatic ecosystems, with particular emphasis on microbial diversity and activity, using traditional and molecular techniques.

She is also interested in ecotoxicological effects of emergent (pharmaceuticals and nanomaterials) and persistent contaminants (metals and PHAs) in freshwaters, studying effects from cells, to populations and communities levels.

# Session MULTI-STRESS

## Response of microorganisms to pollutants under multi-stress conditions

**Chairs: Mechthild SCHMITT-JANSEN and Stéphane VUILLEUMIER**

A major challenge in environmental risk assessment of pollutants lies in the establishment of causal relationships between chemical exposure and the resulting community response within complex ecosystems which are generally subjected to multi-stress conditions due to multiple chemical and/or physical pressures. To face such a challenge, there is a need to develop microbial ecotoxicological approaches to better predict and evaluate the interactions between multiple pollutants and between pollutants and other environmental parameters, which can modulate both the exposure and the sensitivity of microorganisms to toxicants.

This session will welcome original presentations about experimental and in situ studies aiming at assessing, predicting or modeling the effects of pollutants on microorganisms (at various biological levels) in a context of multiple stresses (e.g. exposure to multi-pollutant mixtures, combined exposure to pollutants and physical disturbance…).

Keywords: Pollutants vs. other environmental parameters, Exposure (including bioavailibility), Multivariate statistical analysis, Modelisation

### Keynote conference: ***Combined chemical and non-chemical stress: towards a mechanistic understanding of stressor interactions***, Dr. Mechthild SCHMITT-JANSEN, Helmholtz Centre for Environmental Research, Leipzig, Germany.

Dr. Mechthild Schmitt-Jansen received her PhD in Hydrobiology at the Brandenburg University of Cottbus, Germany in the Department of Freshwater Conservation studying plankton input and dynamics in lowland streams. She continued her work as a postdoctoral researcher in the Department Chemical Ecotoxicology and the Department Effect Propagation at the Helmholtz Centre for Environmental Research – UFZ in the research areas of community ecotoxicology and site-specific toxicity assessment of aquatic systems. Since 2005 she has been working as a senior scientist at the Department Bioanalytical Ecotoxicology - UFZ leading the research group aquatic stress ecology. Since 2011 she has been holding a teaching assignment at the Technical University Bergakademie Freiberg with lectures related to aquatic ecology and ecotoxicology.

Dr Schmitt-Jansen`s research interests concern community responses to multiple stressors and contaminants with special focus on biofilm ecology. Her work is based on ecological concepts like stress/pollution-induced community tolerance (SICT/PICT). In order to understand the underlying processes of stress responses, she investigates molecular responses like metabolomics and links them to phenotypic observations including physiologically-based (photosynthesis) and ecologically-based parameters (dynamics of species traits, community tolerance). Model systems she is working with rang from algal cultures to periphyton communities.

# Session RISK ASSESSEMENT

## Microorganisms as a tool for a priori and a posteriori environmental risk assessment

**Chairs: Dimitrios KARPOUZAS and Fabrice MARTIN-LAURENT**

One of the primary objectives of ecotoxicology is to contribute to the development of innovative methods and tools for environmental risk assessment, both including predictive hazard and risk assessment as well as the in situ evaluation of environmental quality and ecotoxicological impacts in contaminated environments. Despite the recognized importance of microbial communities in supporting a range of ecosystemic functions and services and their ubiquity in all ecosystems, microorganisms are only rarely considered in the a priori environmental risk assessment of pollutants and they are typically not protected by any regulations or legislations. Moreover, whereas chemical analysis remains essential to assess the nature and extent of a contamination, the use of bioindicators can provide valuable complementary information on both the impact and fate of pollutants. The ubiquity of microorganisms in the environment and their capacity to adapt in many ways to a large range of pollutants make them good candidates as exposure and effects indicators, allowing them to contribute to the a posteriori environmental risk assessment. This session will focus on the development and/or application of microbial bioindicators/biomarkers/bioassays for environmental risk assessment including pollutant availability and transfer, evaluation of environmental quality and ecological health in various ecosystems. In this session we also intend to discuss about needs and possible strategies to promote the use of microorganisms (at different levels of biological organization) in a priori and a posteriori environmental risk assessment.

Keywords: Model organisms, sentinel species, Bioavailability, Bioindication, Bioassays, Biosensors, Environmental Legislation, Quality guidelines, Indices, Monitoring

### Keynote conference: ***Assessing the soil microbial ecotoxicity of pesticides: Advances, limitations and new risk assessment schemes***, Dr. Dimitrios KARPOUZAS, University of Thessaly, Volos, Greece.

Dr. Dimitrios Karpouzas studied Crop Protection in the Aristotle University of Thessaly and then he moved to UK where he was awarded an MSc in Crop Protection from the University of Reading and a PhD on the microbial degradation of pesticides by the same University in collaboration with HRI-Wellesbourne. He is currently Associate Prof. in Environmental Microbiology and Head of the Lab of Plant and Environmental Biotechnology in the Department of Biochemistry and Biotechnology, University of Thessaly. His main research focus is on the interactions of pesticides with soil microorganisms which could lead either to biodegradation of the pesticides by soil microorganisms or to toxicity effects on the soil microbial community. On the former his focus is on the metabolic pathways involved and the genes/enzymes controlling each step of the process. In recent years within the frame of a series of EU-funded projects Dr. Karpouzas focused on the development of tools and procedures for the comprehensive assessment of the toxicity of pesticides on soil microorganisms which could be the basis for a forthcoming revision of the regulatory framework regarding pesticides soil microbial toxicity assessment.

# Session TROPHIC INTERACTIONS

# Impact of pollutants on the interactions among micro - or between micro- and macro-organisms

**Chairs: Rick RELYEA and Jean-François GHIGLIONE**

In many ecosystems, phototrophic organisms (including microoganisms) are at the base of “green” food webs supported by primary production while heterotrophic microorganisms play a pivotal function in detritus-based (“brown”) food webs, whatever the kind of ecosystems. Generally, the brown and the green pathways are not separated and microbial communities are playing a key role in the between-pathways interactions, which are a major determinant of ecosystem functioning. Microorganisms are also part of living animals and plants (the ‘holobiont’ and ‘hologenome’ concept) which are no longer viewed as autonomous entities, a concept relatively new that challenged the general life science concepts. Moreover, the life of microorganisms themselves are closely linked to nutrient availability (“bottom-up control”) and to viral lysis or predation by ciliates and flagellates (“top-down control”), which control their growth and activities. Pollutants are acting at different trophic levels, and the emerging discipline of microbial ecotoxicology is now facing the challenge of evaluating the role of microbial communities in ecosystem homeostasis by considering the complex array of interactions between micro- or micro-macro-organisms and their environment under pollution pressure.

This session welcome original presentations addressing both the indirect effects of pollutants on living organisms and the alteration of interactions between organisms in all kinds of ecosystems (fresh or marine water, soil...) . Indirect effects could arise from modification of consumer-prey balance (top-down) or competition for resources (bottom-up) due to direct toxic pressure against one partner in the frame of community ecology. Interactions such as mutualism, cooperation, and symbiosis can be modified under pollution stress, either on one or several components of the interaction. Presentations assessing ecological modeling to predict the effects of pollutants on the interactions among micro- and between micro-macro-organisms are also fostered.

Keywords: Trophic interaction, Top-down (predation, lysis) vs. bottom-up (nutrients) controls, Host-microorganisms, Microbiota, Modelisation

## Keynote conference: ***The complexities of pollutants and ecological interactions: Lessons from freshwater ecosystems***, Dr. Rick RELYEA, Rensselaer Polytechnic Institute, Troy, NY, USA.

Rick Relyea received his PhD from the University of Michigan in 1998 and is currently the David D. Darrin '40 Senior Endowed Chair in Biology at Rensselaer Polytechnic Institute (RPI). He has spent his career working to merge the fields of ecology, evolution, and ecotoxicology using a diversity of species and a wide range of experimental venues from the laboratory to outdoor field experiments. His research group has made a number of important discoveries regarding the synergistic effects of contaminants and natural stressors and how contaminant impacts on one species can have substantial impacts on the entire ecosystem due to the pervasive interconnectedness of food webs.

# Session ECOTOXICOMIC

## EcotoxicoMic: towards an International Network on Microbial Ecotoxicology?

**Chairs: Philippe GARRIGUES, Jean-François GHIGLIONE, Stéphane PESCE, Fabrice MARTIN-LAURENT, Wilfried SANCHEZ**

**Presentations and discussion regarding the creation of an international Network on Microbial Ecotoxicology.**

The general objective of the French EcotoxicoMic Multidisciplinary Thematic Network is to improve the visibility and federate the community of microbial ecotoxicologists, with a desire to promote cross-functional actions between the ecosystems studied and between researchers belonging to different research organizations.

**What about the extension of such objectives at an International Level?**

### Keynote conference: ***Ecotoxicomic: A team player part of the global game in Ecotoxicology***, Dr. Philippe GARRIGUES, National Center for Scientific Research, Bordeaux, France.

Dr. Philippe Garrigues is a CNRS Research Director and currently at the University of Bordeaux. His research interests are the analytical aspects (chromatographies and spectroscopies) related to the detection of organic pollutants and the environmental fate and the toxicological effects of these compounds. He has been involved in the development of biochemical markers as early warning systems for the toxicological evaluation of marine ecosystems through the coordination of large research projects supported by the DG Research (EC, Brussels). His recent research interest deal with REACh regulation and how analytical chemistry is developed for its implementation.

Dr. Garrigues has authored about 180 publications. He has been member of the Executive Committee of SETAC-Europe (1997-1999) and Chair of the EuCheMS Division «Chemistry and the Environment» (2003-2008). He is presently Deputy-Chair of the French Chemical Society (SCF) and elected member of the Executive Board of EuCheMS (European Association of Chemical societies). He is/has been member of scientific committees in French institutions (CNRS, IFREMER, INERIS, Rovaltain Foundation). He has organized various Scientific Conferences dealing with environmental chemistry and toxicology, most of them related to scientific societies (SETAC, ISPAC, EuCheMS). He has also various editor's responsibilities (Polycyclic Aromatic Compounds, Environmental Science and Pollution Research, Analytical and Bioanalytical Chemistry).

### Introduction to the Round Table: ***Towards an international EcotoxicoMic network for an emerging discipline facing contemporary environmental threats worlwide*** Dr. Jean-François GHIGLIONE, National Center for Scientific Research, Banyuls sur mer, France.

# Communication Abstracts

## TALKS

# Improving ecotoxicological assessment of chemicals using soil microbial communities

Kristian K. Brandt*[1]

[1]Department of Plant and Environmental Sciences, University of Copenhagen – Thorvaldsensvej 40
DK-1871 Frederiksberg, Denmark

## Abstract

Current environmental risk assessment (ERA) schemes aim to protect the biodiversity and ecological functioning of ecosystems, but ecosystem protection goals are only vaguely described in current ERA guidelines. Ongoing revisions of these guidelines focus on the needs for more specific ecosystem protection goals based on the ecosystem service concept. Consequently, key ecosystem services provided by microorganisms and their key drivers must be identified and experimentally assessed in order to establish improved microbial ecotoxicological assessment protocols useful for ERA. Based on recent and ongoing work performed in my research group in collaboration with other groups in Denmark, Sweden and France, I here propose a new approach for microbial community-based toxicity testing of chemicals (toxic metals, petroleum-derived hydrocarbons, antibiotics, biocides, and fungicides) in soil. The test scheme can be carried out in soil microcosms as outlined in current OECD protocols, but with a significantly expanded set of endpoints to evaluate effects on microbial community function, community structure, and pollution-induced community tolerance while at the same time accounting for chemical fate of the test compound(s). In essence, the proposed test scheme offers a tiered, polyphasic systems approach using sensitive techniques for measurement of bacterial and fungal *in situ* growth dynamics (1st tier) and comprehensive microbial community characterization (2nd tier) to account for effects on microbial diversity and resistance development. Our results reveal that different classes of toxicants, and sometimes even closely related compounds (e.g. isothiazolinones), can affect soil microbial communities in highly contrasting ways highlighting the need for the proposed polyphasic community approach.

**Keywords:** Antibiotics, Biocides, Copper, Zinc, Pesticides, Petroleum hydrocarbons, Soil microbial ecology, Molecular community analysis, Leucine incorporation, Acetate, in, ergosterol incorporation, PICT

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*Speaker

# Dissipation of chloroacetanilides in agricultural soil microcosms: insights from stable isotope fractionation and bacterial community response

Ehssan Torabi[1], Charline Wiegert[2], Stéphane Vuilleumier*[3], and Gwenaël Imfeld†[4]

[1]Department of Plant Protection, University College of Agricultural Natural Resources, University of Tehran, Karaj – Iran

[2]Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), UMR 7517 CNRS, and Department of Microbiology, Genomics and the Environment, UMR 7156 CNRS – université de Strasbourg – France

[3]Department of Microbiology, Genomics and the Environment, UMR 7156 CNRS, Université de Strasbourg, France (UMR 7156 CNRS) – université de Strasbourg – 28 rue Goethe 67000 Strasbourg, France

[4]Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), UMR 7517 CNRS – université de Strasbourg – France

## Abstract

Studying the degradation of pesticides and their effects on microbial populations in agricultural soils promises to provide insights into predicting the fate and ecotoxicity of agrochemicals. Dissipation and biodegradation of two widely applied chloroacetanilide herbicides butachlor and *S*-metolachlor were evaluated in two different agricultural soils (paddy and crop) using compound-specific isotope analysis (CSIA) and 16S rRNA gene based bacterial community analysis (Illumina MiSeq). CSIA results revealed that most dissipation (80-90%) and carbon fractionation of butachlor and *S*-metolachlor occurred within day 0-30 (bulk= -1.8 to -3.2) and 15-60 (bulk= -2.3 to -4.8) days following pesticide treatment, respectively, regardless of soil type or treatment dose (15 and 150 $\mu$g.g-1). Half-lives determined for the two pesticides at the two application rates of 15 $\mu$g g-1 and 150 $\mu$g.g-1 ranged between 7-17 and 12-42 days, respectively. Changes in bacterial community profiles were investigated by NDMS and analysis of similarities (ANOSIM). Soil type and incubation time were the main drivers of bacterial community composition, and no significant effect of pesticide type or spiking dose could be observed ($p < 0.01$). In paddy soil (flooded/anoxic conditions), the bacterial community was mainly dominated by anaerobic populations of Clostridiales and sulfate/sulfur reducers such as Desulfuromonadales and Syntrophobacterales. In the crop soil (20% moisture/oxic conditions), the majority of taxa belonged to Alphaproteobacteria, Actinobacteria and Bacillales. Obtained results provide new knowledge about the biodegradation of butachlor and *S*-metolachlor and corresponding changes in bacterial soil communities under different conditions and pesticide application regimes.

**Keywords:** Chloroacetanilide, CSIA, enrichment factor, bacterial communities, half, life

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*Speaker

†Corresponding author: imfeld@unistra.fr

# Insights into flexibility of Rhodococci in transformation of PCBs

Ines Petric*†[1], Vlasta Drevenkar[2], and Fabrice Martin-Laurent[3]

[1]Rudjer Bosković Institute (RBI) – POB 180, 10002 Zagreb, Croatia
[2]Institute for Medical Research and Occupational Health (IMI) – Ksaver street 2, Zagreb, Croatia
[3]Agroécologie, AgroSup Dijon, INRA, Université de Bourgogne Franche-Comté (INRA Dijon) – Institut national de la recherche agronomique (INRA) – 17 Rue Sully, 21000 Dijon, France

## Abstract

Perhaps one of the most known POPs, polychlorinated biphenyls (PCBs), comprise a family of about 200 related compounds. Even though the manufacture and sale of PCBs were banned decades ago, they are continuously entering environment, mainly *via* point source pollution due to inadequately managed hazardous waste sites, by leaching from electrical equipment or during treatment of contaminated wastes. Microbial biodegradation of PCBs has been a subject of intensive research but until now, most of our knowledge on PCBs metabolism come from studies carried out on *Burkholderia xenovorans* LB400 and *Rhodococcus* sp. RHA1, two PCBs degraders whose genomes have been fully sequenced. Our knowledge on enzymes involved in PCB metabolism remains fragmentary and diversity of bacterial metabolisms in transforming PCBs is highly underestimated. In order to explore the metabolic pleiotropy of the *Rhodococci* we settled an experiment aiming at monitoring the transformation of structurally different PCB congeners (2,4,4'-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl and 2,4,3'-trichlorobiphenyl) in four *Rhodococci* isolated from PCB-contaminated environments. These strains showed strong ability to transform various PCBs. Transformation products were detected by gas-chromatography and identified by mass spectrometry on aliquots of microbial cultures. We propose that bacteria belonging to the genus *Rhodococcus* do not only transform PCBs using the classical *bph* pathway, but also through a complex interplay of different catabolic pathway modules. Our study suggests that the PCB catabolic potential of *Rhodococci* is not yet fully understood.

**Keywords:** PCBs, biotransformation, catabolic pathway, intermediates, Rhodococcus, GC, MS

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*Speaker

†Corresponding author: ipetric@irb.hr

# Capacity of leaf-associated microorganisms to biotransform pesticides in rivers

Louis Carles*[1,2], Florent Rossi[1], Muriel Joly[1,2], Pascale Besse-Hoggan[2], Isabelle Batisson[1], and Joan Artigas[1]

[1]Laboratoire Microorganismes : génome et environnement (LMGE) – CNRS : UMR6023, Université Clermont Auvergne – 63000 Clermont-Ferrand, France
[2]Institut de Chimie de Clermont-Ferrand (ICCF) – CNRS : UMR6296, Université Clermont Auvergne, SIGMA Clermont – 63000 Clermont-Ferrand, France

## Abstract

Leaf-associated microbial communities, and especially fungi, possess a large panel of enzymes permitting the breakdown of leaf polymers as well as the transformation of xenobiotic compounds. This study aims (i) to assess the potential of leaf-associated microbial communities, exhibiting different exposure histories (upstream site, less polluted *versus* downstream site, more polluted), to biotransform three maize herbicides (mesotrione, S-metolachlor and nicosulfuron) in single and cocktail molecule exposures, and (ii) to identify the microorganisms involved in these biotransformations. The results showed a significant dissipation of nicosulfuron (29 % to 66 %, day 40) in both single and cocktail exposures, respectively, but not of mesotrione and S-metolachlor. The formation of nicosulfuron metabolites such as ASDM (2-(aminosulfonyl)-N,N-dimethyl-3-pyridinecarboxamide) and ADMP (2-amino-4,6-dimethoxypyrimidine) confirmed the transformation of this molecule by leaf-associated microorganisms. In addition, the downstream communities showed a greater ability to transform nicosulfuron than the upstream communities suggesting that exposure history to pesticides can enhance the biotransformation potential of microorganisms. After 40-day single exposure to nicosulfuron, the downstream communities were also those experiencing the greatest shifts in fungal and bacterial community diversity. Specifically, the fungal strain (*Plectosphaerella cucumerina*) isolated from leaves was able to biotransform 100 % of nicosulfuron (25 mg L-1, in 14 days of incubation). Although leaf-associated fungal communities are known to mostly feed on litter constituents, the alternative use of xenobiotic compounds transported by flowing waters place fungi as good candidates for pesticide removal in polluted rivers.

**Keywords:** leaf, associated microorganisms, biodegradation, mesotrione, nicosulfuron, S, metolachlor, herbicide mixture

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*Speaker

# Micropollutant biotransformation potential of natural river biofilms

Werner Desiante*†[1], Bernadette Vogler[1], Cresten Mansfeldt[1], Stephanie Merbt[1], and Kathrin Fenner‡[1]

[1]Eawag – Überlandstrasse 133 8600 Dübendorf, Switzerland

## Abstract

Micropollutants, such as pesticides and pharmaceuticals, are released through several pathways into our environment and can pose non-negligible risks to aquatic organisms. Aquatic microbial communities exposed chronically to micropollutants can change significantly in terms of biodiversity and can gain an increase in community-tolerance. These findings raise the question what driving factors lead to such community changes. Since an improved ability to rid themselves of micropollutants could be a possible tolerance mechanism, in our research we investigate the potential of differently adapted natural river biofilms to biotransform a large mixture of environmentally relevant polar micropollutants.
Biofilm has been cultivated in an indoor channel system, fed with river water from a suburban, treated wastewater-receiving stream in the Zurich area. Suspensions of this biofilm were used to seed a series of batch reactors and spiked with a mixture of 43 micropollutants (1 $\mu$g/L). Micropollutant concentrations were monitored over four days.

Seventeen of the 43 micropollutants showed decreasing concentrations over time, of which the abatement of eleven parent compounds could be attributed to biotransformation, whereas for the remaining six compounds, which all contained amine functional groups, concentration time patterns pointed towards intracellular ion-trapping.

The eleven chemicals showing potential biotransformation were found to belong to distinct chemical classes, including sulfonamide antibiotics, primary or secondary amide-containing compounds and substances containing carboxylic ester groups.
In an on-going study, the same approach is applied to investigate the biotransformation ability of biofilms grown up- and downstream of treated wastewater effluents, respectively, for two medium-sized rivers in central Switzerland.

**Keywords:** Biotransformation, Natural river biofilm, Periphyton, Micropollutants, Pollutant fate

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*Speaker

†Corresponding author: werner.desiante@eawag.ch

‡Corresponding author: kathrin.fenner@eawag.ch

# Hydrocarbon impact on microbial communities in marine coastal sediments

Robert Duran*[1], Cyril Noel , Cristiana Cravo-Laureau , and Christine Cagnon

[1]Equipe Environnement et Microbiologie - IPREM UMR CNRS 5254 (EEM) – CNRS : UMR5254, Université de Pau et des Pays de l'Adour [UPPA] – IBEAS - UFR Sciences BP 1155 64013 PAU CEDEX, France

## Abstract

To date studies of hydrocarbon biodegradation have focused mainly on simplified systems (e.g. pure strains, individual molecules), which are not representative of natural environments. As a result, little is known about whole microbial communities in their actual context, and the impact of environmental fluctuations on pollutant degradation and microbial community structure is insufficiently investigated. Notably, little is known as yet about degradation within the transitional zone from oxic to anoxic conditions. It is thus important to determine the behavior of microbial communities in such oscillating conditions, especially the microbial populations involved on anaerobic metabolisms such as sulfate-reducing microorganisms.
In order to decipher the mechanisms underlying microbial community organization after an oil spill, we develop experimental ecology approaches to manipulate the microbial communities. After an adaptive stage, the modification of the microbial community structure occurred, concomitant with the beginning of the degradation of hydrocarbon compounds, followed by a succession of bacterial community structures along the degradation process. Submitting sediments to different oxygenation regimes in bioreactors showed the influence of the oxygenation on hydrocarbonoclastic microbial-assemblages, which was favoured by oxygenation after a period of anoxia. Surprisingly, sulfate-reducers were not affected by oscillating conditions.

Applying different strategies of experimental ecology, our studies highlight the functional redundancy of microbial communities involved in hydrocarbons degradation. The description of the microbial communities functioning under different oxygenation regimes by omics approaches will allow to answer important ecological questions for understanding the mechanisms underpinning the bacterial communities assemblages.

**Keywords:** mesocosm, microbial ecology, oil biodegradation, intertidal sediments, experimental ecology, omic's approaches

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*Speaker

# Selective colonization processes of plastic-loving bacteria in an abandoned landfill site

Edoardo Puglisi*†[1], Serena Galletti*[1], Francesco Romaniello*[1], Jie Feng[2], Alberto Frache[2], and Piersandro Cocconcelli‡[1]

[1]Istituto di Microbiologia, Università Cattolica del Sacro Cuore (UCSC) – Via Emilia Parmense 84, 29122 Piacenza, Italy, Italy

[2]Dipartimento Scienza Applicata e Tecnologia, Politecnico di Torino – Viale Teresa Michel 5, Alessandria, Italy, Italy

## Abstract

Polyethylene (PE), the most common petroleum-based plastic, is strongly recalcitrant to degradation. Despite this, recent evidences showed that a few microbial strains can partially degrade PE pre-oxidized by abiotic processes.
Here, we investigated plastic samples from an abandoned landfill, with the aim of elucidating the ecology of soil microorganisms able to grow on the plastics and to correlate them with plastic degradation levels.

Isolations were carried after detachment of the biofilm from the plastic surfaces and serial dilutions on selective agar plates of mineral medium with paraffin as the only carbon source. Microbial DNA derived from the plastic surfaces, the landfill soil and an uncontaminated neighboring agricultural soil was also analyzed by Illumina sequencing of 16S rRNA amplicons.

A total of 49 strains growing on paraffin were identified: the most representative genus is *Bacillus* genus with 12 different species represented, followed by *Pseudomonas*, *Lysinibacillus*, *Comanomonas* and *Acinetobacter*.
Differential scanning calorimetric analysis of the plastic samples showed an additional shoulder melting peak with various enthalpies besides the main melting peak, implying the plastic exhibited different degrees of degradation. In parallel, Illumina 16S rRNA analyses showed that the different plastics hosted different microbial communities, and that the bacterial community derived from plastic sample with the highest degradation level was the most similar to that of the soil derived from contaminated landfill. Results point to an interesting correlation between the level of plastic degradation and the composition of the microbial biofilm.

**Keywords:** polyethylene, biofilm, colonisation, Illumina

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*Speaker

†Corresponding author: edoardo.puglisi@unicatt.it

‡Corresponding author: pier.cocconcelli@unicatt.it

# Dynamics of bacterial communities in a field-scale pilot treating As-rich acid mine drainage

Elia Laroche*[1,2], Corinne Casiot[1], Lidia Fernandez-Rojo[1], Vincent Tardy[1], Angélique Désoeuvre[1], Gérard Grapin[3], Julie Savignac[3], Jolanda Boisson[4], Fabienne Battaglia-Brunet[2], Catherine Joulian[2], and Marina Héry[1]

[1]HydroSciences Montpellier (HSM) – CNRS : UMR5569, IRD, Université Montpellier II - Sciences et techniques – 163, rue Auguste Broussonet, 34090, Montpellier, France

[2]Unité Biogéochimie environnementale et qualité de l'eau (BRGM) – Bureau de Recherches Géologiques et Minières (BRGM) – 3 avenue Claude-Guillemin BP 36009 45060 Orléans Cedex 2, France

[3]Institut des Ressources Humaines Ingénieur Conseil – IRH – 427 rue Lavoisier - CS 50155, 54714, Ludres Cedex, France

[4]Institut des Ressources Humaines Ingénieur Conseil – IRH – 197 avenue de Fronton, 31200, Toulouse, France

## Abstract

Arsenic rich AMDs (Acid Mine Drainages) represent a major source of pollution for aquatic ecosystems. Microbially driven iron (Fe) and arsenic (As) oxidation and precipitation represent a promising strategy to treat this pollution. A better understanding of the biogeochemical mechanisms involved is required prior any further exploitation of this microbial potential. A field-scale pilot was implemented at the Carnoulès mine (France) for the treatment of AMD. It is an ergonomic and passive aerobic system: five treatment units of 1.5 m2 are combined vertically in series and fed with the AMD water by gravitation flow. Biogenic precipitates (corresponding to Fe- and As-rich biofilms) covered the bottom of the units. Inlet water and biogenic precipitates were collected over a 7 months period. We determined the bacterial community structure in the precipitates by fingerprint (ARISA), metabarcoding (16S rRNA gene) and qPCR targeting arsenite oxidase gene *aioA*. Chemical and mineralogical analyses were conducted on the precipitates and on the feed water. The bacterial communities in the precipitates developed from the indigenous communities of the AMD used to feed the pilot. Our results showed an evolution of these communities over time associated with an increase of the potential genetic for As oxidation. The proportion of As(V) in the precipitates and arsenic removal efficiency fluctuated, with maximum levels of 99% and 97 % respectively. This work provided information about microbial dynamics and pollution removal efficiency in a treatment pilot under field conditions. It will serve for future design of a bioremediation system to treat As-rich AMD.

**Keywords:** bioremediation, arsenic, acid mine drainage, engineering, iron, arsenic, oxidizing bacteria

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[*] Speaker

# Insights into uranium tolerance of Microbacterium oleivorans A9 by proteogenomic analyses

Nicolas Gallois*†[1], Béatrice Alpha-Bazin[2], Philippe Ortet[3], Mohamed Barakat[3], Justine Long[3], Laurie Piette[1], Séverine Zirah[4], Adrienne Kish[4], Jean Armengaud[2], Catherine Berthomieu[1], and Virginie Chapon[1]

[1]Laboratoire des Interactions Protéine-Métal (CEA/BIAM/LIPM) – CEA Cadarache (Saint Paul-lez-Durance) – France
[2]Laboratoire Innovations technologiques pour la Détection et le Diagnostic (Li2D) – Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble – France
[3]Laboratoire d'écologie microbienne de la rhizosphère et d'environnements extrêmes (LEMiRE) – CEA Cadarache (Saint Paul-lez-Durance) – France
[4]Molécules de Communication et Adaptation des Micro-Organismes (MCAM) – CNRS : UMR7245, Musée National d'Histoire Naturelle - MNHN (France) – 57 rue Cuvier 75005 Paris, France

## Abstract

*Microbacterium oleivorans* A9 is a uranium-tolerant actinobacteria isolated from the trench T22 located near the Chernobyl nuclear power plant (Chapon *et al.* 2012). This site is contaminated with different radionuclides including uranium. Under uranium exposure, cells of *Microbacterium oleivorans* A9 exhibit three sequential mechanisms involved in uranium detoxification: a rapid metal removal within the first 30 minutes, then an active U(VI) release in the exposure medium and a final biomineralization step of uranium in autunite-like mineral phases (Theodorakopoulos *et al.* 2015). Using an innovative proteogenomic approach, we explored the molecular changes at the proteome level occurring in this strain upon uranyl exposure. We interpreted high-throughput proteomic data (1,221,282 spectra) against a six-reading frame ORF database deduced from the draft genome (Ortet *et al.* 2017), validated the identification of 1,532 proteins, identified these proteins and compared protein abundances from cells exposed or not to uranyl stress. These data show that a complex cellular response to uranium occurs in *Microbacterium oleivorans* A9. The abundance of 591 proteins was significantly different between conditions. In particular, the uranyl stress perturbed the phosphate and iron metabolisms pathways. A large ratio of proteins more abundant upon uranyl stress, are distant from functionally-annotated proteins, highlighting the lack of fundamental knowledge regarding numerous key molecular players from soil bacteria. Furthermore, several transporters have been identified to be specifically associated to uranyl stress, paving the way to the development of biotechnological tools for uranium decontamination.

**Keywords:** uranyl stress, Microbacterium, proteogenomic, radionuclides toxicity

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*Speaker

†Corresponding author: nicolas.gallois@cea.fr

# Influence of plastic litters on marine microbial life

Claire Dussud* , Leila Meistertzheim , Matthieu Georges , Pascale Fabre , Maria-Luiza Pedrotti , Gaby Gorsky , and Jean-François Ghiglione*†[1]

[1]Laboratoire d'Océanographie Microbienne (LOMIC) – CNRS : UMR7621, Université Pierre et Marie Curie (UPMC) - Paris VI – Observatoire Océanologique, Banyuls/mer, France

## Abstract

The Mediterranean Sea is one of the most plastic-polluted zone and offer a unique opportunity to evaluate the ecotoxicity of plastic marine debris (PMD) on bacterioplankton lifestyle. The large set of samples taken during the TARA expedition revealed for the first time a clear niche partitioning between the PMD compared to the organic particle-attached (PA) and the free-living (FL) bacteria. Bacterial counts in PMD presented higher enrichment factors than generally observed in PA, as compared to the abundance of FL bacteria in the surrounding waters. New generation sequencing showed that taxonomic diversity was also higher in the PMD communities, where higher evenness indicated a favorable environment for a very large number of species. Cyanobacteria were particularly overrepresented in PMD, together with essential functions for biofilm formation and maturation. The consistent distinction of community structure and functional traits between the three lifestyles, that exceeded the large-scale geographical variation in the Western Mediterranean basin, support a new trade-off for the maintenance of a large biodiversity of bacterioplankton in the Oceans. Bacterial production and ectoenzymes revealed also a higher activity on PMD, thus suggesting that this community take significant part of the global biogeochemical cycles in the Oceans.

**Keywords:** marine plastic pollution / microbial ecotoxicology / biofouling

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*Speaker

†Corresponding author: ghiglione@obs-banyuls.fr

# Transfer and distribution of diuron in biofilms and joint toxic effects

Betty Chaumet*[1], Julie Neury-Ormanni[1], Caroline Doose[2], Brigitte Delest[1], Joan Artigas[3], Nicolas Mazzella[1], and Soizic Morin[1]

[1]Laboratoire Ecosystèmes aquatiques et changements globaux (UR EABX) – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA – 50 avenue de Verdun, 33612 Cestas cedex, France

[2]Institut National de la Recherche Scientifique - INRS (CANADA) – Canada

[3]Microorganismes : génome et environnement (LMGE) – Université Blaise Pascal - Clermont-Ferrand II – Université Blaise Pascal, Campus des Cézeaux, 24, avenue des Landais BP 80026 63 170 AUBIERE, France

## Abstract

Biofilms are composed of microalgae and other microorganisms embedded in a polysaccharidic matrix (EPS). We developed a new approach dedicated to the determination of pesticide transfer and distribution into the matrix. This approach is based on the physical separation, then analysis of the different matrix compartments: pore water, EPS and microorganisms. The aim of this study is to obtain a better understanding of mechanisms taking part into pesticide bioaccumulation on biofilms. In parallel, toxicokinetic are coupled with toxicodynamic studies, in order to investigate the relationship between accumulation rate and joint effects of herbicides.

In this study, we determined the accumulation and distribution of diuron, a photosynthesis inhibitor. Simultaneously, we characterized biofilm biomass (dry weight, total organic carbon), protein and polysaccharide contents, coupled with the assessment of functional effects on enzymatic activities ($\beta$-Glucosidase and $\beta$-Glucosaminidase) and photosynthetic activity. After one month of colonization in Gazinet-Cestas pond (France) on glass slides, biofilms were exposed for 21 days in channels with three conditions: control, and 2 contaminated with 5 and 50 $\mu$g.L-1 of diuron, respectively.

Pesticide quantification in biofilm showed that diuron was quickly accumulated (i.e. after 15 minutes of exposure) in both EPS and cells, highlighting early distribution in the whole biofilm, followed by cell internalization. However, photosynthesis was clearly inhibited from 15 minutes until 21 days in the biofilms exposed to 50 $\mu$g.L-1 of diuron, as an expectable result of its mode of action. All these results provide an innovative basis to develop a pesticide toxicokinetic and toxicodynamic model for biofilms.

**Keywords:** Biofilm, Diuron, Bioaccumulation

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*Speaker

# Morphological plasticity in Chlamydomonas reinhardtii: an adaptive response to micropollutant stress

Giulia Cheloni*[1], Michel Goldschmidt-Clermont[1], and Vera Slaveykova[1]

[1]University of Geneva (UNIGE) – Switzerland

## Abstract

Phytoplankton are characterized by a great phenotypic plasticity and amazing morphological variability, both playing a primary role in the acclimation to changing environments. However only few data are available about morphological variability as adaptive responses to stress. The results that we present here highlight palmelloid colony formation of the green alga *Chlamydomonas reinhardtii* upon exposure to micropollutants.
*C. reinhardtii* was exposed to four micropollutants with different modes of action (copper, cadmium, PFOS and paraquat) for up to 96h. Effects on growth, cell traits (size, chlorophyll fluorescence), membrane integrity and oxidative stress were monitored daily via flow cytometry and fluorescence microscopy.

Colony formation was observed after 48h exposure to the four micropollutants. The number of cells forming the colony increased daily to reach up to 16 cells after 96 hours incubation. Microscopic observation showed the cells to be encased in a multilayer envelope, suggesting that the colony formation might be associated to the lack of hatching from the mother cell wall. When colonies were harvested, rinsed and inoculated in fresh medium without micropollutants *C. reinhardtii* cells reverted to their unicellular lifestyle. No effects on chlorophyll fluorescence, membrane integrity or oxidative stress were observed in cells forming colonies. In contrast such effects were observed at higher concentrations of the same micropollutants, suggesting that colony formation might be a stress response activated to face sub lethal micropollutant concentration.
From an ecotoxicological perspective, such influences on phytoplankton morphological plasticity might have important consequences on feeding dynamics at higher levels of the trophic chain.

**Keywords:** Phenotypic plasticity, adaptive stress response, palmelloid colonies, C. reinhardtii

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*Speaker

# Genome-wide analyses of rare earth elements responsive genes in microbial model organisms

Nicolas Grosjean*[1,2], Marie Le Jean[2], Elisabeth Gross[2], and Damien Blaudez[1]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Faculté des Sciences et Technologies, Université de Lorraine F-54506 VANDOEUVRE-LES-NANCY, France

[2]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Campus Bridoux - Rue du Général Delestraint, Université de Lorraine F-57070 METZ, France

## Abstract

The Rare Earth Elements (REEs) group gather 17 chemical elements covering 15 lanthanides (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu), Yttrium and Scandium. As part of the Technical Critical Elements (TCEs), they are of high importance in high-tech domains such as renewable energies, aeronautics, new communication technologies, and medicine. Although many investigations have been carried out to find innovative ways to supply the rising demand for these elements, the effects of the increasing release of these new emerging contaminants in the environment is poorly documented. Furthermore, the few existing studies on biota often mention controversial effects and also highlight the lack of knowledge on the impact of REEs at the cellular level. Among others, REE-mediated oxidative stress and modification of membrane permeability have been reported while almost nothing is known at the molecular level. Thus, we adopted high throughput strategies to study REE stress responses on several model organisms. These strategies consisted, on the one hand, of the screening of genome-wide mutant collections on several REEs, and on the other hand of transcriptional analyses (RNA-seq) under REE stress. These two large-scale methods allowed the identification of key genes, proteins or processes involved in a REE stress and/or their translocation in organisms (e.g. membrane transport). The results obtained from yeast and bacteria exposed to various REEs will be discussed, highlighting the potential impact of REEs on these organisms.

**Keywords:** Rare Earth Elements, Emerging contaminants, Bacteria, Yeast, RNA seq, Genomic phenotyping

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*Speaker

# Novel nanopesticides introduce new challenges to microbes in agro-ecosystems and downstream wetlands

Marie Simonin*[1], Steven Anderson[1], Christina Bergemann[1], Jennifer Rocca[1], Benjamin Colman[2], and Emily Bernhardt[1]

[1]Duke university – Durham, NC 27708, United States
[2]University of Montana – United States

## Abstract

Widespread application of engineered nanoparticles for the purpose of crop protection – termed nanopesticides – are useful for agriculture, but little is known about the potential environmental impact on agro-ecosystems and downstream ecosystems, including wetlands. Here, we assessed the impact of a copper hydroxide nanopesticide on microbial community function and diversity in two large-scale mesocosm experiments. The first mesocosm experiment simulated the realistic application of the nanopesticide to an agro-ecosystem over a 6-month growing season. The second mesocosm study tested nanopesticide effects on wetlands with chronic weekly additions of low realistic concentrations over nine months. In these experiments, we tested the interactive effects of nanopesticides and nutrient enrichment treatment, as nanopesticide exposures will often co-occur with fertilizer enrichment. We hypothesized that resource availability will influence the resistance and resilience of microbial function to nanopesticides.

In the agro-ecosystem mesocosms, the eight measured microbial extracellular enzyme activities (EEAs) on soils were suppressed (-41 to -71%, $P< 0.01$) within 15 days under ambient nutrient conditions. In contrast, no negative effects were detected in fertilized mesocosms, but instead a stimulation of EEAs (+20 to +51%, $P< 0.01$) was observed after the third application at five months. In the wetland mesocosms, the microbial EEAs were decreased in the sediment and periphyton samples in both nutrient conditions after 6 months of exposure, but recovered after 9 months.

These findings suggest that nanopesticides could have more lasting effects in downstream ecosystems than in agro-ecosystems and that nutrient availability influence the ecosystem impacts of this emerging contaminant.

**Keywords:** Agro, ecosystem, nanomaterials, aquatic ecosystems, nutrient pollution, chronic exposure, Mesocosms

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*Speaker

# Diversity and functions of microbial communities in historically multi-contaminated soils

Florian Lemmel*†[1], Florence Maunoury-Danger[2], Corinne Leyval[1], and Aurélie Cebron[1]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360 – Faculté des Sciences et Technologies, BP70239, 54506 Vandoeuvre-lès-Nancy, France
[2]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Campus Bridoux, Avenue du général Delestraint, 57070 Metz, France

## Abstract

The intensive industrial activities of the 20th century in Lorraine Region (France), conducted to highly contaminated wasteland soils after coking plant dismantling. The organic and metallic multi-contamination may affect the microbial communities that are key actors of soil functioning and biogeochemical cycles. The aims of our study was to: i) evaluate microbial functional diversity in historically multi-contaminated soils, focusing on functions of the carbon cycle, and ii) determine how edaphic properties or pollutant concentrations contribute to shape microbial community. We hypothesized that high soil contamination would conduct to lower microbial diversity.

We worked on a range of 10 soils that were characterized for physicochemical (C, N and pollutant concentrations) and microbiological (microbial abundance using MPN and qPCR and microbial taxonomic diversity through Illumina MiSeq sequencing) properties. Microbial metabolic capacity, i.e. carbon substrate degradation rates using Biolog$^{\text{TM}}$ and MicroResp$^{\text{TM}}$ tools, were used as indicators of the functional diversity. Pollution induced community tolerance (PICT) was also tested after zinc input to soils.

Physicochemical properties discriminated the soils in 3 groups corresponding to their history (steel crusher; sludge ponds; old gravel pit and forest) while microbiological properties did not discriminate the soils in the same way. The functional diversity partly depends on the edaphic parameters such as the C/N ratio that was correlated with the basal mineralization and the substrate induced respiration yields. The pollution also shapes the microbial community because the highly polluted soils harboured the lowest metabolic diversity and were less sensitive to a metallic stress induced by zinc input.

**Keywords:** soils, wastelands, multipollution, microbial functions, biolog, microResp

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*Speaker

†Corresponding author: florian.lemmel@univ-lorraine.fr

# Long-term effects of triclosan on community function, community tolerance and metagenomic composition in marine periphyton communities

K. Martin Eriksson*[1], Tobias österlund , Sanna Abrahamsson, , Henrik Johansson , Natàlia Corcoll , Kemal Sanli , Thomas Backhaus , Hans Blanck , and Erik Kristiansson

[1]K. Martin Eriksson (K.M. Eriksson) – Chalmers University of Technology Dept. of Shipping and Marine Technology 41296 Gothenburg Sweden, Sweden

## Abstract

This study presents data from a long-term flow-through microcosm experiment, describing the toxicity of triclosan (TCS) to natural marine periphyton communities. We measured the functional endpoints photosynthesis, bacterial carbon utilization, photosynthetic pigment content, as well as Pollution-Induced Community Tolerance (PICT) to TCS. In addition, we performed large-scale shotgun DNA sequencing (metagenomics), which resulted in detailed descriptions of TCS effects on taxonomic composition (16S and 18S marker genes) and composition of biological functions (protein-coding genes). These measurements enables us to compare how community functions, metabolic functions and taxonomic composition were affected by TCS. Results from functional endpoints showed that TCS stimulated photosynthesis and pigment content at concentrations of 31.6 nM, a change that co-occurred with an increase in PICT. TCS did not, however, affect the functional diversity or the composition of bacterial carbon utilization. In contrast to these results, the DNA sequencing results showed that both bacterial and eukaryote community composition was changed by TCS concentrations above 10 nM. These changes included *e.g.* increases in *Gammaproteobacteria* and decreases of other bacterial groups, as well as increases and decreases of specific diatom taxa. Also, changes in abundance of specific biological functions was detected at concentrations of 3.16 nM and higher. These included, for example, increases carbohydrate metabolism and transport genes and decreases photosynthesis genes. Overall, the study shows that the endpoints employed have different capabilities to detect effects from TCS. It also shows that functional redundancy and indirect effects from TCS are present in the complex biofilm communities studied here.

**Keywords:** metagenomics, shotgun sequencing, 16S, 18S, protein coding genes, PICT, pigment, triclosan, biofilm

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*Speaker

# Responses of microbial communities in mangrove ecosystem under the influence of pretreated wastewater discharges

Cécile Capdeville*†[1], Jonathan Gervaix[2], François Fromard[1], Jean-Luc Rols[1], Thomas Pommier[2], and Joséphine Leflaive[1]

[1]Laboratoire Ecologie Fonctionnelle et Environnement (EcoLab) – Université Paul Sabatier - Toulouse III – France

[2]Ecologie microbienne – Université Claude Bernard - Lyon I – France

## Abstract

Mangrove forests are coastal ecosystems continuously affected by various environmental stresses but also under the influence of anthropogenic pressures. The aim of this study was to evaluate the capabilities of microbial communities in sediments to respond to these anthropogenic pressures in function of the level of natural stress they are subjected to. We used an *in situ* experimental system set up in Mayotte Island where mangrove is exposed since 2007 to pretreated wastewater discharges (WD) to study its bio-epuration capability. Six plots in two mangrove zones with different environmental constraints were exposed to WD either for several years or since the beginning of the present study or not exposed. In two additional plots WD have been stopped to assess the resilience capacities of microbial communities. Sediments have been sampled after 1, 3, 8 and 12 months in 4 subplots per plot. Since wastewaters were concentrated in ammonium, we focused on communities involved in N-cycle. Microorganisms densities have been estimated by qPCR and the denitrification activity (DEA) evaluated by potential activity measurements. The composition of microbial photosynthetic communities at sediment surface was assessed by HPLC.

Long-term WD significantly impacted the composition of phototrophic microorganisms and increased the density of microorganisms and DEA in both mangrove zones. The resilience seemed to be faster on the less constrained mangrove zone. Within less than 8 months, the DEA returned to the same level than control plots. These results confirm the high potential of mangrove for bio-epuration, associated with strong resilience properties of microbial communities.

**Keywords:** Mangrove ecosystem, multiple disturbance, in situ experimental system, microbial community, Ncycle, resilience, wastewater discharge, natural environmental stress

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*Speaker

†Corresponding author: cecile.capdeville1@univ-tlse3.fr

# Do anthropogenic stressors impact the functional performance of leaf-degrading aquatic fungi? A case study using microcosms, fungicides, and modern biochemical tools

Patrick Baudy*[1], Nina Röder[1], Marco Konschak[1], Jochen Zubrod[1], Christiane Baschien[2], Ralf Schulz[1], and Mirco Bundschuh[3]

[1]Institute for Environmental Sciences, University of Koblenz-Landau – Fortstraße 7, 76829 Landau, Germany

[2]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH – Inhoffenstraße 7B, 38124 Braunschweig, Germany

[3]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences – Lennart Hjelms väg 9, 75007 Uppsala, Sweden

Session
FUNCTION
Talk

## Abstract

Aquatic hyphomycetes – a polyphyletic group of true fungi – play a key role in leaf-litter breakdown as they contribute substantially to the leaf mass loss while increasing the nutritional value of leaf material as food source for detritivorous invertebrates. Despite their ecological significance, information about their communities' functional composition is scarce, particularly under stress. In order to address this knowledge gap, we conducted a microcosm experiment with four common aquatic hyphomycete species (*Alatospora acuminata, Heliscella stellata, Neonectria lugdunensis*, and *Tetracladium marchalianum*) in every possible mono, binary, and quaternary combination. These combinations were exposed towards a model stressor, namely a five-component fungicide mixture over 14 days. Surprisingly, in monocultures, only *N. lugdunensis* and *T. marchalianum* were capable of degrading leaf material and they even maintained this capability up to high fungicide concentrations of 500 and 2,500 $\mu$g/L, respectively. Moreover, preliminary analyses of the decomposition data indicated non-additive species interactions in binary and quaternary culture combinations, which were influenced in presence of different fungicide-mixture concentrations. To gain a mechanistic understanding of the responses at the functional level, we are currently using modern biochemical tools including species-specific quantitative real-time polymerase chain reaction assays as well as enzyme activity assays (diverse hydrolases and phenol oxidase). Our preliminary results, nonetheless, point towards stressors' potential to alter aquatic hyphomycete communities' functional composition (due to an altered functional potential of single species and/or shifts in the structure of the communities). Thus, consequences for heterotrophic food webs due to their bottom-up directed regulation are likely.

**Keywords:** aquatic hyphomycetes, extracellular enzymes, fungicides, leaf decomposition, qPCR

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*Speaker

# Proteomic responses to nanoparticulate and ionic silver in fungi from metal-polluted and non-polluted streams

Diana Barros*†[1], Arunava Pradhan[1], Cláudia Pascoal[1], and Fernanda Cássio[1]

[1]Centre of Molecular and Environmental Biology (CBMA) – Institute for Science and Innovation for Bio-sustainability (IB-S) University of Minho, 4710-057 Braga, Portugal

## Abstract

Enhanced production and usage of silver nanoparticles (AgNPs) raise concerns about their potential impacts in aquatic ecosystems. Effects of AgNPs and Ag+ were assessed based on the variations in the overall proteome and the activities of selected antioxidant enzymes in two fungal strains of *Articulospora tetracladia,* one isolated from a non-polluted stream (At72) and the other from a metal-polluted stream (At61). For that, fungi were exposed to concentrations of AgNPs and Ag+ affecting 20% of growth (EC20). A total of 432 proteins were identified, of which 172 belonged to At72 and 260 to At61; 71 were expressed in both strains. At72 had 58% and 52% of the proteins induced by AgNPs and Ag+, respectively. For At61, the percentages were higher (_˜77%). The higher percentages of proteins suppressed by either Ag forms suggest higher stress in At72 than in At61, which is consistent with the background of this fungal strain. Major groups of proteins were related to carbohydrate metabolism, amino-acid and protein biosynthesis. Both Ag forms also induced stress-responsive proteins, including catalase and superoxide dismutase which remained consistent with the profile of their enzymatic activities. These results supported the ability of these fungi in initiating an efficient antioxidant response to cope with Ag-induced toxicity. Overall, the functional proteomic approach can be useful to get a mechanistic insight on the stress induced by AgNPs or Ag+ in aquatic fungi that play a key role in plant litter decomposition in streams.

**Keywords:** AgNPs and Ag+, stress response, functional proteomics, antioxidant enzymes, aquatic fungi.

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*Speaker

†Corresponding author: dianamcbarros@gmail.com

# The Microbiome Stress Project: A database for identifying stressor impacts on microbial community structure across multiple environments

Jennifer Rocca*†[1], Joanna Blaszczak[1], Firas Midani[1], Marie Simonin[1], and Alex Washburne[2]

[1]Duke university [Durham] – Durham, NC 27708, United States
[2]Montana State University – Bozeman, Montana 59717, United States

## Abstract

Understanding the responses of microbial communities to natural and anthropogenic stressors and their interactions is vital for determining ecosystem and host health. The impact of natural stressors (e.g. pH, temperature, storm, fire) and anthropogenic stressors (e.g. synthetic chemicals, land use) on microbial community structure have been extensively studied in various ecosystems. These studies highlight that microbial community composition is extremely sensitive to environmental change but despite an immense amount of data generated, cross-study identification of sensitive and/or tolerant microorganisms has not yet been performed. Therefore, we have constructed an extensive open-source database - The Microbiome Stress Project - to investigate how all types of stressors (natural and anthropogenic) impact microbial community structure across multiple environments (soil, biofilm, water, reactor, gut, sediment). This database is built with published/unpublished microbial data sets (using high-throughput sequencing only) and we are developing a computational pipeline using the R package 'Phylofactor' to identify microbial taxa specifically sensitive or resistant to these stressors. To test the full capacity of the Microbiome Stress Project, we preliminarily set up a smaller proof of concept dataset (8 studies). Specifically, we compared the influence of copper contamination on bacterial community structure in the soil, gut, and an activated-sludge reactor. Additionally, we examined the response of soil bacterial communities to a comprehensive set of stressors (Cu, Hg, antibiotics, PAHs, drought, nanoparticles, fire). This preliminary analysis tested if the relatedness of stressors holds true for their impact on microbial community structure and aimed at identifying indicator species responding ubiquitously to stress.

**Keywords:** Stress, Microbial Community, Microbiome, High, Throughput Sequencing, Diversity, Open Database

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*Speaker

†Corresponding author: jennifer.rocca@duke.edu

# Changes in bacterial communities in response to multicontamination phenomena and other anthropogenic forcing during different hydrological regimes in mediterranean watercourses.

Brice Reoyo-Prats*†[1], Dominique Aubert[1], Olivier Verneu[1,2], and Carmen Palacios[1]

[1]Centre de Formation et de Recherche sur les Environnements Méditérranéens - CEFREM (Perpignan, France) (CEFREM) – Université de Perpignan : VIADOMITIA - UPVD (FRANCE), Centre national de la recherche scientifique - CNRS (France) – France

[2]Univ. for Environmental Sciences and Management – ZA-2520, Potchefstroom, South Africa

## Abstract

Mediterranean contrasted climate affects river dynamics and therefore the concentration of micropollutants encountered in freshwaters. Indeed, contaminants get concentrated in rivers watersheds during dry periods, while, at storm events, they get remobilized from soils and sediments or arrive from combined sewer overflows, leading to recurrent multipollution phenomena. Besides, microorganisms are capable of responding very rapidly to variations in the environment. In this study, we aim at understanding more particularly how aquatic bacteria attached to particles respond to changes in pollutants concentrations. To do so, a total of 60 samplings were carried out on a typically Mediterranean coastal watercourse, the Têt River, from upstream to coastal waters, in two complete drought-flood-drought hydrological cycles along 2013-2014 and 2015-2016. A high frequency monitoring at most impacted stations was also conducted during heavy rain periods, with a total of 27 samplings. Among physico-chemical parameters measurements, up to 350 contaminants have been analyzed including fecal indicators, nutrients, metals, pesticides and pharmaceuticals. A targeted metagenomics analysis with Illumina-based 16S rRNA gene sequencing was performed to study how the diversity of bacterial communities shifts with changes in contaminants concentrations. Multipollution phenomena occurring during storm events had a remarkable impact on bacterial alpha and beta diversity with an increment of the opportunistic phylum Proteobacteria among other changes. Thus, bacteria biofilms attached to suspended matter are able to quickly react to sudden changes in pollutants concentrations, being therefore potential good early signals of multipollution stress.

**Keywords:** Microbial ecotoxicology, multipollution, aquatic ecosystems, Mediterranean, bacterial communities, structural diversity.

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*Speaker

†Corresponding author: brice.reoyo@univ-perp.fr

sciencesconf.org:ecotoxicomic:149743

# Interactive effects of pesticides and nutrients on microbial communities responsible of litter decomposition in streams

Florent Rossi*†1, Joan Artigas1, Clarisse Mallet1, Christelle Margoum2, Matthieu Masson2, and Stéphane Pesce2

1Microorganismes : génome et environnement (LMGE) – CNRS : UMR6023, Université d'Auvergne - Clermont-Ferrand – 24, avenue des Landais BP 80026 63 170 Aubiere, France

2Milieux aquatiques, écologie et pollutions (UR MALY) – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA – 5 rue de la Doua, BP 32108, 69616 Villeurbanne cedex, France

## Abstract

Streams contamination by pesticides and nutrients, resulting from human activities, is widespread. However, the interactive effect of these two stressors on heterotrophic microbial communities and their activity of organic matter decomposition is still poorly understood. In this context, a microcosm experiment was performed to evaluate the single and interactive effects of environmental concentrations of the herbicide S-metolachlor and the fungicide tebuconazole, on the decomposition ability of leaf-associated microbial communities, in either eutrophic or mesotrophic conditions. Eight treatments were tested in triplicate to assess, in laboratory channels, the resulting effects on natural microbial communities from a pristine forested stream (Couze d'Ardes, Centre France) developing on *Alnus glutinosa* leaf species. Every week for 40-days, water was renewed and samplings were performed to measure *Alnus* leaves' decomposition rates ($K$day), as well as the structure (biomass and diversity) and enzymatic activities of the leaf-associated microbial communities. Results showed a stronger influence of nutrients compared to that of pesticides. Indeed, while decomposition rates, microbial biomass, fungal diversity and ligninolytic activities (laccase, phenol oxidase) were higher in eutrophic channels compared to mesotrophic ones, only a three-fold increase of laccase activity by tebuconazole was observed in eutrophic condition. Surprisingly, such activity increase was not observed in eutrophic conditions when the fungicide and the herbicide were mixed. Overall, our study shows that the effect of realistic environmental concentrations of pesticides on leaf-associated microbial communities can be negligible compared to the effect of nutrients. However, the tested tebuconazole concentration was sufficient to trigger physiological response in fungal community.

**Keywords:** streams, microcosms, pesticides, nutrients interaction, leaf litter, microbial communities.

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*Speaker

†Corresponding author: florent.rossi@univ-bpclermont.fr

# Monitoring the impact of multi-stress contamination on an environmental opportunistic pathogen, Stenotrophomonas maltophilia: application to antibiotics and metals

Armelle Crouzet*† , Géraldine Depret[1], Justine Thiot[2], David Lejon[2], Alain Hartmann[1], Lorenzo Spadini[3], Sabine Favre-Bonté[4], and Sylvie Nazaret[4]

[1]Agroécologie – Agrosup, Institut national de la recherche agronomique (INRA) : UMR1347, Université de Bourgogne – BP 86510, F-21000 Dijon, France
[2]Rovaltain Research Company – Rovaltain Research Company – France
[3]Institut des Géosciences de l'Environnement (IGE) – Institut de Recherche pour le Développement, Institut Polytechnique de Grenoble - Grenoble Institute of Technology, Centre National de la Recherche Scientifique, Université Grenoble Alpes – UGA - IGECS 4070038 058 Grenoble Cedex 9, France
[4]Ecologie Micorbienne – Universite Claude Bernard Lyon1, CNRS : UMR5564 – Campus de la Doua 43 Bd du 11 Novembre 1918 69622 Villeurbanne, France

## Abstract

*Stenotrophomonas maltophilia* is a ubiquitous bacterial species known to be a soil and rhizospheric inhabitant as well as a human opportunistic pathogen. Multi-drug resistant strains are frequently found in hospitals because of its ability to acquire resistance genes through horizontal gene transfer and its possession of efflux pumps. Recent data also showed the link between antibiotic resistance and metal resistance among both clinical and environmental strains. In this study, we evaluated over a 3 month study the impact of a cocktail of antibiotics (ciprofloxacin and sulfamethoxazole) and metals (Cu and Zn) on indigenous *S. maltophilia* in soil using an experimental design of soil columns exposed to manure containing various concentrations of the cocktail. In parallel, the toxicant adsorption, distribution, and migration in the soil matrix were measured to interpret the observed biological response. Data showed that regardless of the concentration of the cocktail i.e. from low (1X PEC) to high (40X PEC) doses, no changes in population abundance were observed after 7 and 100 days of exposure. Genetic diversity (REP-profile) analysis among more than 650 isolates showed that metals were the dominant stressors. Antibiotic resistance gene abundance (sulfonamide and quinolone) was unaltered and no resistance gene transfer was observed. Finally, no changes were observed on the phenotypic (antibiotic resistance) properties at low or high antibiotic doses. These data suggest the lack of a dose/response effect as well as the lack of synergic effects between metals and antibiotics on the genotypic and phenotypic evolution of *S. maltophilia* populations.

**Keywords:** Antibiotic resistance, human pathogen, bacteria, soil, multistress, dose/response

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*Speaker

†Corresponding author: armellecrouzet@gmail.com

# Biofilm phosphorus uptake capacity responds to chemical and environmental stressors in river ecosystems.

Lorenzo Proia*[1], Anna Maria Romaní[2], and Sergi Sabater[2,3]

[1]Ecology of Aquatic Systems - University of Bruxelles (ESA - ULB) – Belgium
[2]Institute of Aquatic Ecology - University of Girona (IEA - UdG) – Spain
[3]Catalan Institute for Water Research (ICRA) – Spain

## Abstract

Biofilms are a key component in the nutrient removal from the water column. However, nutrient uptake by biofilms may be hampered by the occurrence of pollutants or other stressors. We investigated the responses of biofilm phosphorus uptake capacity (PUC) to different chemical and environmental stressors by using chamber experiments. PUC was calculated by measuring P temporal decay after performing controlled P-spikes in chambers with biofilm-colonized tiles. Four different experiments were conducted to evaluate the response of PUC to: (a) several river waters from increasing polluted sites; (b) the effect of the bactericide triclosan (TCS); (c) the combined effect of TCS and grazers; and (d) the effect of TCS after a drought episode that affected the biofilms. These experiments showed that biofilms decreased their PUC along the pollution gradient. The biofilm PUC was significantly reduced after receiving higher TCS concentrations, though lower TCS concentrations also affected the biofilm when this was submitted to grazing pressure. PUC decrease was induced by flow interruption which further enhanced the TCS negative effects. Overall, PUC was sensitive to the effects of pollutants like TCS as well as to the action of biological (grazing) and environmental (drought) factors. The study also showed that multiple stressors enhance the negative effects of pollutants on the PUC of biofilms. Our study values the use of biofilms' PUC as a sensitive ecological-based tool to assess the effects of chemicals on freshwater communities and their derived functioning in river ecosystems.

**Keywords:** Phosphorus, Uptake, Biofilm, River, Selfdepuration, Pollution

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*Speaker

# Spatio-temporal dynamics of TPMT-harboring bacteria among urban deposits reveal significant shifts in Pseudomonas and Aeromonas populations matching pollutant concentrations

Emilie Bourgeois*[1], Romain Marti[1], Claire Bernardin[1], Céline Bécouze[2], Sébastien Ribun[1], Laurence Marjolet[1], Audrey Gleizal[1], Sylvie Barraud[2], Gislain Lipeme Kouyi[2], Laure Wiest[2], Didier Blaha[1], and Benoit Cournoyer[1]

[1]Ecologie Microbienne - Bacterial Opportunistic Pathogens and Environment – CNRS 5557, INRA 1418, Université Lyon 1, VetAgro Sup – France

[2]Laboratoire Déchets, Eaux, Environnement, Pollutions (DEEP) – INSA - Institut National des Sciences Appliquées – 34 avenue des Arts Bâtiment Bât. JCA Coulomb 69621 Villeurbanne cedex, France

## Abstract

Urban activities can generate surface deposits made, among others, of urban soils, fecal matter, and pyrogenic contaminants. These deposits harbor specific microbiota indicative of their origin and ecotoxicity. Here, the bacteriome of these deposits was investigated by an analysis of their TPMT harboring bacteria. The TPMT (thiopurine methyltransferase) can detoxify several toxic metalloid derivatives. It was found to have a distribution limited to 120 bacterial genera including major pathogens such as *Pseudomonas aeruginosa*, *Legionella pneumophila*, and *Vibrio cholerae*. A *tpm* (encoding TPMTs) meta-taxogenomics approach was developed and used to investigate shifts in TPMT-bacterial populations. Deposits were sampled over a 6 year-period from a detention basin. They had been washed off during rainfall events occurring over an industrial urban watershed in Lyon. The *tpm* genetic structures were compared between sampling years, and related to measured PAH and MTE concentrations. An important diversity of TPMT bacteria was observed (over 88 species). Bacterial structures were found impacted by the accumulation time-periods, with higher numbers of reads for opportunistic pathogens (e.g. *P. stutzeri, P. mendocina, Aeromonas caviae, A. hydrophila*) among recently trapped sediments. Reads of the phytopathogen *P. syringae* were enriched in old sediments, where significant plant developments had occurred. *P. aeruginosa* reads were found but were not enriched over the study period. TPMT bacterial structures were correlated to pollutant concentrations (e.g. fluorene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene). The *tpm* meta-taxogenomics approach was found highly reliable to infer the incidence of pollutants on urban bacteriomes but also for performing a tracking of pathogenic bacterial species.

**Keywords:** urban ecosystem, bacterial opportunistic pathogens, Pseudomonas, Aeromonas, pollutant concentrations

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*Speaker

# Ecotoxicological effects on the interactions of fullerenes and organic micro-contaminants in stream biofilms

Anna Freixa[*,†,1], Vicenç Acuña[1], Marina Gutierrez[1], Ferran Romero[1], Josep Sanchís[2], Lucia-Helena Santos[1], Sara Rodriguez-Mozaz[1], Damià Barceló[1,2], Marinel•la Farré[2], and Sergi Sabater[1,3]

[1]Catalan Institute for Water Research (ICRA) – Spain
[2]Institute of Environmental Assessment and Water Research (IDAEA-CSIC) – Spain
[3]Institute of Aquatic Ecology - University of Girona (IEA - UdG) – Spain

## Abstract

An increased amount of carbon nanomaterials such as fullerenes (C60) is recently observed in rivers, which co-occur with multiple organic micro-contaminants, becoming a problem of unknown consequences for aquatic systems. Due to their high surface area, C60 make good compounds for the adsorption of organic micro-contaminants, their interaction potentially affecting the toxicity for stream biofilms. We designed several experiments with the main objective of defining the toxicity of fullerenes in the presence of 3 different organic micro-contaminants in biofilms.

We performed 3 different experiments with epilithic biofilm exposed to environmental relevant concentrations of C60 (1ppb), one micro-contaminant (triclosan; 10 ppb diuron 10 ppb or venlafaxine 100 ppb) and a mixture of each compound (C60 + micro-contaminant) during 72 h. Nutrients, micro-contaminants and C60 concentrations were analysed in water. Functional (extracellular enzyme activities, photosynthetic efficiency and respiration) and structural endpoints (extracellular polymeric substances (EPS) and chlorophyll-a content) were analysed in biofilm. Finally, quantitative RT-PCR was used for absolute quantification of 4 different genes (16S rRNA, 18S rRNA, *psaA* and *psbA*).

Our results showed a significant decrease on algal biomass fluorescence (F0) after the exposure of C60, but no significant responses in all other endpoints. The exposure of diuron and triclosan significant altered the photosynthetic efficiency, respiration and the EPS content in biofilms whereas no evidences were detected after the exposure of venlafaxine compound. Finally, when nanomaterials and contaminants were exposed together an antagonistic response (i.e. decrease) was produced to the toxicity of the micro-pollutants in biofilms after the interaction with fullerenes.

**Keywords:** Microcontaminants, carbon nanoparticles, biofilms, ecotoxicity, mixtures.

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[*] Speaker

[†] Corresponding author: afreixa@icra.cat

# Influence of natural dissolved organic matter on herbicide toxicity to two marine microalgae

Sabine Stachowski*[1], Nathalie Coquillé†[2,3,4], Dominique Ménard[1], Julien Rouxel[1], Valentin Dupraz[1], Mélissa Eon[5], Hélène Budzinski[6], Edith Parlanti[6], and Soizic Morin[2]

[1]Ifremer – Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Institut Français de Recherche pour l'Exploitation de la MER - IFREMER – France
[2]Irstea – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA (FRANCE) – France
[3]Ifremer – Ifrer – France
[4]EPOC-LPTC – EPOC-LPTC – France
[5]IRSTEA (IRSTEA) – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA (FRANCE) – France
[6]EPOC-LPTC – CNRS – France

## Abstract

The continuum between ecosystems leads to herbicide transfer up to coastal areas, where they can affect microalgae. In addition, the dissolved organic matter (DOM) can also interact with herbicides and microalgae. In this study, the influence of natural DOM was assessed in controlled conditions, on the toxicity of three herbicides (diuron (D), irgarol (I) and S-metolachlor (S)) towards cultures of two marine microalgae, *Chaetoceros calcitrans* and *Tetraselmis suecica*. The microalgae were exposed during 6 days to each herbicide alone (D0.05 and D0.5 $\mu$g.L-1; I0.05 and I0.5 $\mu$g.L-1; S0.5 and S5 $\mu$g.L-1) and to mixtures (M1: I0.05+D0.05+S0.5 and M2: I0.5+D0.5+S5), with and without DOM. The effects were assessed on growth, photosynthetic efficiency and relative lipid content. The chemical environment (herbicides, nutrients, DOM quantity and quality) was also characterized.

Results showed that irgarol (I0.5) was the most toxic, without and with DOM. When M2 effects were similar to I0.5 effects in *C. calcitrans*, they were amplified in *T. suecica.* The addition of DOM to the cultures induced a decreased and increased toxicity in *C. calcitrans* and *T. suecica*, respectively. Chemical analyses in the cultures exposed to I0.5 and M2 showed a higher production of dissolved organic carbon, with more aromatic molecules and a higher molecular weight, than in control cultures.

The toxicity modulation with DOM could be explained by (i) a modification of herbicide bioavailability (complexation), (ii) a different composition of cell walls depending on species and/or (iii) a higher detoxification ability for *C. calcitrans* made possible by DOM utilization.

**Keywords:** Microalgae, Herbicides, Natural dissolved organic matter, Toxicity, Interactions, Modulation

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*Speaker

†Corresponding author: nathalie.coquille@gmail.com

# How climate change enhances nanoparticle toxicity towards freshwater biofilms – NanoTox project

Berta Bonet*†[1], Stefan Krause[1], and Iseult Lynch[1]

[1]The School of Geography, Earth and Environmental Sciences - The University of Birmnningham (GEES - UoB) – School of Geography, Earth and Environmental Sciences University of Birmingham Edgbaston Birmingham B15 2TT United Kingdom, United Kingdom

## Abstract

Aquatic ecosystems are threatened by multiple environmental stressors. Release of toxicants such as engineered nanoparticles (ENPs) into aquatic systems and their effects on these ecosystems remain poorly understood. This, combined with the acceleration of the global climate warming (ENP + ΔT°C), could have significant consequences for aquatic life. The NanoTox project focuses on responses of fluvial biofilms, as key points of ENP entry into aquatic food webs, and examines the effects of combined stressors at ecosystem level. Preliminary results from recirculating stream mesocosms measurements on how river warming, the presence of environmental concentrations of ENPs and the combination of both stresses may further stress biofilms at different levels (e.g. functional, community structural and ecosystem) will be presented. To achieve this aim, outdoor microcosm studies are being performed at different temperatures (i.e. 15°C, 20°C and 25°C) representing realistic warming scenarios (i.e. up to 4°C increase from current average UK summer temperatures). After biofilm colonization at different temperatures, ENP will be added obtaining 4 treatments: (i) control (only T°C), (ii) AgNP, (ii) Ag2S NP to represent the ”environmentally aged” form of silver, known to have lower dissolution potential, and (iv) AgNO3 as a positive (ionic) control. Sampling will be at the beginning of ENP exposition (0h), after 6, 24 and 72 hours (addressing acute effects) and after 7, 14 and 30 days (addressing chronic effects). Endpoints assessed include: uptake/localisation of ENP in biofilms, changes in community structure and ecosystems services provision, quantification/identification of secreted biomolecules and resulting ENP-coronas, in an integrated approach.

**Keywords:** Climate change impact, engineered nanoparticles (ENPs), engineered nanoparticle toxicity, contamination, biofilms, microbial metabolic activity.

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*Speaker

†Corresponding author: b.bonet@bham.ac.uk

# Real-time and in situ monitoring of aquatic environments using indigenous microbial community-based biosensors

Jean-Michel Monier*[1] and Olivier Sibourg[1]

[1]ENOVEO – ENOVEO – 7 place Antonin Poncet - 69002 Lyon, France

### Abstract

Increasing contamination of aquatic environments by organic loads or toxic chemicals has resulted in the need for real-time monitoring tools to be used in pollution detection and risk assessment. While monitoring aquatic environments is critical to ensure their quality and sustainability, monitoring strategies often relies on the collection of individual spot samples. Such approach, usually costly and time-consuming, only provides a snapshot that may often fail to detect localized transient contamination events and describe the dynamic changes of the monitored environment. The objective of our work was to develop a cost-effective solution to monitor aquatic environment in real-time, inform targeted sampling by conventional means (*ie,* spot sampling) and act as a decision support tool. This novel biosensor uses microorganisms naturally occurring in the targeted environment as bioindicators and exploits their ability to convert chemical energy into an exploitable electrical signal. The electrical signal generated by the sensor is proportional to the organic load or affected by the presence of toxic compounds, as it directly reflects microbial activity at the surface of the colonized electrodes. Biosensors have been deployed at different sites in order to estimate either the organic load or BOD in real time (*eg*, groundwater, sewage networks...) or detect toxic compounds and assess their impact on their environmental sink (*eg*, industrial processes and wastewater treatment plants...). Through real case studies, the presentation will address the principle, information provided and advantages of such devices and how they can provide unique information to act as a decision support tool.

**Keywords:** biosensor, microbial communities, aquatic environments, in situ, real, time, toxicity, BOD, organic load

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*Speaker

# Tolerance of stream biofilms to micropollutants indicates causality and reflects community recovery

Ahmed Tlili*†[1], Natàlia Corcoll[2], åsa Arrhenius[2], Thomas Backhaus[2], Juliane Hollender[1], Bettina Wagner[1], and Renata Behra[1]

[1]Swiss Federal Institute of Aquatic Science and Technology - EAWAG (SWITZERLAND) – Switzerland
[2]Dept. Biological and Environmental Sciences, University of Gothenburg – Sweden

## Abstract

In Switzerland, wastewater treatment plants (WWTPs) are currently upgraded to reduce input of micropollutants into the receiving streams. This, offers the opportunity to study in real environments consequences of a reduction in micropollutants. Therefore, this study aims to examine tolerance to micropollutant mixtures of periphyton that has been sampled one year before and after the upgrade, in comparison to impacts on periphyton from non upgraded WWTPs. Results showed that one year after the upgrade, difference in micropollutant concentrations between up- and downstream of the effluent decreased. These results concurred with the tolerance measurements at this site, since no difference between up- and downstream periphyton was observed, unlike before the upgrade. Increased tolerance to the extracts, on the other hand, was detected at the non-upgraded sites in both sampling times. These results point to the strong relationship between microbial tolerance and the intensity of contamination by micropollutants. Furthermore, microbial diversity was consistent with tolerance measurements. This was the case at the non-upgraded sites in both sampling times, where bacterial and algal diversity of up- and downstream periphyton were different. On the contrary, differences at the upgraded site were less pronounced after than before the upgrade, indicating that micropollutants were a driver for the microbial diversity downstream of the WWTPs. Overall, our findings prove the relevance of using community tolerance for retrospective risk assessment of micropollutants, and to assess ecosystem recovery, while capturing the biological and chemical complexity in the environment.

**Keywords:** wastewater, periphyton, pollution, induced community tolerance, passive sampler

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*Speaker

†Corresponding author: ahmed.tlili@eawag.ch

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# Unrecognized soil algal and cyanobacterial communities as a model, for herbicide risk assessment in agricultural soils.

Olivier Crouzet*[1], Laurent Consentino[1], Clarisse Mallet[2], Jean-Pierre Aguer[2], Pierre-Alain Maron[3], and Annette Bérard[4]

[1]UMR 1402 ECOSYS – Institut National de la Recherche Agronomique - INRA, AgroParisTech, Université Paris-Saclay – 78026 Versailles cedex, France

[2]UMR 6023 LMGE – Centre National de la Recherche Scientifique - CNRS, Université Clermont Auvergne – Campus universitaire des Cézeaux Avenue des Landais 63622 AUBIERE cedex, France

[3]UMR 1347 Agroecologie – Institut national de la recherche agronomique (INRA) : UMR1347, Agrosup Dijon, Université de Bourgogne – 21000 Dijon, France

[4]UMR 1114 EMMAH – Institut National de la Recherche Agronomique - INRA – 84914 Avignon, France

Session
RISK ASSESSEMENT
Talk

## Abstract

Soil algae and cyanobacteria form microbiotic photosynthetic crusts, not only on desert soils, but also in temperate cropped soils. Despite a scarce literature about their ecology in agricultural soils, they could constitute an original model to improve the consideration of microbial component in the environmental risk assessment of herbicides.
This work focused on the suitability of biochemical and molecular methods to characterize structural and functional responses of algae and cyanobacteria, to herbicides, in agricultural soils under different cropping systems, throughout laboratory and field approaches.

Cultural approaches are still helpful to isolate edaphic species for further ecotoxicological tests at the individual level. Photosynthetic pigments can provide biomass (chlorophyll a) or structural (pigment diversity) indicators. Based on chlorophyll *a* biomass, a modified pollution-induced community tolerance (PICT) approach was developed. Several genetic markers were successfully applied, to estimate their community composition and diversity. Toxic effects on algal and cyanobacterial biomasses and diversities have been evidenced at doses sometimes below recommended field rates, in microcosm experiment. Overall, edaphic algal and cyanobacterial communities showed a higher sensitivity to herbicides, compared to commonly studied soil bacterial and fungal community, in field monitoring. The PICT approach highlighted a higher tolerance to phenyl urea herbicides of the photosynthetic microbial communities in conventional versus organic soils. Characterization of the diversity of the algal and cyanobacterial communities, unravel links between some taxa and shift in tolerance levels. The structural stability of soil surface aggregates, considered as a functional output of algae and cyanobacteria crusts, were disturbed by herbicide treatments.

**Keywords:** soil, pesticides, algae, communities, functions, diversity, tolerance

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*Speaker

# Towards the implementation of Pollution Induced Community Tolerance (PICT) approaches in water regulatory frameworks: Developing standard procedures for the sediment compartment

Stéphane Pesce*†[1], Anaïs Charton[1], Aymeric Dabrin[1], Emilie Lyautey[2], Ayanleh Mahamoud Ahmed[2], Emmanuel Naffrechoux[3], Olivier Perceval[4], and Chloé Bonnineau[1]

[1]UR MALY – Irstea – 5 rue de la Doua, BP32108, 69616 Villeurbanne Cedex, France
[2]Centre Alpin de Recherche sur les Réseaux Trophiques des Ecosystèmes Limniques (CARRTEL) – Université de Savoie, Institut national de la recherche agronomique (INRA) – Domaine Universitaire, 73376 Le Bourget-du-Lac Cedex, France
[3]Laboratoire de Chimie Moléculaire et Environnement (LCME) – Université Savoie Mont Blanc, Université Grenoble Alpes – Campus Scientifique de Savoie-Technolac Le Bourget du Lac Cedex, France
[4]Agence Française pour la Biodiversité – AFB, France – 5 Square Félix Nadar, 94300 Vincennes, France

## Abstract

Assessing the ecological effects of pollutants is an important aspect of regulations dealing with the sustainable management of water resources. In this context, scientists and regulators face the challenge of going beyond the estimation of pollutant concentrations to take into account the ecotoxicological effects on exposed aquatic communities. Among microbial ecotoxicological approaches, the pollution induced community tolerance (PICT) concept has been proven to be a suitable biomonitoring tool for *in situ* assessmentof either the response of aquatic microbial communities to toxicant exposure *[1]* or of their recovery following the improvement of water chemical quality *[2,3]*. The PICT is a powerful microbial metric to better link the assessment of ecological and chemical status of ecosystems by providing causal analysis in effect-based monitoring of impacted field sites *[4]*. However, prior its implementation in regulatory frameworks, further work is required to standardize PICT measurement and define baseline tolerance levels at large geographical scales. In this context, we will present the first results of the application of PICT approaches in the sediment compartment (with copper as model compound). We will specifically focus on both the development of standardized protocols (from sampling to dose-response modeling) and on the evaluation and control of potential confounding factors to make measurements comparable in space and time by considering robust reference conditions. *[1]* Tlili et al., 2017. *Water Res* 111; *[2]* Larras et al., 2016. *ESPR* 23; *[3]* Pesce et al., 2016. *Agr Ecosyst Environ* 221; *[4]* Tlili et al., 2016. *Freshwater Biol* **61**

**Keywords:** Benthic communities, microbial ecotoxicology, PICT, sediments, copper, standardization

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*Speaker

†Corresponding author: stephane.pesce@irstea.fr

# Abiotic, biotic (microflora and fauna) and functional parameters of derelict soils - towards a potential use of derelict soils as a ressource.

Quentin Vincent*†[2,1], Apolline Auclerc[2], Thierry Beguiristain[1], and Corinne Leyval[1]

[2]Laboratoire Sols et Environnements (LSE) – Institut national de la recherche agronomique (INRA) : UMR1120, Université de Lorraine – 2 avenue de la Forêt de Haye, 54500 Vandoeuvre-lès-Nancy, France
[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Faculté des Sciences et Technologies, 54500 Vandoeuvre-lès-Nancy, France

## Abstract

The intensification and closure of industrial activities during the last century led to large surfaces of derelict lands. The derelict soils have a low fertility and might be contaminated, and remain unused. However, with the increasing demand of soil surface, they might be considered as a resource for non-food biomass production. The study of their biodiversity and biological activity may be an indication for a potential re-use of derelict soils. The objective of our study was to investigate the quality of six derelict soils, considering abiotic, biotic and functional approaches. Soil bacteria, fungi, plant, meso- and macro-fauna communities were studied in a set of six different derelict soils (coking plant, settling pond, constructed, inert waste storage soils) as well as the decomposition process, considering the decomposer trophic network, enzyme activity, mineralization activity and organic pollutant degradation. Biodiversity levels in the derelict soils were rather high but depended on the considered biotic component. Multivariate analysis showed that biotic parameters co-varied more with fertility proxies than with soil contamination parameters. Similarly, functional parameters significantly co-varied with abiotic parameters. Co-inertia analysis showed that several structural and functional components of biota were useful to discriminate the derelict soils and evaluate their quality. We found that the compost amended constructed soil had the highest quality while the settling pond soil and the contaminated constructed soil had the lowest one. Although differences between the derelict soils were observed, this study showed that derelict soils may provide a biodiversity ecosystem service and are functional for decomposition.

**Keywords:** Derelict soil, Microflora, Fauna, Pollution, Mineralization, Function, Soil quality

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*Speaker
†Corresponding author: quentin.vincent@univ-lorraine.fr

sciencesconf.org:ecotoxicomic:149593

# Are fungicides a threat for heterotrophic aquatic food webs?

Jochen Zubrod*[1], Ralf Schulz[1], and Mirco Bundschuh[2]

[1]University of Koblenz-Landau – Germany
[2]Swedish University of Agricultural Sciences – Sweden

### Abstract

Fungicides can affect heterotrophic organisms and the functions they provide via multiple pathways. Here we discuss the impact of fungicides on leaf-associated biofilms (i.e., aquatic fungi and bacteria) and how these modifications modulate the nutritional quality of leaf material for leaf-shredding invertebrates. When leaves were exposed to the inorganic fungicide copper during microbial conditioning, an increased palatability of the leaves for the amphipod shredder *Gammarus fossarum* was observed. This preference was attributed to an increased fungal biomass accrual and a shift in fungal community composition favoring species considered as more palatable. When, however, fed with these preferred leaves over multiple weeks, *Gammarus* showed impairments in growth and energy reserves. Organic fungicides, in contrast, favored leaf-associated fungal species considered less palatable, and thus induced a rejection of this leaf material, while feeding on these leaves for several weeks again induced impairments in gammarids' growth. These insights, thus, suggest that the food choice of selectively feeding detritivores may not be predictive for indirect effects on their physiological level. Nonetheless, it was shown that fungicides can modify the physiological fitness of detritivores via bottom-up directed effect pathways indicating long-term impacts in the population development of these species at field relevant levels.

**Keywords:** fungicides, leaf, associated microorganisms, shredders, trophic interactions

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*Speaker

# Evaluation of the ecotoxicologycal impact of municipal wastewaters on wildlife: a case study in Tuscany

Federica Spina*[1], Laura Palli , Mario Aragno , A Bonari , A Bossi , I Pompilio , S Dugheri , D Santianni , S Caffaz , M Vincenzi , Riccardo Gori , and Giovanna Cristina Varese

[1]Mycotheca Universitatis Taurinensis, University of Turin – Turin, Italy

**Abstract**

As effect of human activities, persistent organic pollutants are discharged in the environment and can be found in surface waters. They could ultimately accumulate in wastewaters treatment plants (WWTPs) which unfortunately are rarely design to face this problem. The aim of this study was to evaluate the quality of waters in Tuscany region, in term of pharmaceutical active compounds (PhAC) concentration and environmental risk assessment. Three WWTPs managed by Publiacqua SpA located in Pistoia, Figline Valdarno and Lastra a Signa were taken into consideration. The process efficiency was studied, evaluating also the effect of seasonal variability: samples were collected from both the influents and the effluents in spring, summer and winter. The chemical characterization revealed the presence of nine PhAC including three non-steroidal anti-inflammatory drugs (diclofenac, ketoprofen, paracetamol), three antibiotics (amoxicillin, clarithromycin, doxycycline), one $\beta$-blocker (atenolol), one antiepileptic drug (carbamazepine) and one hormone ($\beta$-estradiol). As expected, their concentration was strongly affected by the season, with high antibiotics concentration detected during cold periods. As regards the wastewaters ecotoxicity, several bioassays were performed based on three seeds (watercress, sorghum and cucumber), algae, crustaceous and luminescent bacteria. *Raphidocelis subcapitata* was often the most sensitive organism, but data confirmed the importance of a battery of tests with model organisms representative of different trophic levels of the aquatic ecosystem. The WWTPs did not always reduce the toxicity indicating that the waters discharged in the receiving basin still had relevant effects on the ecosystem. Ecotoxicologycal index will be performed to better define the actual environmental risk.

**Keywords:** ecotoxicologycal bioassays, micropollutants, fungi

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[*] Speaker

# Changes in the interactions between periphytic microalgae and micromeiofauna induced by environmental exposure to diuron and/or imidacloprid

Julie Neury-Ormanni*[1], Jacky Vedrenne[1], Gwilherm Jan[1], Betty Chaumet[1], Mélissa Eon[1], and Soizic Morin†[1]

[1]Irstea-Bordeaux – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture – 50 Avenue de Verdun 33612 Gazinet Cestas, France

## Abstract

Only a little part of agricultural pesticides reach their target organisms; the remaining is transferred by phenomena like leaching to aquatic ecosystems, where they are found in mixtures. Chemical stressors induce direct and indirect changes on the fauna and/or the flora, thus complicating pesticide risk assessment.

We exposed mature biofilms for 14 days in aquaria to four treatments: control, the herbicide diuron (priority substance in the Water Framework Directive) and the insecticide imidacloprid (included in the Watchlist), singly and in mixture, at environmental concentrations (5$\mu$g/L). Our aim was to understand biofilm community responses when exposed to pesticides, based on changes in structure (species composition, diversity and density) and community tolerance, within a PICT (Pollution-Induced Community Tolerance) framework. We quantified weekly microalgal and micromeiofaunal composition, biofilm dry weight, chlorophyll a concentration and exopolysaccharide content.

In the controls, biofilm dry weight increased over time; whereas in the three pesticide treatments the biomass did not increase after 7 days of exposure. PICT approaches based on photosynthesis inhibition revealed pre-exposure to diuron, while imidacloprid didn't affect directly the microalgae. Contrastingly, the autotrophy index increased with imidacloprid pressure, in agreement with its known target action on animals. These impacts were confirmed by taxonomy analyses. The binary mixture had the same effects on microalgae as diuron, decreasing photosynthetic efficiency and chlorophyll concentration.

Altogether the results of this study highlighted changes in the proportions of microalgae and micromeiofauna reflecting their interactions and exposure history. They also demonstrate that micromeiofauna should not be overlooked in microbial ecotoxicology.

**Keywords:** biofilm, micromeiofauna, microalgae, diuron, imidaclopride, community response, PICT

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*Speaker

†Corresponding author: soizic.morin@irstea.fr

# Impacts of silver nanoparticles on aquatic detritus-based food webs

Claudia Pascoal*[1], Daniela Batista[1], and Fernanda Cassio[1]

[1]CBMA, University of Minho – Centre of Molecular and Environmental Biology, University of Minho, Campus de Gualtar, Braga, Portugal

## Abstract

The production and use of silver nanoparticles (AgNPs) has significantly grown over the last decade, increasing the chances of these NPs entering in freshwaters. In these ecosystems, AgNPs and ionic silver released from dissolution of NPs can have toxic effects on aquatic species and can compromise important ecosystem processes. We used a microcosm approach, to assess the impacts of environmentally-realistic concentrations of AgNPs and ion Ag across a freshwater detrital food web comprising plant litter, microbes and invertebrate detritivore species. Exposure to increasing concentrations of AgNPs and ionic Ag led to a decrease in fungal diversity and reproduction and inhibited plant litter decomposition. The toxicity of AgNP to microbial decomposers may be related to AgNP absorption on leaves, where the fungi are growing, preventing the release of spores, limiting fungal growth and inhibiting the activity of microbial decomposers. Moreover, AgNPs and ionic Ag were able to trigger oxidative and neuronal stress in invertebrate detritivores. The consumption of leaves by detritivores was lower in treatments with leaves pre-exposed to nano and ionic Ag compared to water exposure treatments, which can be related to a food avoidance behavior with impacts to detrital-based food webs.

**Keywords:** Nanoparticles, Silver, Detritus foodwebs, Freshwaters

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*Speaker

# Dysbiosis in plant-endophyte partnership: repeated short exposures of sulfametho- xazole & trimethoprim at microconcen- trations can disturb the microbial community in soft rush, Juncus effusus

Muhammad Arslan*[1], Marcello Santoni , Arndt Wiessner[1], Thomas Neu[2], Dietmar Pieper[3], and Jochen Müller[1]

[1]Helmholtz Centre for Environmental Research, Department of Environmental Biotechnology (UFZ) – Permoserstr. 15, Leipzig, Germany
[2]Helmholtz Centre for Environmental Research, Department of River Ecology – Brückstr. 3A, 39114 Magdeburg, Germany
[3]Helmholtz Center for Infection Research – Microbial Interactions and Processes Research Group (HZI) – 38124 Braunschweig, Germany

## Abstract

Plants harbour complex endophytic communities that play beneficial role for growth of their host. A disturbance of these interactions can be detrimental for the plant. In this study, our objectives were to query whether certain organic micropollutants might interfere with the plant-bacteria partnership and, if so, how the endophytic community responds. Towards these aims, a wetland plant *Juncus effusus* was repeatedly exposed to low aqueous concentrations of sulfamethaxazole and trimethoprim, antibiotics frequently found in the environment. Plant status was assessed based on evapotransporation rates as indicator of plant fitness and by visual inspection. Changes of endophytic communities in roots and shoots of exposed and unexposed plants were tracked via culture-dependent (MPN), and culture-independent (qPCR, FISH, Illumina 16S rRNA sequencing) approaches. The *in planta* physiological response was evaluated via detection of reactive species (ROS/RNS) using confocal laser scanning microscopy (CLSM). During the first dose of antibiotics, evapotransporation rates decreased, and plants became infested with insects. After omitting the antibiotics, evapotransporation recovered to some extent, but after repeated exposures it was almost zero. Eventually, plant tissue turned necrotic and CLSM analysis showed increased generation of ROS/RNS in stressed plants, i.e., evidence for infection. Endophytic fungi were never observed microscopically. Comparative analyses of bacterial endophytes revealed that post-treated roots, but not shoots, had significantly increased abundances of *Gammaproteobacteria*, *Firmicutes*, and *Actinobacteria*. Nevertheless, community profiles of post-treated roots and shoots differed substantially from their non-treated counterparts. In conclusion, study provides evidence that antibiotics at environmental concentrations can trigger a dysbiosis in a wetland plant.

**Keywords:** plant, endophyte partnership, dose antimicrobials, microbial ecology, organic micropollutants

Session
TROPHIC INTERACTIONS
Talk

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*Speaker

# RECOTOX: a network of research sites to implement multiple and multi-scale approaches for a posteriori environmental risk assessment in agricultural systems

Christian Mougin*[1], Véronique Gouy†[2], and Vincent Bretagnolle‡[3]

[1]Ecologie fonctionnelle et écotoxicologie des agroécosystèmes (ECOSYS) – Institut National de la Recherche Agronomique : UMR1402 – Pôle Ecotoxicologie, Route de St-Cyr, 78016 Versailles, France
[2]Milieux Aquatiques, Écologie et Pollutions (MAEP) – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture – 5 rue de la Doua, 69626, Villeurbanne, France
[3]Centre d'Etudes Biologiques de Chizé (CEBC) – CNRS : UMR7372 – 405 Route de La Canauderie, 79360, Villiers-en-Bois, France

## Abstract

One of the greatest current challenges is the ever increasing environmental pollution with its concomitant environmental and human impacts. For many years, the national scientific community has pointed out the lack of long-term field and large-scale monitoring of the effects of chemicals on natural media such as water, soil and air. In that context, the network RECOTOX aims at promoting transversal, spatially explicit and integrated research to meet the scientific challenges of understanding and anticipating the environmental and health impacts of pesticides and biocides (organics, biopesticides, metals). RECOTOX includes 15 sites in metropolitan France and French West Indies, which are mostly already endorsed by national organizations (AllEnvi, INRA, CNRS, IRSTEA, universities) and instrumented networks (SOERE RBV, network of catchment area; SOERE RZA, network of long term ecological research observatories; long-term cropping system trials...). These sites allow *in natura* observation and experimentation in ecotoxicology in offering various and complementary agro-pedo-climatic situations. The network aims at: promoting transversal and integrated research, analyzing the whole chain "pressures-exposures-impacts" of pesticides, sharing between its sites a common culture of ecotoxicology. RECOTOX encourages the implementation of multiple and large geographical scale approaches for *a posteriori* environmental risk assessment in agricultural systems. Through its scientific project and its transverse activities, it will contribute to promote the use of bio-indicators as, microorganisms, for environmental risk assessment by ensuring the validity of biological tools and their operational potential. https://www6.inra.fr/ecotox/Animation-nationale/Infrastructures/RECOTOX

**Keywords:** network, sites, in natura, pesticides, ecotoxicology, pressures, fate, exposure, effects, risk assessment, microbial communities, invertebrates, vertebrates, spatial and temporal scales, landscape

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*Speaker

†Corresponding author: veronique.gouy@irstea.fr

‡Corresponding author: Vincent.BRETAGNOLLE@cebc.cnrs.fr

# Communication Abstracts

## POSTER CORNERS

# Bioremediation of an aged dioxins and furans contaminated soil by saprotrophic telluric fungi and a mild chemical oxidation

Isabelle Delsarte*[1], Catherine Rafin*[1], Yann Landkocz[1], and Etienne Veignie[1]

[1]Unité de chimie environnementale et interactions sur le vivant (UCEIV) – Université du Littoral Côte d'Opale : EA4492 – Maison de la Recherche en Environnement Industriel 145 avenue Maurice Schumann 59140 Dunkerque, France

## Abstract

An innovative method for the bioremediation of contaminated soils by dioxins and furans (PCDD/F) by telluric saprotrophic fungi is developed. The degradation of PCDD/F has two main obstacles to efficient biological treatments by fungi: their low bioavailability and the difficulty of initiating the oxidation of so stable molecules. To overcome these technological obstacles, we propose to couple a mild chemical oxidation to a biological one by saprotrophic telluric fungi.
A collection of 33 fungal strains is built up using a PCDD/F historically contaminated soil originating from an experimental site (Halluin) in the North of France. A simplified study system in mineral medium under axenic conditions allowed us to determine the best parameters i.e. the fungal strains (*Penicillium brasilianum* and *Fusarium solani*), the type and the dose of chemical oxidant (the Fenton's reagent) for the degradation lab experiment conducted in historically PCDD/F contaminated soil microcosms.

Twelve treatments (biological and/or chemical) are realized in triplicate for only two months of incubation and the concentrations of 17 PCDD/F congeners are then analysed. The inoculation of the endogen strain *P. brasilianum* in presence of cardboard chips as a bulking agent led to a decrease in PCDD/F toxicity of 40.5 % in comparison with the control.

This work draws new perspectives for remediation techniques of contaminated soils by PCDD/F that are cost-effective and environmentally friendly.
Acknowledgement: Financial support for this research was provided by Region Hauts de France, Pôle Métropolitain Côte d'Opale (PMCO) and the European Funds for Regional Development (ERDF).

**Keywords:** Bioremediation, Dioxins, Fenton's reagent, Furans, Saprotrophic fungi, Soil

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*Speaker

# On-farm bioremediation of leftover pesticide solutions using biobed

Talaat Elsebai*[1]

[1]National Research Centre (NRC) – National Research Centre, 12622 Dokki, Giza, Egypt., Egypt

## Abstract

Emissions of pesticides from agricultural soils to adjacent environmental compartments are deriving from direct (point source) and indirect (non-point source) losses. Direct losses of pesticides from point sources are of different origins among which (leftover pesticide solution, spillages during filling and cleaning of the spraying equipment, leakages of spraying equipment, etc.). In Egypt, leftover pesticide solution represents the main source of pesticides emissions due to the small size of Egyptian farm. Biobed is a smart system designed to receive and dissipate pesticide contained in wastewaters, it is of a prime interest. The biomixture (mix of straw, compost or peatmoss and top soil at a volumetric proportion of 2:1:1 respectively) is the key component of biobed. This study aimed to remediate a complex of leftover pesticide mixtures (methomyl, pirimiphos methyl, diniconazole, pyridaben, hexythiozox, hymexazole and abamectin) at recommendation rate. Four biobeds were prepared; ;*(i)* pesticide solution was replaced by water (control) , *(ii)*, for insecticides remediation *(iii),* for fungicides remediation (iv) for remediation of complex of insecti- and fungi-cide, the moisture content was maintained around 60% of WHC. Samples of the biobed material at different depths were withdrawn at the end of experiment for pesticide residues analysis (6 months after the last addition). Our data indicated that more than 90% of all tested pesticides were dissipated. In addition, dehydrogenase activity of all treatment was comparable of this of the control treatment. It could be suggested from our findings that biobeds are an efficient technique to limit pesticide release in the environment.

**Keywords:** pesticide pollutions, point source, biobed, bioremediation

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*Speaker

# Dichloromethane degradation in multi-contaminated groundwater: coupling biomolecular and compound-specific isotope analyses

Louis Hermon*[1,2], Jérémie Denonfoux[3], Jennifer Hellal[1], Catherine Joulian[1], Stéphanie Ferreira[3], Stéphane Vuilleumier[2], and Gwenaël Imfeld†[4]

[1]Département Eau Environnement Ecotechnologies (D3E/BGE) – Bureau de Recherches Géologiques et Minières (BRGM) – France
[2]Department of Microbiology, Genomics and the Environment – université de Strasbourg, CNRS : UMR7156 – 28 Rue Goethe 67000 Strasbourg, France
[3]Communautés microbiennes – genoscreen – 1 rue du Professeur Calmette, Lille, France
[4]Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS) – université de Strasbourg, CNRS : UMR7517 – 1 rue Blessig, 67084 Strasbourg Cedex, France

## Abstract

*In situ* and *in vitro* biodegradation of DCM were investigated in groundwater from a former solvent-processing industrial site where DCM is a major contaminant together with chlorinated ethenes and ethanes. Compound-specific isotope analysis (CSIA) of DCM enabled to evaluate and quantify *in situ* biodegradation at the field scale. Applying DCM-specific isotopic enrichment factors ($\epsilon$C) from reference strains, biodegradation extent (B%) varied across the plume and through time from 4 to 50%. Carbon isotope enrichment factors of-21.8 ± 3 and -31.0 ± 3 for aerobic and anaerobic degradation of DCM by *Hyphomicrobium* sp. GJ21 grown in contaminated site groundwater and in synthetic medium were obtained. Under aerobic conditions, significant differences in $\epsilon$c were observed for contaminated site groundwater and culture mineral medium, suggesting an effect of field physico-chemical conditions on the degradation mechanism of DCM. High-throughput sequencing of the 16S *rrnA* gene amplified from groundwater samples along the contamination plume revealed the occurrence of several genus-level taxa previously associated with DCM biodegradation under both aerobic and under anaerobic conditions. The abundance of dehalogenase genes associated with DCM biodegradation was quantified by qPCR of samples along the contamination plume, totalling 4102-1107 copies/L depending on the location. Highest abundances of dehalogenase genes were detected in the source zone where the highest co-contaminant concentrations were measured. Obtained results highlight the potential for on-site DCM biodegradation. The proposed integrative approach coupling biomolecular and CSIA may help identify microorganisms, microbial pathways and suitable redox conditions associated for bioremediation of the investigated site.

**Keywords:** Bioremediation, contaminated groundwater, dichloromethane, degradation, CSIA, biomarkers

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*Speaker
†Corresponding author: imfeld@unistra.fr

# Microbiology of Acid Mine Drainage: from natural attenuation to bioremediation

Marina Héry*[1], Odile Bruneel[1], and Corinne Casiot[1]

[1]Hydrosciences Montpellier (HSM) – Institut de recherche pour le développement [IRD], CNRS : UMR5569, Université Montpellier II - Sciences et techniques – CC57, 163, rue Auguste Broussonet, 34090, Montpellier, France, France

### Abstract

Acid mine drainages (AMD) resulting from the oxidation of sulfides contained in mining wastes lead to the release of toxic elements into the aquatic environment. They represent a severe treat for the ecosystems and public health. Arsenic (As) is one of the most toxic elements commonly associated with AMD. In the abandonned Carnoulès mine (Gard, France), the formation of an AMD containing hundred of mg.L-1 of As coincides with the spring of the Reigous stream. HydroSciences Montpellier lab has conducted for almost 20 years interdisciplinary work aiming at deciphering the biogeochemical processes controlling the fate of As in the Reigous waters. Along this stream, natural attenuation leads to the removal of up to 90% of the dissolved As from the water. This natural process is based on microbially driven oxydation of iron and arsenic followed by their co-precipitation in the streambed. Such capacities offer a promising strategy for the development of passive biological treatments of As-rich AMD. The IngECOST-DMA ANR project proposed a multidisciplinary approach associating microbiologists, process engineers, geochemists, mineralogists, materials engineers, to develop an integrated strategy for the management of As-rich AMD. Biotreatment options were first studied on a pilot-scale system and then tested *in situ* with in a field-scale pilot implemented at the Carnoulès former mine. This presentation will provide a review of (i) the results obtained on the biogeochemistry of the Carnoulès AMD and of (ii) the recent work conducted on innovative biotreatment options of this extreme pollution.

**Keywords:** acid mine drainage, natural attenuation, iron and arsenic oxidation, bacterial communities, bioremediation

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*Speaker

# Microbial resources management for bioremediation

Olivier Sibourg[†1] , Cedric Malandain[1], Celine Baguelin[1], Sandra Entresangles[1], and Jean-Michel Monier[*1]

[1]ENOVEO – ENOVEO – 7 place Antonin Poncet - 69002 Lyon, France

## Abstract

Successful site remediation through microbial degradation requires a thorough understanding of the microbial mechanisms implicated in the biodegradation of the pollutant in question. Regardless of the nature of the pollutant or environmental matrix, the success of a bioremediation project hinges on the consideration of two essential components: the presence of a microbial community which can be stimulated in order to promote biodegradation activity and the bioavailability of the pollutant (often influenced by the chemical and physical characteristics of the site*)*. Understanding microbial degradation processes is therefore a major contribution to the success of such projects. ENOVEO's biotechnological solutions include the characterization of the microbial degradation potential of a site and the evolution of the activity of indigenous bacteria. On-going technological advancements in the fields of metagenomics, metatranscriptomics and metaproteomics continue to bring new perspectives to our understanding of microbial mechanisms. Our presentation will illustrate how different molecular biology tools can be utilized to characterize and manage microbial resources, from the development of the remedial action plan to site treatment and closure, to identify any potential difficulties associated with up-scaling and to enable the optimization of depollution strategies.

**Keywords:** bioremediation, polluted sites, microorganisms, molecular biology tools

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[*] Speaker

[†] Corresponding author: o.sibourg@enoveo.com

# Metal pollution effects on ammonia oxidation

Maria Argudo*[1], Helena Guasch[1], Berta Bonet†[2], Carmen Espinosa‡[3], and Frederic Gich§[1]

[1]Universitat de Girona – Spain
[2]University of Birmingham – United Kingdom
[3]Universitat de Vic – Spain

## Abstract

Metals from mining are common chemical stressor pollutants from human activities that cause a high impact in natural communities in the Mediterranean rivers. Key biological processes carried out in fluvial biofilms are affected by environmental stressors. In consequence natural communities can be altered both in diversity and activity. The aims of this study are to determine the toxicity of Zn on growth, photosynthetic efficiency and changes on structure and function of the ammonia oxidizing prokaryotes in fluvial epilithic biofilms along three rivers. Accordingly an experiment was set up based on biofilm colonization on artificial substrate during spring along Ter, a river larger and impacted by human activities and two of its tributaries, Osor a contaminated river by an effluent of a blende ((Zn,Fe)S) and galena (PbS) and a control river (Llémena). The effects of Zn on algae and on the abundance of ammonia oxidizing prokaryotes were determined by using amplitude modulated fluorimetry (PAM) and reverse transcription quantitative PCR (qPCR) for 16S rRNA, 16S AOB structural genes and functional AmoA genes. RNA abundances in the biofilm were higher than DNA abundances. Bacteria are more abundant in polluted sites whereas algal growth and photosynthetic activities are greater in the less polluted ones. In relation to structure and function of ammonia oxidizers prokaryotes, the ammonia oxidizing bacteria (AOB) are more abundant than the ammonia oxidizing archaea (AOA). Moreover AOB relative abundance, decrease because of pollution indicating that ammonia oxidation may also be impaired. Effects of pollution on nitrification activities will also be discussed.

**Keywords:** Ter river, pollution, Zn, algal growth, ammonia oxidizing prokaryotes, reverse transcription quantitative PCR.

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*Speaker

†Corresponding author: berta.bonet@udg.edu

‡Corresponding author: u1917287@campus.udg.edu

§Corresponding author: frederic.gich@udg.edu

# Do plant traits matter in the microbial ecotoxicity of metal nanoparticles in soil?

Amélie Cantarel[1], Jonathan Gervaix[1], Jean Martins[2], and Agnès Richaume*†[1]

[1]Ecologie microbienne (EM) – Institut national de la recherche agronomique (INRA) : UMR1418, CNRS : UMR5557, Université Claude Bernard - Lyon I (UCBL), Ecole Nationale Vétérinaire de Lyon – 43 Bvd du 11 Novembre 1918, 69622 Villeurbanne cedex, France

[2]Institute Geosciences Environment (IGE) – CNRS : UMR5001, Université Grenoble Alpes – Université Grenoble Alpes CS 40 700. F-38058 Grenoble cedex, France

## Abstract

The use of nanoparticles (NPs) in many common products led to assess their impact on living organisms and microorganisms in various environments. Several studies showed the deleterious effects of metal nanoparticles (NPs) on soil microbial communities and pointed out the importance of organic matter (OM) content in the ecotoxicity of NPs due to its role as dispersing and stabilizing agent . In this study, we assessed the role of plant root exudation in NPs ecotoxicity in soil to- wards the microbial communities involved in the nitrogen cycle assuming that plant root exudation reduces NPs ecotoxicity in the rhizosphere due to a higher dissolved OM content compared to bulk soil. The experimental design consisted in planted and unplanted soil microcosms contaminated or not with CuO-NPs (1mg and 100 mg/Kg) often entering in the composition of nano- fertilizers or pesticides. Control microcosms spiked with soluble Cu were included. Since the measurement of root exudation is hardly possible in soil, we compared the effect of two types of wheat (Triticum aestivum) exhibiting contrasted traits reflecting contrasted exudation. Ecotoxicological NPs effects were assessed after 30 and 50 days of incubation in a greenhouse by measuring microbial activities (nitrification and denitrification) and microbial abundances by qPCR targeting function genes in rhizosphere and bullk soil. NPs characterizations (sur- face charges and hydrodynamic diameter) were realized in solutions of rhizosphere and bulk soils. Dissolved OM was measured in both solutions. The microbial ecotoxicological effects of NPs are discussed in the light of plant traits and NPs characteristics.

**Keywords:** metal nanoparticles/ nitrification/ denitrification/ plant traits/ rhizosphere

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*Speaker

†Corresponding author: agnes.richaume@univ-lyon1.fr

# Mixtures effect of antibiotics on soil nitrification and denitrification - an experimental and modelling approach

Viviane David[1,2], Celine Roose-Amsaleg[2], Fabrice Alliot[2], Marjolaine Bourdat-Deschamps[1], and Olivier Crouzet*†[1]

[1]UMR 1402 ECOSYS – Institut National de la Recherche Agronomique - INRA, AgroParisTech, Université Paris-Saclay – 78026 Versailles cedex, France

[2]Milieux Environnementaux, Transferts et Interactions dans les hydrosystèmes et les Sols (METIS) – École Pratique des Hautes Études [EPHE], CNRS : UMR7619, Université Pierre et Marie Curie (UPMC) - Paris VI – UPMC, Case courrier 105, 4 place Jussieu, 75005 Paris, France

## Abstract

Impacts of antibiotic mixtures on microbial ecosystem functions need to be unraveled to improve the environmental risk assessment. This study aims to characterize and predict the toxicity of antibiotic mixtures on soil nitrification and denitrification processes.
Different antibiotics (tetracycline, sulfonamide, macrolide, fluoroquinolone) with distinct mechanisms of action, were tested alone and in mixtures by dose-response approaches on soil substrate-induced nitrification (SIN) and minimum inhibitory concentration of soil denitrifier enrichment (MIC-E). Abundance of ammonia-oxidizers (archeal and bacterial) and denitrifiers (clade I and II of N2O-reducers) were investigated by molecular approach. For nitrification, the experimental results for different antibiotic mixture ratio were further analyzed regarding mathematical modeling interpretation based on the concepts of concentration addition (CA) or independent action (IA).

The SIN bioassay highlighted bacteriostatic effects at sulfonamide or macrolides concentrations lower than observed environmental concentrations. The differences in toxicities of antibiotics individually tested were explained by the sorption coefficient of the various antibiotics. Surprisingly, despite different cellular targets of the tested antibiotics, modelling mixture analyses revealed few differences in toxicity prediction between IA or CA concepts. Denitrification showed a higher tolerance than nitrification to the antibiotics and the lowest MIC-E for tetracycline. For nitrification and denitrification, the mixture toxicity was mainly driven by the more toxic compound.
The archeal/bacterial nitrifiers ratio was positively related to the magnitude of nitrification inhibition; archaea showing invariant abundances while the antibiotic doses increased. The abundances of the denitrifier *nosZ* clade II greatly decreased under any antibiotic exposures unlike the *nosZ* clade I guilds.

**Keywords:** Microbial ecotoxicology, antibiotics, mixture effect, N, cycling microbial activities

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*Speaker

†Corresponding author: olivier.crouzet@versailles.inra.fr

# Impact of pesticides on denitrification in groundwaters

Caroline Michel*[1], Nicole Baran[1], Laurent Andre[1], and Catherine Joulian[1]

[1]BRGM – Bureau de Recherches Géologiques et Minières (BRGM) – 3 avenue Claude Guillemin, B.P. 36009, Orléans cedex 2, France

### Abstract

Denitrification processes are involved in the decrease of nitrate (NO3-) concentrations in groundwaters impacted by human activities. Non-target/side effects of pesticides on soil microbial denitrification have been demonstrated. It is however now well established that groundwater is an ecosystem in itself with specific microbial communities different from soils' ones. If the impact of pesticides on groundwater bacterial communities has already been studed, the impact of pesticides on the denitrification activity in groundwater has still to be investigated. Groundwater samples are collected from several wells on a catchment submitted to agricultural practices with fertilizers and pesticides uses. Groundwaters contain nitrate (lower than limit of quantification up to 80 mg/l), pesticides and some of their metabolites in low concentrations. The abundance of genes involved in denitrification (*narG*, *napA*, *nirS*) shows the occurrence of bacteria able to perform, at least, the two first steps of denitrification. Three groundwaters distributed within the catchment and exhibiting different denitrification signatures based on genes identification and physic-chemical properties are selected. They are used in laboratory to perform batch experiments in conditions boosting microbial denitrification (addition of KNO3 and acetate, anaerobic conditions, 25$\circ$C). The microbial ecotoxicology study is conducted in the presence or absence of pesticides spiked as single or cocktail pesticides. Several parameters (pH, concentrations of acetate, NO3, NO2, N2O, biomass) are recorded over time to quantify denitrification rate according to experimental conditions. This experimental work constitutes a first step in the understanding of the inhibitor/catalyzer role of pesticides on denitrification process.

**Keywords:** denitrification, groundwater microbial community, pesticides, side effects

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*Speaker

# Long Term Exposure of Agricultural Soil to Veterinary Antibiotics Changes the Population Structure of Symbiotic Nitrogen-Fixing Bacteria Occupying Nodules of Soybeans (Glycine max)

Cécile Revellin[1], Alain Hartmann*[1], Edward Topp[2], and Sébastien Solanas[1]

[1]Agroécologie [Dijon] – Université de Bourgogne, Institut National de la Recherche Agronomique : UMRINRA 1347, Institut National Supérieur des Sciences Agronomiques, de lÁlimentation et de lÉnvironnement, Université Bourgogne Franche-Comté – Université de Bourgogne - 17 rue Sully - BP 86510 - 21000 Dijon, France

[2]London Research and Development Centre (AAFC) – 1391 Sandford St London, Ontario N5V 4T3, Canada

### Abstract

Antibiotics can be entrained onto agricultural land through the application of organic wastes or wastewater for irrigation. In order to evaluate the impacts on soil microorganisms of exposure to antibiotics (ATB), replicated plots treated each year with a mixture of sulfamethazine, chlortetracycline and tylosin at concentrations ranging from 0.1 to 10 mg/kg, were initiated in 1999 at the AAFC research farm in London ON. Plots were seeded with soybeans. Soybean nodules were collected in 2012, after 14 annual ATB treatments. The bradyrhizobia strains were characterized by serology and molecular methods.The sensitivity of isolates to the 3 ATB was also evaluated. Based on molecular characterization the collection of 278 bradyrhizobia was very diverse. The distribution of isolates in RS$\alpha$ fingerprint groups was significantly different in the 3 soils treated with antibiotics compared to control soil, with an increase in proportion of strains belonging to RS$\alpha$ types a, b, c and q. This result confirms those based on serotyping: strains belonging to *B. liaoningense* serogroup 135 are more abundant in soils having received antibiotics (compared to control). Using RS$\alpha$ fingerprinting results, Shannon diversity indexes were computed, T-tests revealed that Shannon diversity indexes were significantly different between control soil and soil having received the lower dose of antibiotics. Overall, the present study indicates that long term treatment with veterinary antibiotics i.e. tylosin, chlortetracycline and sulfamethazine alters the composition of Bradyrhizobial populations occupying soybean nodules. The sensitivity of bradyrhizobia to the three antibiotics was not associated with the treatment from which they were recovered.

**Keywords:** Bradyrhizobium, antibiotics, soil, molecular characterization, serology

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*Speaker

# SYNAQUA: A French-Swiss program to modernize the environmental biomonitoring of Lake Geneva and transboundary rivers

Agnès Bouchez*†[1] and Jan Pawlowski

[1]UMR CARRTEL – Institut National de la Recherche Agronomique - INRA (FRANCE) – 75 av. de Corzent, 74203 Thonon-les-Bains, France

## Abstract

Rivers and lakes play a major role in the environment by providing not only habitat for fauna and flora, but also improving the landscape and therefore the quality of life. The effectiveness of environmental protection measures rests on the early and precise identification of pressure points, which should be facilitated by environmental genomics. To implement measures to preserve or restore aquatic areas, the SYNAQUA project proposes to use the genetic tools based on the recognition of bio-indicator organisms present in the aquatic environment directly from their DNA. The method of environmental DNA analysis, is tested by Swiss and French scientists in collaboration with public and private stakeholders on diatoms, a bioindicator commonly used for water quality assessment.

The aim is to bring together scientists, environment managers as well as public and private partners in Switzerland and France to develop and apply this DNA-based broadband approach to biomonitoring of regional aquatic ecosystems. Collaboration between public and private stakeholders will help to develop a robust and reliable tool that is tailored to their needs and will strengthen the current practice of environmental diagnosis. The innovative tools that will be developed in synergy in France and Switzerland should make it possible to respond to the need for better monitoring and protection of the regional heritage of aquatic environments in a rapidly changing area subject to multiple pressures.

**Keywords:** aquatic ecosystem, biomonitoring, eco, genomic tools, High Throughput Sequencing, DNA, barcoding

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*Speaker

†Corresponding author: agnes.bouchez@inra.fr

# Environmental persistence of pharmaceutical residues from hospital and urban effluents and their impacts on environmental aquatic microbial communities.

Agnès Bouchez*†[1], Teofana Chonova , Benoit Cournoyer , Leslie Mondamert , and Jérôme Labanowski

[1]INRA CARRTEL – Institut National de la Recherche Agronomique - INRA (FRANCE) – 75 bis av. de Corzent, 74200 Thonon-les-Bains, France

## Abstract

Pharmaceutical residues coming from wastewater treatment plant (WWTP) discharges are regularly observed in natural waters. In this respect, the one-health issues associated with this contamination are currently being examined with considerable attention. The lack of knowledge and tools to monitor environmental exposure make their risk assessments hard to perform. The French SIPIBEL pilot site, monitored from 2011, includes a WWTP where urban and hospital wastewaters are treated separately. Treated effluents are delivered into a connected river. On this unique pilot site, the reliability of natural aquatic biofilms for monitoring the ecological impact of pharmaceuticals was investigated. The relations between field parameters, pharmaceutical concentrations, and organization of microbial communities were investigated. Microbiological and chemical tools were used to i) monitor the exposure of aquatic biofilms to pharmaceutical residues (analysis of different matrices: water, passive samplers, biofilms), and ii) assess changes in biofilm communities related to pharmaceuticals (community structure assessed through molecular fingerprinting or High Throughput Sequencing - HTS). Aquatic biofilms were grown from urban and hospital treated wastewaters, and from river surface waters located up- and downstream the WWTP discharge area. The finer the evaluation tools used to investigate the biofilm exposure and changes, the more reliable were the relations observed between pharmaceutical concentrations and community genetic patterns. Thus, the impact of low-level pharmaceutical concentrations could be investigated *in situ*. The combination of these monitoring tools highlighted environmental changes among the investigated aquatic ecosystem that might have impacted its functioning and ecological quality status.

**Keywords:** environemental monitoring, pharmaceutical residues, monitoring tools, biofilm

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*Speaker

†Corresponding author: agnes.bouchez@inra.fr

# Shifts in planktonic and biofilm prokaryotic community composition along a chemical multicontamination gradient in a north-western coastal mediterranean environment (Toulon bay, France)

Coclet Clément*[1], Jean-François Briand[2], Gaël Durrieu[1], Christophe Le Poupon[1], Sébastien D'onofrio[1], Lyria Berdjeb[1], Dario Omanović[3], Cédric Garnier[1], and Benjamin Misson[1]

[1]PROTEE – Université de Toulon : EA3819 – 83041 Toulon, France
[2]MAPIEM – Université de Toulon : EA4323 – 83041 Toulon, France
[3]Ruer Bošković Institute – Bijenička 54, Zagreb, Croatia

## Abstract

Mediterranean coastal environments are currently subjected to various anthropogenic inputs, resulting in multiple chemical contaminations. Microbial communities play an essential role in ecosystem functioning, however their response to such chemical multicontaminations remains poorly characterized. Samples were collected from June 1st to June 29th, 2015 at two depths (top and bottom of the water column) in five chemically contrasted sites along a gradient of multicontamination (in several trace metals and nutrients) in Toulon Bay. By combining flow cytometry and 16S rRNA gene sequencing (Illumina MiSeq), we assessed spatio-temporal variability of planktonic and biofilm microbial abundance and diversity in relation to natural and human-influenced environmental variables (physico-chemical variables, nutrients and trace metals (TM) concentrations). Strong spatial gradients of TM concentrations were observed in both surface and bottom seawater, with up to 115-fold enrichments. On the one hand, samples collected at the most contaminated sites exhibited a significantly higher planktonic prokaryote abundance than those from less contaminated sites, while no difference was observed in biofilms. On the other hand, alpha diversity (richness, Shannon and Chao1) was the lowest at the most contaminated sites. Moreover, microbial assemblages appeared to differ strongly all along the contamination gradient (45 to 57% dissimilarity, unweighted Unifrac distances). The main taxonomic groups identified in contaminated sites were Bacteroidetes (Flavobacteriia) and Proteobacteria (Alpha- and Gammaproteobacteria) while in the less contaminated site Proteobacteria (Alphaproteobacteria) and Cyanobacteria (Synechococcophycideae) were found dominant. Respective contributions of natural and anthropic environmental gradients to biological spatio-temporal shifts remain to be precise.

**Keywords:** Prokaryotic community, Biofilm, Environmental factors, Mediterranean Sea, High throughput sequencing

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*Speaker

# Combination of high-throughput sequencing and geochemistry methods for evaluating the impact of single and multi-metal pollutions in soil at the aggregate scale.

Jean Martins*[1], Frédéric Lehembre , Emilie François , Aline Navel , Erwann Vince , and Lorenzo Spadini

[1]Institut des Géosciences de l'Environnement (IGE) – Institut National Polytechnique de Grenoble (INPG), INSU, OSUG, CNRS : UMR5001, Institut de recherche pour le développement [IRD] : UR252, Université Grenoble Alpes – IGE - HyDRIMZ – Université Grenoble Alpes CS 40 700. F-38058 Grenoble cedex 9, France

## Abstract

J.M.F. Martins, F. Lehembre, E. François, A. Navel, E. Vince and L. Spadini.
IGE UMR5001, UGA-CNRS-GINP-IRD, France
In soils toxic effects of metals, especially in mixtures, disturb and modify competitive balance between species of microorganisms, involving in return a deterioration of the fundamental biogeochemical cycles (C, N, P...). The sensitivity of microorganisms responses to environmental stresses make them relevant ecotoxicological indicators. Soils are spatially heterogeneous environments structured in aggregates of different sizes. Similarly to microorganisms, metals present heterogeneous and specific distribution patterns among these micro-aggregates. The objective of this work was to study the restructuring action of a copper, chromium and cadmium mixture on the microbial communities of a structured-soil at the microaggregate-scale. A grassland-soil contaminated with equitoxic-concentrations of Cu-Cd-Cr, alone or mixed. The contaminated soils were gently physically fractionated during a 2 months period. Each soil size-fraction ($>$ 250$\mu$m, 250-63$\mu$m, 63-20$\mu$m and $<$ 20$\mu$m) and the bulk soil were analyzed for mass-balance, determination of free-soluble-total metals contents and intrinsic properties (pH-Eh and OC-N contents). Microbial communities were characterized by estimating biomass and bacterial density/diversity were estimated by 16SrDNA rtPCR and high-throughput sequencing (MiSeq-Illumina). The results provide better understanding of the spatial impact of heavy-metal mixtures on soil microbial-communities, according to their respective distribution within the different soil-microhabitats, and the temporal evolution of microbial communities. A new vision of the microstructure of soil bacterial community is presented, which also opens the way for the detection and identification of rare-taxa that may be new bio-indicators of soil health

**Keywords:** Metals, mixtures, soil, spatialized impact, aggregates, microhabitats, microbial community, MiSeq, distribution

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*Speaker

# Quick metaproteomics for environmental risk assessment

Jean Armengaud*[1], Sophie Ayrault , Guylaine Miotello , Emilie Avazeri , Gérard Steinmetz , Jean-Charles Gaillard , Charlotte Mappa , Karim Hayoun , Béatrice Alpha-Bazin , and Olivier Pible

[1]Innovative technologies for Detection and Diagnostics (Li2D) – CEA Marcoule – BP17171, F-30207 BAGNOLS-SUR-CEZE, France

### Abstract

Metaproteomics allows in-depth exploration of microbiota through i) establishing an inventory of organisms present in a sample, even for a complex sample, and ii) documenting new insights into functional pathways and interactions between microorganisms. We propose to simplify metaproteomics data acquisition and data treatment for being able to explore any microbiota in a few hours even without sequencing data. This allows quick metaproteomics of water or soil samples become a reality. We designed a new bioinformatics pipeline for quick analysis of samples and a lab-assembled microbiota reference comprising 24 bacterial species covering 20 genera and 5 phyla for evaluating data processing procedures for metaproteomics. We exemplified the possible applications of this procedure for environmental risk assessment on soil samples from the Seine River basin downstream of Paris Megacity. Major changes in biodiversity may be evaluated from a 60 min tandem mass spectrometry run with a broad vision as the method is able to assess bacteria, archaea, fungi, and microalgae at once. Results on diverse environmental samples of interest for monitoring anthropogenic impacts will be commented.

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**Keywords:** Microbiota, Metaproteomics, Tandem mass spectrometry, Bacteria, Archaea, Fungi, Soil, Water

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*Speaker

# From the screening of soil eukaryotic metatransciptomes to the functional characterization of new metallothioneins

Laurence Fraissinet-Tachet*[1], Antoine Ziller[1], Rajiv Yadav[1], Frédéric Lehembre[1], Laurent Vallon[1], Jan Colpaert[2], and Roland Marmeisse[1]

[1]Ecologie microbienne (EM) – Institut national de la recherche agronomique (INRA) : UR1193, CNRS : UMR5557, Université Claude Bernard - Lyon I (UCBL), Ecole Nationale Vétérinaire de Lyon – BAT GREGOR MENDEL (ex 741)-4 et 43 Bvd du 11 Novembre 1918 69622 VILLEURBANNE CEDEX, France

[2]University of Hasselt - Centrum voor Milieukunde – Belgium

## Abstract

Metal-polluted soils are colonised by microbial communities which have developed different adaptations that allow them to resist. Knowledge of eukaryotic resistance mechanisms to pollutants often results from studies on a limited number of model species. The objective of this study was to isolate and characterize new environmental genes expressed directly in soils by eukaryotic microorganisms and involved in metal resistance mechanisms. For this purpose, we have implemented an environmental genomics approach, the functional metatranscriptomics, to isolate resistance genes expressed in soils.

Metatranscriptomics is based on the direct extraction of eukaryotic polyadenylated mRNAs from soil samples followed by their conversion into cDNAs which are cloned in a yeast expression vector. These environmental cDNA libraries were used to complement a Cd-sensitive yeast mutant and resistant genes were recovered and analysed. Fifty percent of these genes were completely new, without homologs in databases. Some of these genes encode cysteine-rich proteins that we have called CRPs. These protein sequences present specific features that relate them to metallothioneins (MTs). They, however, also display unusual features: an "unusual" length (110-132 aa), the presence of a CCC cluster, two conserved His residues and a cysteine-free linker dividing the protein sequence in two parts. After CRP expression in E. coli and purifications, ESI-TOF-MS assays revealed that CRPs are able to chelate Cd, Zn and Cu and belong to a completely new MT family.

These results highlight the power of the functional metatranscriptomic approach which allows the discovery of new metal resistance genes.

**Keywords:** Microbial ecotoxicology. Heavy metals. Functional metatranscriptomics. Metal resistance genes. Metallothioneins. Protein purification and biochemical characterization

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*Speaker

# Volatolomics in microbial ecotoxicology, a pathway for detecting metabolic signatures of exposure to xenobiotics

Kevin Hidalgo*[1], Erwan Engel[1], Jean Louis Bonnet[2], and Philippe Bouchard†[2]

[1]UR 370 Qualité des Produits Animaux, Equipe Micro-contaminants Arôme et Sciences Séparatives (MASS) (QuaPA, MASS) – Institut National de la Recherche Agronomique - INRA (FRANCE) – INRA Centre de recherches Auvergne-Rhône-Alpes Theix 63122 Saint-Genès-Champanelle, France
[2]Microorganismes : génome et environnement (LMGE) – CNRS : UMR6023, Université Clermont Auvergne – Laboratoire Micro-organismes Génome et Environnement, Campus Universitaire des Cézeaux 1 impasse Amélie Murat, TSA 60026, CSA 60026 63178 Aubière Cedex, France

## Abstract

Monitoring the presence of xenobiotics in ecosystems is an important environmental, health and economic challenge. Development of alternative analytical methodologies based on "omics" technologies makes possible to consider an exposure biomarker approach to xenobiotics. It is therefore no longer a matter of quantifying residues of phytosanitary products, but of measuring the level of exposure of sentinel organisms in various compartments of the environment.
The stress tolerance of tissue and/or cell involves response processes whose metabolic signatures are highly volatile metabolites. The objective of this work was to evaluate the feasibility of a novel rapid approach based on a non-targeted analysis of volatile compounds in microorganisms to detect previous exposure to different types of xenobiotic. We seek to detect metabolic fingerprints specifically indicative of an exposure to metabolic response libraries obtained under treated *versus* control conditions of contamination.

Our analytical process is based on the *in situ* concentration of such volatile molecules using solid phase micro-extraction techniques (SPME) and gas chromatography coupled to a mass spectrometer (GC-MS). We highlight that low-dose exposure (NOEC) of two pyrethro´ids (deltamethrin and cypermethrin) and a tricetone (sulcotrione) in soil and water sentinel micro-organisms (*Bacillus megaterium*, *Pseudomonas fluorescens* and the ciliate *Paramecium*) induces specific metabolomic fingerprints, indicative of the exposure.
The toxicogenomics objective is now to feed a database of metabolic biomarkers revealing exposure to phytosanitary agents in micro-organisms then in animal models, consequent of a continuum in food production (*e.g.* bees, fish and poultry).

**Keywords:** Volatolomic, Metabolomic, Biomarker, Toxicogenomic

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*Speaker

†Corresponding author: philippe.bouchard@uca.fr

# Development of a standardized aquatic hyphomycete laboratory assay for fungicide risk assessment

Patrick Baudy*[1], Jochen Zubrod[1], Ralf Schulz[1], and Mirco Bundschuh[2]

[1]Institute for Environmental Sciences, University of Koblenz-Landau – Fortstraße 7, 76829 Landau, Germany

[2]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences – Lennart Hjelms väg 9, 75007 Uppsala, Sweden

## Abstract

During the aquatic environmental risk assessment (ERA) of herbicides and insecticides, the testing of the presumably most sensitive taxa (i.e., aquatic plants and arthropods, respectively) is required. During fungicide ERA, in contrast, no aquatic fungi are tested. The protectiveness of *a priori* ERA for aquatic fungi is thus largely unknown, while recent studies indicate effects on these organisms at field-relevant fungicide concentrations. Furthermore, *a posteriori* ERA is mostly based on algae as benchmark microorganisms, while the correlation between the sensitivity of algae and aquatic fungi was never investigated. To address this fundamental knowledge gap, we are currently developing a suggestion for a standardized laboratory assay using aquatic hyphomycetes – an ecologically important group of fungi. This involves the assessment of the fungi's reproduction (i.e., sporulation) over 4 days as well as their growth on fungicide-spiked agar over 56 days. In preliminary experiments ($n$=3), we exposed three aquatic hyphomycete strains towards eight concentrations of either of the fungicides dimethomorph or tebuconazole (0 to 10,000 $\mu$g/L). Sporulation was three orders of magnitude more sensitive towards fungicide exposure than growth. Respective EC50 values were partly in the low $\mu$g/L-range, which, however, requires further verification due to high variation. Based on these first insights, we conducted a power analysis to optimize the assay's replication and thus statistical performance. Using this adjusted design, we are currently screening a range of fungicides frequently detected in surface waters to check for the protectiveness of fungicide ERA and the correlation of fungal and algal sensitivities towards these pesticides.

**Keywords:** aquatic hyphomycetes, fungicides, growth, reproduction, risk assessment

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*Speaker

# Integrated biomarker and ecological response (IBER) index

Helena Guasch*[1,2], Berta Bonet , Chloé Bonnineau , and Laura Barral

[1]Universitat de Girona – Spain
[2]Institute of Aquatic Ecology (University of Girona,Spain) (IEA) – Faculty of Sciences, C Aurelia Capmany, Spain

## Abstract

Biomarkers, defined in ecotoxicology as functional measures of exposure to chemicals, may not be informative on the consequences of exposure at the scale of interest, which is the entire ecosystem. This drawback is because links and interactions existing between these measures and the biological system at a larger scale are not always sufficiently known. In this presentation, we propose the application of an integrative multi-metric approach providing unequivocal information concerning exposure, early effects, adaptation and ecosystem damage caused by metal pollution. The IBER index is based on biomarkers of effect and/or exposure (e.g. antioxidant enzyme activities, community structure and resistance genes), together with other chemical, biogeochemical and ecological metrics related to microbial metabolism (e.g. bioaccumulation, chemical speciation, organic matter decomposition or nutrient cycling).

**Keywords:** biomarker, integrated response, index, metal pollution, fluvial ecosystem

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*Speaker

# Microbial molecular and isotopic biomarkers for evaluating natural attenuation of chlorinated hydrocarbons in multi-contaminated sites

Jennifer Hellal[*][1], Jérémie Denonfoux[2], Catherine Joulian[1], Stéphane Vuilleumier[3], Stéphanie Ferreira[2], Louis Hermon[1,3], and Gwenaël Imfeld[4]

[1]Environmental Biogeochemistry and Water Quality Unit (D3E/BGE) – Bureau de Recherches Géologiques et Minières (BRGM) – France

[2]Genoscreen [Lille] – Institut Pasteur de Lille – Campus de l'Institut Pasteur, 1 rue du Professeur Calmette, Lille 59000, France

[3]Génétique moléculaire, génomique, microbiologie (GMGM) – université de Strasbourg, Centre National de la Recherche Scientifique : UMR7156 – 28 Rue Goethe 67083 STRASBOURG CEDEX, France

[4]Laboratory of Hydrology and Geochemistry of Strasbourg (LHyGeS), UMR 7517 CNRS – université de Strasbourg – France

## Abstract

Groundwater contamination by aliphatic chlorinated hydrocarbons (*e.g.* tetrachloroethene (PCE) and its degradation products) and dichloromethane (DCM) in the environment is high due to their extensive use as solvents and their persistence in the environment. Their efficient remediation mainly relies on prior plume characterization. Although physico-chemical assessment is indispensable, it can be complemented with an evaluation of bacterial diversity and activity on-site, using molecular biomarkers to evaluate *in situ* natural attenuation. The aim of this collaborative research project funded by the French Environment and Energy Management Agency (ADEME) is to evaluate the occurrence of PCE, TCE, DCE and CV degradation in contaminated groundwater, and how it can be linked to specific genes to evaluate the added value of molecular biomarkers and compound-specific isotope analysis (CSIA) for enhanced diagnosis and monitoring of multi-contaminated groundwater. Groundwater was sampled biannually in 12 piezometers at the source, in the plume and its fringes at a contaminated site (France). Four comparative approaches were used to analyze the samples: i) groundwater physical-chemical parameters, ii) CSIA of the pollutants ($\delta$13C), iii) functional gene abundance, and iv) new generation sequencing of microbial diversity (Illumina MiSeq). In addition to on-site monitoring, the degradation potentials of PCE, DCE, VC and DCM were assessed in different redox conditions in batch assays. These complementary experiments coupling all or several of the 4 comparative approaches contributed to interpreting the onsite data. The expected impact of this project is to validate a set of tools to help define remediation and site management strategies.

**Keywords:** vinyl chloride, dichloroethene, biomarkers, reductive dechlorination, functional genes.

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[*] Speaker

# High throughput microbial array for complex environmental sample assessment

Anna Hua[*,1], Mickael Cregut[1], Sulivan Jouanneau[1], Christophe Cordella[2], Gérald Thouand[†,1], and Marie-José Durand[‡,1]

[1]Laboratoire de génie des procédés - environnement - agroalimentaire (GEPEA) – CNRS : UMR6144, Université de Nantes : UMR6144 – France
[2]Inra, AgroParisTech – AgroParisTech – France

## Abstract

Ecotoxicological assessment using different bioassays for environmental sample are largely used this last 20 years. Among them, recombinant bioluminescent or fluorescent bacterial bioassays have been developed to determine the presence and the toxic effect of pollutants. Chemical analyzes are sensitive and specific to detect pollutant, but they failed in the determination of bioaccessibility and biodisponibility of these contaminants. To overcome this problem, recombinant bioluminescent bacteria have been developed over the past years. These recombinant bacteria carry reporter genes for bioluminescence or fluorescence fused with gene promoters involved in metal resistance mechanisms or with genes involved in the degradation of organic compounds. These bioassays are generally not specific for one compound, in particular those dedicated to Metal Trace Element (MTE). Because of the lack of specificity of these recombinant bacteria, applications for environmental samples are limited. To deal with this problem, we used a set of bioluminescent recombinant bacteria sensitive to MTE, which bioluminescence increases in the presence of MTE, and a collection of 1896 fluorescent recombinant bacteria covering the majority of gene promoters of *Escherichia coli* strain. Results obtained on complex environmental samples (wood-waste leachates, soil) showed variation of bacterial response according to the contamination allowing an interesting method of screening. Because improvement of ecotoxicological assessment needs a large panel of bioassays, we propose a mixture of targeted and non-targeted high throughput arrays of bacteria to describe more realistically complex samples toxicity, combined with data mining interpretation.

**Keywords:** Bioassays, recombinant bacteria, assessment, bioaccessibility

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[*] Speaker
[†] Corresponding author: gerald.thouand@univ-nantes.fr
[‡] Corresponding author: durand-thouand-mj@univ-nantes.fr

# Communication Abstracts

## POSTERS

# Reduction of pesticide contamination in agricultural soils: a bioprophylactic approach.

Louis Carles[1,2], Pascale Besse-Hoggan[2], and Isabelle Batisson*†[1]

[1]Laboratoire Microorganismes : Génome et Environnement, UMR CNRS 6023 (LMGE) – Université Clermont Auvergne – France

[2]Institut de Chimie de Clermont-Ferrand (ICCF) – CNRS : UMR6296, Université Blaise Pascal - Clermont-Ferrand II, Ecole Nationale Supérieure de Chimie de Clermont-Ferrand – 24 Avenue des Landais, 63177 Aubière Cedex, France

## Abstract

The major problem with pesticides is linked to the more or less substantial proportion of the active ingredient quantity spread that does not reach its intended target, and contaminate environmental compartments. We developed a bioprophylactic process based on the simultaneous application of pesticide and pesticide-degrading microorganisms, providing optimal conditions for microbial degradation of pesticides after their specific action and before their transport to environmental compartments. Up to now, studies related to bioprophylaxis rather focused on non-agricultural uses of pesticides [1-2]. The aim of our study was to determine the suitability of this concept in agriculture, with a model of herbicide (2,4-D, 2,4-dichlorophenoxyacetic acid) and one of its bacterial degrading-strain (*Cupriavidus necator* JMP134).

The biodegradation of 2,4-D was studied in soil microcosms planted with sensitive and non-sensitive plants. Simultaneous application of 2,4-D at agricultural recommended doses and inoculation with 105 cells.g-1 dw of soil of JMP134 strain were carried out. The quantification of residual 2,4-D in soil, the survival rate of inoculated bacteria and the assessment of the herbicidal effect on sensitive plants were monitored. In soil inoculated with C. necator JMP134, the herbicidal effect was conserved and the 2,4-D concentration was reduced by 97% within seven days, while only 55% reduction was observed in the non-inoculated control.

By using realistic conditions as close as possible to those of the field (*i.e.* recommended field dose of pesticide, commercial formulation), our study constitutes first bases for the development of bioprophylaxis as a good means to reduce environmental contamination by agricultural pesticides.

**Keywords:** Pesticide, 2, 4, dichlorophenoxyacetic acid, Bioremediation, Cupriavidus necator

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*Speaker

†Corresponding author: isabelle.batisson@uca.fr

# Natural petroleum seepages shapes the microbial community diversity and activities of a forest ecosystem

Aurélie Cebron*†[1], Thierry Beguiristain[1], Kifayatou Rabiou Cholabomi[1,2], Delphine Catteloin[2], Catherine Lorgeoux[2], and Pierre Faure[1]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360 – Faculté des Sciences et Technologies, BP70239, 54506 Vandoeuvre-lès-Nancy, France
[2]GeoRessources – CNRS : UMR7359, Université de Lorraine – Faculté des Sciences et Technologies, BP70239, 54506 Vandoeuvre-lès-Nancy, France

## Abstract

Rapid selection of microbial populations and biodegradation functions after accidental petroleum contaminated is well studied. However, the long-term adaptation of microbial diversity on chronically polluted environment is less documented. The aim of our study was to evaluate how petroleum contamination shapes microbial communities and how it selects for specific microbial taxa or modulates microbial functions. We worked on a unique site (forest of Haguenau, Pechelbronn, France) contaminated for hundreds of years by petroleum seepages inherited from a tertiary source rock. Two seepages presenting different pedological characteristics were studied. We sampled soils at five points (in 3 replicates) along a 20 m gradient from the seepage source to the forest control soil. Hydrocarbon content was quantified (GC-FID) and qualified (GC-MS), microbial enzymatic activities related to C, N and P cycles were measured and microbial abundance and diversity were evaluated. On both sites, results were roughly similar. Hydrocarbon indexes showed lateral gradients of contamination, i.e. petroleum was unaltered at the seepage and biodegradation evidences appeared with increasing distance from the seepage. Moreover, microbial mineralization activities, some enzymatic activities, microbial abundance and occurrence of some hydrocarbon-degrading bacteria were low at the source point, became higher at the closest points (2-3 m from the petroleum source) and then decreased toward the less contaminated area, indicating modifications of the bacterial and fungal community structures.

**Keywords:** petroleum, natural seepages, forest soil, biodegradation, enzymatic activities, microbial diversity

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*Speaker

†Corresponding author: aurelie.cebron@univ-lorraine.fr

# Dissipation of chlorpyrifos, pendimethalin, and thiophanate-methyl pesticides in compost based-versus peatmoss based-biomixtures of biobeds

Talaat El Sebai*†[1], Wafaa Zidan[1], Wafaaabd El Rehim[1], Alaa Marzouk[2], Marion Divers[3], and Fabrice Martin-Laurent[3]

[1]Agricultural Microbiology Dept. National Research Centre – Cairo, Egypt
[2]Laboratory of pesticide analysis, Agriculture Research Centre – Cairo, Egypt
[3]Agroécologie – Institut National de la Recherche Agronomique : UMR1347, Université de Bourgogne, Institut National Supérieur des Sciences Agronomiques, de lÁlimentation et de lÉnvironnement – Université de Bourgogne - 17 rue Sully - BP 86510 - 21000 Dijon, France

## Abstract

Biobeds is a simple and a cost effective way to treat point source pesticide's contamination. In this study, we replaced peat-moss (P) with compost (C) which is locally available to compare the effectiveness of both bio-mixtures (biomix) P and C on pesticide dissipation. Chlorpyrifos, pendimethalin, and thiophanate methyl were added separately (at three concentrations 25, 50, and 75 mg/kg of both biomix P and C). Our results showed that biomix composition influenced the dissipation of pesticides. For chlorpyrifos, the highest dissipation rate was recorded in biomix P. This result was confirmed by the mineralization kinetics, since, 25% of 14C-chlorpyrifos initially added to biomix evolved to 14CO2 in biomix P while only 14% evolved to 14CO2 in biomix C. In addition, chlorpyrifos dissipation rate was found to be influenced by the initial concentration applied to biomix P and C. In contrary, highest rate of dissipation of pendimethalin was observed in biomix C. More than 76% of pendimethalin was dissipated in biomix C while only 67 % were dissipated in biomix P. The efficiency of both biomix (C and P) was similar in the dissipation of thiophanate-methyl but less efficient compared to other tested pesticides. The addition of the three tested pesticides as mixture at 25 mg/kg of biomix C had a positive effect on both chlorpyrifos and thiophanate-methyl dissipation, while pendimethalin dissipation was similar when applied separately or mixed. In both biomixtures, dehydrogenase activity of microorganisms was stimulated by the addition of pesticides.

**Keywords:** biobeds, pesticid, contamination, point source, bioremediation

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*Speaker

†Corresponding author:

# Analysis of the extracellular matrix of a monospecific benthic biofilm exposed to different forms of carbon nanoparticles

Marion Garacci*[1], Jérôme Silvestre[1], Florence Mouchet[1], Pierre Lonchambon[2], Emmanuel Flahaut[2], Laury Gauthier[1], Maïalen Barret[1], and Eric Pinelli†[1]

[1]Laboratoire Ecologie Fonctionnelle et Environnement (EcoLab (équipe ECI)) – Institut national de la recherche agronomique (INRA), CNRS : UMR5245, Ecole Nationale Supérieure Agronomique de Toulouse, Université Paul Sabatier (UPS) - Toulouse III, Institut National Polytechnique de Toulouse - INPT, PRES Université de Toulouse – ENSAT, Avenue de l'Agrobiopole, 31326 Castanet Tolosan, France

[2]Centre interuniversitaire de recherche et d'ingenierie des matériaux (CIRIMAT) – Université Paul Sabatier (UPS) - Toulouse III, CNRS : UMR5085, Institut National Polytechnique de Toulouse - INPT – Laboratoire de Physique des Poly 118,route de Narbonne 31062 TOULOUSE CEDEX 4, France

## Abstract

Carbon-based nanoparticles such as Few Layer Graphene (FLG) and Carbon NanoTubes (CNT) present a rising interest in several fields as aerospace. Then, at the end of their life cycle, carbon nanoparticles are likely to be found in aquatic ecosystems, where phototrophic microorganisms such as diatoms represent the first level in the food chain.
In this study, the effect of different forms of carbon nanoparticles on the benthic freshwater diatom *Nitzschia palea* was assessed distinguishing shading and total effect induced by carbon nanoparticles.

The cellular response of *N. palea* exposed to carbon nanoparticles was assessed by monitoring the cellular growth. After 48 hours of exposure, an impact of high FLG and CNT concentration (50 mg.L-1) on the growth rate was observed. Nevertheless, a recovery of growth was observed after 144 hours of exposure, linked to a sticking of nanoparticles in the biofilm formed by the extracellular substances secreted by diatoms.

To bring the role of extracellular proteins to light, the molecular response of *N. palea* exposed to CNT and FLG has been assessed carrying out the extraction of the extracellular matrix. At 48h of exposure, the effect of CNT was more pronounced than FLG inducing an overproduction of 50% of hydrophobic proteins at high concentration. At 144h of exposure, the effect of FLG and CNT was similar and lead to a ten-fold overproduction of ionic proteins. Our results demonstrate that carbon nanoparticles can modify the characteristics of benthic biofilms, which is likely to disturb the functioning of natural ecosystems.

**Keywords:** benthic biofilm, carbon nanoparticles, exopolymeric substances, protein overproduction

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*Speaker

†Corresponding author: pinelli@ensat.fr

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# Does compost addition affect the mineralisation of 14C-pesticides non-extractable residues in soil ?

Thomas Lerch*†[1] and Enrique Barriuso[2]

[1]Institute of Ecology and Environmental Sciences (IEES-Paris) – CNRS : UMR7618 – Université Paris-Est Créteil Val de Marne (UPEC) Faculté de sciences et technologie Bâtiment P 61, avenue du Général de Gaulle 94010 Créteil Cedex, France

[2]Ecologie fonctionnelle et écotoxicologie des agroécosystèmes (ECOSYS) – Institut national de la recherche agronomique (INRA) : UMR1402 – Campus INRA-AgroParisTech 78850 Thiverval-Grignon, France

## Abstract

The formation and the fate of pesticide non-extractable residues (NER) in soil are important processes that should be taken into account when assessing pollution risks. However, the underlying mechanisms are still poorly understood. In particular, the compost often applied on agricultural soils to sustain the content in soil organic matter (SOM) is likely to alter the biodegradation of such xenobiotics residues, either by reducing their availability or stimulating the microbial biomass. The main objective of this study was to assess the long and short terms effects of compost on the mineralisation of pesticide NER. To this aim, we set up an incubation with a 5x2x3 factorial design using NER coming from 5 different 14C-labelled pesticides (atrazine, simazine, pendimethaline, metsulfuron-methyl and 2,4-dichlorophenoxy acetic acid), initially formed in a soil amended or not with compost (Barriuso et al., 1996), and incubated with 3 different doses (0, 10 or 20%) of composts during 386 days at 28∘C and 80% WHC. During the incubation, total CO2 and 14C-CO2 were trapped in NaOH solution and measured with colorimetric or scintillation counting, respectively. The mineralisation kinetics showed differences among NER, simazine- and pendimethaline-NER being the least mineralised (< 6%) and metsulfuron NER the most (> 12%). The short term effect of compost on NER mineralisation was minor compared to long term effect, especially for 2,4-D and atrazine. These results suggest that the addition of compost strongly affects NER fate when applied at the same time as the pesticides in soil, but has only small effects years after.

**Keywords:** Soil, Pesticides, Bound, residues, Mineralisation, Compost

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*Speaker

†Corresponding author: thomas.lerch@u-pec.fr

# Isolation of rhizobacteria from agricultural soils treated with pesticides in the Meknes region, Morocco

Mohamed Maldani*†[1], Laila Nassiri[1], and Jamal Ibijbijen[1]

[1]Environment Soil Microbiology Unit – Faculty of Sciences, Moulay Ismail University, Meknes,, Morocco

## Abstract

Soil quality covers the physical, chemical and biological. the interactions between them result in efficient operation in terms of production. In agricultural soils, contamination by pesticides has become an alarming environmental problem. This problem influences primarily on the biological properties of soils particularly on the microbial community in these soils. several studies have shown that the excessive use of pesticides has a toxic effect on the microbial flora of the soil. The objective of our study was to isolate rhizobacteria from 17 agricultural soils, previously cultivated by legumes and cereals, from the agricultural zone of Moulay Idriss (Meknes, Morocco), and subsequently tested their tolerances and degradation of pesticides. From these soils we isolated 300 strains of bacteria, after the tolerance test we isolated 57 bacterial strains that tolerated high concentration of pesticide. These strains will be identified.

**Keywords:** Rhizobacteria, Soil, Pesticides, Degradation, Morocco

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*Speaker

†Corresponding author: maldani.mohamed@hotmail.com

# Dispersion of microplastic in various media and its quantification in periphyton by flow cytometry and viSNE data analysis

Stephanie N. Merbt*[1], Linn Sgier[1], Ahmed Tlili†[1], Kristin Schirmer[1], and Renata Behra[1]

[1]Eawag – Department of Environmental Toxicology, Überlandstrasse 133, 8600 Dübendorf, Switzerland

## Abstract

Microplastic, defined as particles smaller than 1 mm, occurs ubiquitously in aquatic environments. Microplastic can sink in natural waters and potentially accumulates in periphyton, which is an assemblage of embedded algae and bacteria in a matrix of extracellular polysaccharide substances (EPS). With the objective to analyse interactions of microplastic with periphyton, we first aimed to achieve controlled exposure conditions. Therefore, we examined various dispersion media, and developed a method to quantify microplastic within periphyton by flow cytometry. We used various polyethylene microplastics, differing in size and density, and compared the time-dependent stability of the particle suspensions, using a cell counter. For flow cytometry measurements, the particles were dispersed in a periphyton suspension and their abundance was determined using viSNE data analysis. Microplastic was easily dispersed in Tween80, BSA and EPS extracts, unlike in water and fulvic acid where a sonication and strong mixing were needed. As expected, particles with low density buoyed upwards in all media, while particles with high density sedimented. Size of the particles also determined the temporal dynamics of the observed processes. Small particles remained dispersed more than 10 min, while biggest particles accumulated within 1 minute. Based on distinct fluorescence and scattering characteristics of the particles and periphyton organisms, flow cytometry coupled with viSNE data analyses allowed detection and quantification of the microplastic in a highly complex sample. Altogether, this work helps to guide experiments with microplastic under controlled conditions, and adds knowledge to their behaviour in fresh waters.

**Keywords:** Microplastic, periphyton, flow cytometry, fluorescence

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[*] Corresponding author:

[†] Speaker

# Avoidance behaviour of oligochaetes in metal-based fungicide spiked soils: Effect of bacterial consortia

Oluwatosin Oladipo*[1], Pierre De Kock[1], Marco Engelbrecht[1], Carlos Bezuidenhout[1], and Mark Maboeta[1]

[1]Unit for Environmental Sciences and Management, North-West University, (NWU) – Private Bag X6001, Potchefstroom 2520, South Africa

## Abstract

Metal-based fungicides may influence oligochaetes' behaviour and result in inhibiting habitat function. The presence of bacterial consortia in such polluted soils may be of bioremediative advantage. This study investigated the effect of *Achromobacter spanius* and *Bacillus cereus* isolated from mining sites on the avoidance/preference behaviour of *Eisenia andrei* and *Enchytraeus buchholzi* in copper oxychloride (CO) and mancozeb (MZ) spiked soils. Broth cultures (24-H) of *A. spanius* and *B. cereus* were inoculated into MZ (44 and 1250 mg kg-1) and CO (200 and 1000 mg kg-1) spiked soils. Standardized protocols (ISO, 2007) were used for experimental set-ups. Avoidance behaviour revealed that *Eisenia andrei* and *Enchytraeus buchholzi* demonstrated preference for *A. spanius-B. cereus* inoculated compared to non-inoculated spiked soils. Higher preference was also exhibited by *Enchytraeus buchholzi* than *Eisenia andrei* and MZ compared to CO spiked substrates. In 44 mg kg-1 MZ inoculated substrates, a net response of -76 and -88 % were observed for *Eisenia andrei* and *Enchytraeus buchholzi* while 72 and 68 % were obtained in non-inoculated substrates. For 200 mg kg-1 CO spiked substrates, a net response of 28.57 and -98 % were observed respectively in inoculated substrates while in non-inoculated, 100 and 6.1 % avoidance was obtained. At 1250 and 1000 mg kg-1 MZ and CO concentrations, reduced habitat function was observed, with both oligochaetes revealing higher avoidance especially in non-inoculated and CO substrates. *Achromobacter spanius-Bacillus cereus* consortia thus enhanced *Eisenia andrei* and *Enchytraeus buchholzi* preference behaviour towards fungicide spiked soils.

**Keywords:** Soil pollution, metal, based fungicides, avoidance behaviour, oligochaetes, Achromobacter spanius, Bacillus cereus consortia

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[*] Speaker

# Ecology of bacterial communities during petroleum biodegradation: a molecular analysis on degrading consortia isolated from hydrocarbon polluted soils, sediment and seawater samples

Rahma Omrani*†[1], Edoardo Puglisi*‡[2], Giulia Spini[2], and Dalila Saidane[1]

[1]Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy, Monastir University, Tunisia – Tunisia

[2]Istituto di Microbiologia, Università Cattolica del Sacro Cuore (UCSC) – Via Emilia Parmense 84, 29122 Piacenza, Italy, Italy

### Abstract

Environmental microbial communities are key players in the transformation and mineralization of hydrocarbon pollutants. The aim of this study was to assess changes in bacterial abundance and diversity during the degradation of Tunisian Zarzatine oil by four indigenous bacterial consortia isolated from different polluted environmental matrix (a petroleum station soil, refinery reservoir soil, harbor sediment and harbor seawater). The microcosms were inoculated for two months with either a defined bacterial consortium and Zarzatine crude oil as only carbon and energy source. The microbial community dynamics was evaluated weekly in microcosms using Illumina 16S rRNA gene sequencing. An average of 7020 sequences per samples were obtained, the measured coverage was higher than 97% and more than 60% of sequence were correctly classified at species level. Degrading consortia enriched from soil and sediments were dominated by species belonging to *Pseudomonas* and *Acinetobacter* genera, while in the seawater-derived consortia *Dietzia*, *Fusobacterium* and *Dicoplana* emerged as dominant genera. We identified specific OTUs whose relative abundances bloomed from small to high percentages and were significantly correlated to total petroleum degradation: an OTU classified as *Dietzia daqingensis* in the seawater microcosms, and three OTUs classified as *Acinetobacter venetianus* in all two soils and sediment derived microcosms. Interestingly, a comparative analysis showed that this was the same OTU in all 3 microcosms which were derived from consortia isolated from different types (soil or sediment) and location. The work provides detailed insights about the modulation of bacterial communities involved in petroleum biodegradation.

**Keywords:** petroleum, degrading consortia, Illumina, bacterial ecology

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[*] Speaker

[†] Corresponding author: rahmaomrani-114@hotmail.fr

[‡] Corresponding author: edoardo.puglisi@unicatt.it

# From biodegradation of organic pollutants in soils by saprotrophic fungi ... to bioremediation.

Catherine Rafin*[1] and Etienne Veignie[1]

[1]Unité de chimie environnementale et interactions sur le vivant (UCEIV) – Université du Littoral Côte d'Opale : EA4492 – Maison de la Recherche en Environnement Industriel 145 avenue Maurice Schumann 59140 Dunkerque, France

## Abstract

Fungi constitute an important group in soil biological diversity and functioning. From the last two decades, our team has explored the natural competence of saprotrophic telluric fungi to degrade persistent organic pollutants (POPs) i.e. polycyclic aromatic hydrocarbons (PAHs) and dioxins/furans for developing soft bioremediation technologies of polluted soils. For bioremediation to be an effective tool for the clean-up of POP-contaminated soils, a greater optimization of factors such as bioavailability and mass transfer of POPs which were once thought to be recalcitrant is required. A collection of filamentous saprotrophic fungi was isolated from various historical contaminated soils (UCEIV mycology collection, Dunkerque, France). We focused our attention on fungi belonging to Ascomycota, Sordariomycetes, Hypocreales, with *Fusarium solani* as a fungal model. The metabolic pathways involved in the degradation of benzo[a]pyrene (BaP) as a model of high molecular weight PAH was explored. Lipid bodies constitute the intracellular storage sites of BaP in *F. solani* in which an energy-dependent uptake of BaP and a cytoskeleton dependent intracellular transport have been demonstrated. The degradative ability of other fungal isolates at laboratory scale (either in mineral medium or in soil microcosms) was also studied. The use of polysaccharides (potato or corn starch) with surfactant properties was considered for enhancing BaP degradation by oxidation. An overview of our current knowledge of saprotrophic telluric fungi mediated degradation/transformation of POPs will be presented. This outlined research performed at different levels of biological organization (from single-species to communities) can be transferred to other polluted ecosystems and other pollutants.

**Keywords:** Benzo[a]pyrene, Biodegradation, Bioremediation, Persistent Organic Pollutants, saprotrophic fungi, soil

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*Speaker

# Environmental impact of natural and synthetic $\beta$-triketone herbicides

Sana Romdhane[1], Marion Devers-Lamrani , Christophe Calvayrac , Franck Dayan , Cedric Bertrand , Fabrice Martin-Laurent , and Lise Barthelmebs*[1]

[1]Biocapteurs - Analyse- Environnement (BAE) – Université de Perignan Via Domitia – Université de Perpignan Via Domitia, 52 Av Paul Alduy, 66860 Perpignan cedex, France

## Abstract

In order to limit potential impacts on the use of plant protection products in agriculture, new generation molecules have been developed such as low impact pesticides and pesticides derived from natural substances.Nevertheless, many issues remain to be studied, including the assessment of potential risks and adverse effects related to their use on the environment and human health.

In the frame of the TRICETOX project, we focused on the $\beta$-triketone herbicide family, a post-emergence maize selective herbicides belonging to this new generation of molecules, and we worked on sulcotrione, one of the synthetic $\beta$-triketone herbicides placed on the market in 1993, and on leptospermone, an allelopathic compound isolated from the bottlebrush plant *Callistemon citrinus*.

A combination of chemical and biological approaches was followed in order to gain new insight for these two molecules, by addressing their soil behaviour, by studying their impact on soil microbial communities, by studying their biotic and abiotic degradation, and by developing new analytical methods.

In this context, our degradation studies revealed the existence of several transformation products. For the first time, we isolated for an arable soil a bacterial strain able to degrade leptospermone, and also, another bacterial strain capable to biodegrade two synthetic $\beta$-triketones herbicides. Microcosm studies revealed that leptospermone has a significant ecotoxicological impact on the abundance and diversity of soil bacterial community, similar to this of sulcotrione. We also succeeded in the development of a new bioassay method, based on the use of recombinant *E. coli* strains to quantify $\beta$-triketones herbicides in soil.

**Keywords:** $\beta$, triketone herbicides, impact, ecotoxicology, biodegradation, analysis, soil.

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*Speaker

# Enrichment procedures for the isolation of xenobiotic-degrading bacterial and fungal strains: a focus on microbial ecology

Giulia Spini*†[1], Federica Spina[2], Anne-Laure Blieux[3], Tiffanie Regnier[3], Abdelwahad Echairi[3], Ilaria Re[4], Giovanna Varese[2], and Edoardo Puglisi*‡[1]

[1]Istituto di Microbiologia, Università Cattolica del Sacro Cuore (UCSC) – Via Emilia Parmense 84, 29122 Piacenza, Italy, Italy
[2]Mycotheca Universitatis Taurinensis, University of Turin, Turin, Italy – Italy
[3]SATT Grand-est - Welience Agro-Environnement, Breteniѐre, France – Societe d'Acceleration du Transfert de Technologie (SATT) Grand Est - Welience – France
[4]Consorzio Italbiotec – Italy

## Abstract

LIFE-BIOREST (www.lifebiorest.com) is a EU-project aimed at the remediation of a soil polluted by PHAs, BTEX and alkanes through the application of selected consortia of fungi and bacteria.
In the first phase of the project, we have isolated from the polluted soil about 220 fungal and 140 bacterial strains with degrading abilities. Most of the fungal strains belonged to Ascomycetes (mainly *Aspergillus, Cladosporium, Fusarium* and *Scedosporium* genera) even though about 20 Basidiomycetes were also isolated. Regarding bacteria, the most abundant genus was *Pseudomonas* followed by *Achromobacter*, *Stenotrophomonas* and *Ochrobactrum.*

The isolates were obtained by means of a liquid enrichment approach on target contaminants (naphthalene, pyrene, phenanthrene, benzene, alkanes and oil extracted from the soil): the enrichment lasted 3 weeks, and sub-culturing was carried out once a week. Since the pollution in the test soil involved several meters of depth, enrichments were carried out separately using as inoculum homogenized soil samples from the 0-1, the 1-2 and 2-3 m depth. A total of 18 enrichment consortia were produced (6 target contaminants X 3 soil depths) for bacteria and other 18 for fungi, and sampled at day 0, 7, 14 and 21, giving a total of 144 samples. For each of these samples, DNA was extracted, amplified respectively for bacterial (16S) and fungal (ITS) biomarkers, and the resulting amplicons sequenced in Illumina.
Results will be presented with the aim of assessing the ecological evolution of the microbial communities (bacteria and fungi) during the selective enrichment procedure in presence of different pollutants.

**Keywords:** selective enrichment, microbial diversity, fungal and bacterial consortia

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*Speaker
[†]Corresponding author: giulia.spini@unicatt.it
[‡]Corresponding author: edoardo.puglisi@unicatt.it

# The dynamic degradation of pollutants by Nocardia in the infiltration basins

Florian Vautrin*[1]

[1]Laboratoire d'Ecologie Microbienne (LEM) – Université Claude Bernard - Lyon I (UCBL) – 16 rue Raphael Dubois, 69100 Villeurbanne, France

## Abstract

Infiltration basins concentrate pollutants derived from petroleum of the anthropised catchment basin. Some bacteria degrade these pollutants to grow in these extreme environments, especially actinobacteria, whose *Nocardia* genus belongs to. Some species of this ubiquitous bacteria are pathogen for frail populations and cause nocardiosis, which would affect about a million people each year in the world.
To date, the degradation ability of a given *Nocardia* species for a pollutant in particular remains unknown. The aim of this work is to assess which pollutants that most stimulate the growth of *Nocardia* pathogen species. This will lead to better understand the relationship between pollutants and infectious risk due to pathogenic *Nocardia* species.

We hypothesize that i) non pathogen species better degrade pollutants than pathogenic ones and ii) environmental strains of pathogenic species better degrade pollutants than clinical ones of the same species.
All the environmental and clinical species are able to use pyrene, fluoranthene and nonylphenol as sole carbon source and diuron as sole nitrogen source. These pollutants are degraded in other metabolism byproducts with enhance development of other environmental bacterial species. Bacteria of nonpathogenic species are most abundant in the sediments, which lower the dissemination risk of the pathogenic ones, and impact human health.

**Keywords:** Actinobacteria, Nocardia, urban sediment, infiltration basin, pollutant

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*Speaker

# Bacterial fungal interactions and their role for the degradation of contaminants

Lukas Yvo Wick*[1]

[1]Helmholtz Centre for Environmental Research - UFZ (GERMANY) (UFZ) – Permoserstrasse 15
D-04318 Leipzig, Germany

## Abstract

Fungal bacterial interactions (FBI) are highly diverse and contribute to many ecosystem processes. Their emergence in structurally heterogeneous soil structures or in environmental stress scenarios still remains mainly elusive. Here we exemplify FBI by the ecosystem function of contaminant biodegradation in soil. The structural heterogeneity of soil results in the formation of a high variety of soil micro-environments and hotspots of microbial diversity and activity. In heterogeneous environments microbial activity can be considered as a 'logistic' problem, as effective biotransformation will evolve only if water, chemicals and nutrients are sufficiently available to actively degrading microbial communities. In order to cope with heterogeneous environments mycelial soil fungi have developed a unique network-based growth form. Unlike bacteria, hyphae spread efficiently in the soil, penetrate air-water interfaces and cross over air-filled pores. We demonstrate the role of mycelial networks for preferential bacterial colonization of subsurface interfaces and discuss its effects on contaminant degradation. In particular, we show that mycelia (i) act as hotspots for horizontal gene transfer, (ii) shape predator-prey interactions and concomitant compound turnover, and (iii) passively and actively enable the functionality of bacterial ecosystems when stressed by low nutrient availability or at low matric potentials. Given the ubiquity and length of up to 500-1000 m g-1 dry soil of hyphae, we conclude that transport and dispersal processes by mycelia not only play a significant role for the ecosystem service of biodegradation of chemicals but also for nutrient and carbon turnover in soil, and, hence, the functional stability of soil ecosystems.

**Keywords:** bacterial fungal interactions, horizontal gene transfer, bioavailability, water, nutrient availability, contaminant

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*Speaker

# Metal pollution effects on ammonia oxidation

Maria Argudo*[1], Helena Guasch[1], Berta Bonet†[2], Carmen Espinosa‡[3], and Frederic Gich§[1]

[1]Universitat de Girona – Spain
[2]University of Birmingham – United Kingdom
[3]Universitat de Vic – Spain

## Abstract

Metals from mining are common chemical stressor pollutants from human activities that cause a high impact in natural communities in the Mediterranean rivers. Key biological processes carried out in fluvial biofilms are affected by environmental stressors. In consequence natural communities can be altered both in diversity and activity. The aims of this study are to determine the toxicity of Zn on growth, photosynthetic efficiency and changes on structure and function of the ammonia oxidizing prokaryotes in fluvial epilithic biofilms along three rivers. Accordingly an experiment was set up based on biofilm colonization on artificial substrate during spring along Ter, a river larger and impacted by human activities and two of its tributaries, Osor a contaminated river by an effluent of a blende ((Zn,Fe)S) and galena (PbS) and a control river (Llémena). The effects of Zn on algae and on the abundance of ammonia oxidizing prokaryotes were determined by using amplitude modulated fluorimetry (PAM) and reverse transcription quantitative PCR (qPCR) for 16S rRNA, 16S AOB structural genes and functional AmoA genes. RNA abundances in the biofilm were higher than DNA abundances. Bacteria are more abundant in polluted sites whereas algal growth and photosynthetic activities are greater in the less polluted ones. In relation to structure and function of ammonia oxidizers prokaryotes, the ammonia oxidizing bacteria (AOB) are more abundant than the ammonia oxidizing archaea (AOA). Moreover AOB relative abundance, decrease because of pollution indicating that ammonia oxidation may also be impaired. Effects of pollution on nitrification activities will also be discussed.

**Keywords:** Ter river, pollution, Zn, algal growth, ammonia oxidizing prokaryotes, reverse transcription quantitative PCR.

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*Speaker
†Corresponding author: berta.bonet@udg.edu
‡Corresponding author: u1917287@campus.udg.edu
§Corresponding author: frederic.gich@udg.edu

# Do plant traits matter in the microbial ecotoxicity of metal nanoparticles in soil?

Amélie Cantarel[1], Jonathan Gervaix[1], Jean Martins[2], and Agnès Richaume*†[1]

[1]Ecologie microbienne (EM) – Institut national de la recherche agronomique (INRA) : UMR1418, CNRS : UMR5557, Université Claude Bernard - Lyon I (UCBL), Ecole Nationale Vétérinaire de Lyon – 43 Bvd du 11 Novembre 1918, 69622 Villeurbanne cedex, France

[2]Institute Geosciences Environment (IGE) – CNRS : UMR5001, Université Grenoble Alpes – Université Grenoble Alpes CS 40 700. F-38058 Grenoble cedex, France

## Abstract

The use of nanoparticles (NPs) in many common products led to assess their impact on living organisms and microorganisms in various environments. Several studies showed the deleterious effects of metal nanoparticles (NPs) on soil microbial communities and pointed out the importance of organic matter (OM) content in the ecotoxicity of NPs due to its role as dispersing and stabilizing agent . In this study, we assessed the role of plant root exudation in NPs ecotoxicity in soil to- wards the microbial communities involved in the nitrogen cycle assuming that plant root exudation reduces NPs ecotoxicity in the rhizosphere due to a higher dissolved OM content compared to bulk soil. The experimental design consisted in planted and unplanted soil microcosms contaminated or not with CuO-NPs (1mg and 100 mg/Kg) often entering in the composition of nano- fertilizers or pesticides. Control microcosms spiked with soluble Cu were included. Since the measurement of root exudation is hardly possible in soil, we compared the effect of two types of wheat (Triticum aestivum) exhibiting contrasted traits reflecting contrasted exudation. Ecotoxicological NPs effects were assessed after 30 and 50 days of incubation in a greenhouse by measuring microbial activities (nitrification and denitrification) and microbial abundances by qPCR targeting function genes in rhizosphere and bullk soil. NPs characterizations (sur- face charges and hydrodynamic diameter) were realized in solutions of rhizosphere and bulk soils. Dissolved OM was measured in both solutions. The microbial ecotoxicological effects of NPs are discussed in the light of plant traits and NPs characteristics.

**Keywords:** metal nanoparticles/ nitrification/ denitrification/ plant traits/ rhizosphere

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*Speaker

†Corresponding author: agnes.richaume@univ-lyon1.fr

# Mixtures effect of antibiotics on soil nitrification and denitrification - an experimental and modelling approach

Viviane David[1,2], Celine Roose-Amsaleg[2], Fabrice Alliot[2], Marjolaine Bourdat-Deschamps[1], and Olivier Crouzet*†[1]

[1]UMR 1402 ECOSYS – Institut National de la Recherche Agronomique - INRA, AgroParisTech, Université Paris-Saclay – 78026 Versailles cedex, France

[2]Milieux Environnementaux, Transferts et Interactions dans les hydrosystèmes et les Sols (METIS) – École Pratique des Hautes Études [EPHE], CNRS : UMR7619, Université Pierre et Marie Curie (UPMC) - Paris VI – UPMC, Case courrier 105, 4 place Jussieu, 75005 Paris, France

## Abstract

Impacts of antibiotic mixtures on microbial ecosystem functions need to be unraveled to improve the environmental risk assessment. This study aims to characterize and predict the toxicity of antibiotic mixtures on soil nitrification and denitrification processes.
Different antibiotics (tetracycline, sulfonamide, macrolide, fluoroquinolone) with distinct mechanisms of action, were tested alone and in mixtures by dose-response approaches on soil substrate-induced nitrification (SIN) and minimum inhibitory concentration of soil denitrifier enrichment (MIC-E). Abundance of ammonia-oxidizers (archeal and bacterial) and denitrifiers (clade I and II of N2O-reducers) were investigated by molecular approach. For nitrification, the experimental results for different antibiotic mixture ratio were further analyzed regarding mathematical modeling interpretation based on the concepts of concentration addition (CA) or independent action (IA).

The SIN bioassay highlighted bacteriostatic effects at sulfonamide or macrolides concentrations lower than observed environmental concentrations. The differences in toxicities of antibiotics individually tested were explained by the sorption coefficient of the various antibiotics. Surprisingly, despite different cellular targets of the tested antibiotics, modelling mixture analyses revealed few differences in toxicity prediction between IA or CA concepts. Denitrification showed a higher tolerance than nitrification to the antibiotics and the lowest MIC-E for tetracycline. For nitrification and denitrification, the mixture toxicity was mainly driven by the more toxic compound.
The archeal/bacterial nitrifiers ratio was positively related to the magnitude of nitrification inhibition; archaea showing invariant abundances while the antibiotic doses increased. The abundances of the denitrifier *nosZ* clade II greatly decreased under any antibiotic exposures unlike the *nosZ* clade I guilds.

**Keywords:** Microbial ecotoxicology, antibiotics, mixture effect, N, cycling microbial activities

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*Speaker

†Corresponding author: olivier.crouzet@versailles.inra.fr

# Assessment of antibiotic resistance of bacteria isolated from Mugil cephalus of the bay of algiers

Souhila Alouache*[1,2], Nawal Touahir[1], and Rabah Bakour[2]

[1]High national school of marine sciences and coastal management, LCVRM laboratory (ENSSMAL) – BP19, campus universitaire, Bois des cars, Algiers, Algeria

[2]University of sciences and technology Houari boumedienne, laboratory of cellular and molecular biology (USTHB) – BP32 El Alia - Bab ezzouar, Algeria

## Abstract

The study of antibiotic resistance in aquatic organisms is important because it could indicate the extent of change in aquatic ecosystems due to anthropogenic actions. Our study focused on the bacterial flora of *Mugil cephalus*; a fish that frequents coastal waters and mouths ; caught in the bay of algiers between May and October 2015.
The evaluation of the resistance of the total flora to antibiotics revealed high rate of resistance to amoxicillin, mean rate to cefotaxime, ceftazidime, ciprofloxacin and tetracycline and low to imipenem and gentamicin.

The identification of 22 gram negative bacteria resistant to amoxicillin or cefotaxime revealed that strains were assigned to: *E.coli (7), K.oxytoca (3), E. cloacae (2), C. freundii (2), A. hyrophila (2), E. aerogenes (1), K. pneumoniae (1), R.ornithinolytica (1), R.terrigena (1), Moell.wisconsensis (1) and P.aeruginosa (1).* The antibiogram results were : AMX (100%) , TE (31.8%), PIP (27%) , CAZ (27%) , NA(27%), FOX (22.7%), CTX (18%), ATM :(18%), CFP (13.6%), SXT (13.6%), TMP (9%), CIP (4.5%) , GM (4.5%) , C (4.5%) et IMP (0%). The antibiotic resistance profiles of these strains range from 1 to 11 antibiotics. The genotyping analysis by PCR showed the presence of the *bla*CTX-M in *K. pneumoniae* (1), the *bla*TEM in *E.coli* (4), *K. pneumoniae* (1), and *C. freundii* (1) and the *bla*SHV in *K. pneumoniae* (2).

The results suggest that anthropogenic input influences the emergence and dessimination of resistant bacteria in coastal waters and in fish that live there, constituting a health and ecological risk.

**Keywords:** antibiotic resistance, bacteria, Mugil cephalus

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*Speaker

# New Finding on the Anti-biofilm Activity of Silk Sericin Against Streptococcus mutans Bacteria Residing within Biofilm

Pornanong Aramwit*†[1] and Nipaporn Bang[1]

[1]Bioactive Resources for Innovative Clinical Applications Research Unit and Department of Pharmacy Practice – Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

## Abstract

Sericin is considered as a waste from silk industry and causes pollution when discarded into the environment. Sericin is a water-soluble protein component in silk which, recently, shows various biological activities such as anti-oxidation, anti-inflammation, and anti-aging. In this study, sericin was extracted by 4 different methods including heat, acid, alkali, and urea and the anti-biofilm effects and cytotoxicity of each sericin against *Streptococcus mutans* bacteria residing within biofilm were investigated. *S. mutans* bacteria were cultivated in the presence of various concentrations of sericins to evaluate the anti-biofilm formation using cell density assay (inhibition effect before biofilm formed). On the other hand, various concentrations of sericins were added to the biofilm already formed by *S. mutans* bacteria and the viability of bacteria was assessed by MTT assay (disruption effects after biofilm formed). We found that the urea-extracted sericin at all concentrations (12.5-100 mg/mL) showed the highest potential anti-biofilm activity in terms of both inhibition and disruption effects, comparing to the sericins extracted by heat, acid, and alkali. The heat-extracted and acid-extracted sericin were found to reduce the biofilm formation dose-dependently while the alkali-extracted sericin did not show either inhibition or disruption effect on the bacterial biofilm. Due to strong negative charged of urea-extracted sericin, it may instabilize the bacterial cell wall, leading to membrane disintegration and finally to cell death. This new finding on the anti-biofilm activity of sericin would establish further researches on the mechanism and application of sericin as novel anti-biofilm agent.

**Keywords:** Silk protein, sericin, biofilm, S. mutans

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*Speaker

†Corresponding author: aramwit@gmail.com

# Infection lithiasis inhibition by citrate-aluminum complex

Aissa Belouatek*†[1]

[1]Laboratoire Structure, Elaboration et Application des Matériaux Moléculaires – Faculté des sciences exactes, Université Abdelhamid Ibn Badis, Mostaganem, 27000, Algeria

## Abstract

The Struvite form during urinary infection with urea-splitting bacteria. The precipitation of the phosphates phase from artificial urine at different initial pH values was the object of our investigation. We chose the classical model for the study of phosphate crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Two solutions of following composition were mixed. In the absence of inhibitor, the crystallization of phosphates at $pH = 6.5$, led to the formation of brushite and amorphous carbonated calcium phosphates (ACCP), after 6 hours. The struvite and carbapatite are favorites at $pH \geq 7$. The capacity of inhibitor was 100% starting from a concentration of mixture (0.05 mM of citrate, and 0.01 mM of the aluminum ions) at $pH = 6.5$. On the other hand at $pH = 7$, the addition of mixture concentration (1mM citrate and 0.01 mM of the ions aluminum) caused the total disappearance of struvite and the brushite. The complex citrate-aluminum acts on the growth and the aggregation of the struvite crystals with various concentrations at $pH = 8$. The complete disappearance of struvite is obtained starting from a concentration of the mixture (6mM ions citrates and 0.07 mm ions aluminium). In this investigation, complex citrate- aluminum proved to be good inhibitors of microorganism. Their effect increases with solution pH but they are more efficient in less acidic or neutral urine than in alkaline one.

**Keywords:** inhibition, pH, citrate, aluminum, brushite, struvite

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*Speaker

†Corresponding author: abelouatek@gmail.com

# Effect of lanthanides on soil microbial communities

Charlotte Berthelot*†[1], Asfaw Zegeye[1], and Patrick Billard[1]

[1]LIEC – Université de Lorraine, CNRS : UMR7360, Université de Lorraine – France

## Abstract

Lanthanides (Ln3+) are a group of metals potentially harmful when concentrated in soils such as in mining and industrial areas. However, due to their low bioavailability in soils, Ln3+ have long been considered as biologically inert at low concentration. Recent studies contradict this view, showing that Ln3+ may act as beneficial cofactor in plants and certain bacteria. In the present study, we investigated the promotion role of Ln3+ on soil bacterial communities.
The effect of La3+ (Lanthanum - a model lanthanide) was examined in three soils exhibiting diverse physico-chemical characteristics. Soil spiking was performed to reach concentrations of 50 and 500mg/kg of La3+. Soil parameters (*e.g.* pH, La3+ availability), enzyme activities related to biogeochemical cycling of C, N and P and bacterial communities (abundance and diversity) were evaluated. Application of La3+ did not alter the soil pH, whereas bioavailability of La3+ was largely dependent on soil properties. Nevertheless, the presence of La3+ at low concentration (50mg/kg) significantly increased microbial respiration (from 129 % to 135% of control). Likewise, laccase and cellulase activities were stimulated by the 50 mg/kg treatment whereas $\beta$-glucosidase and leucine-amino peptidase were inhibited by 500mg/kg of soil

The impact of La3+ on carbon substrate utilization by microbial communities was studied using NPP method and Biolog® microplates. Consumption of organic acids (*e.g.* citrate, gluconate) or alcohols (*e.g.* ethanol) was promoted as a result of La3+ application. Overall, our study provides a first insight into the beneficial effect of lanthanides for bacterial metabolism in soil environments.

**Keywords:** lanthanides, bacterial communities, Lanthanum

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*Speaker

†Corresponding author: charlotte.berthelot@univ-lorraine.fr

# Developing a transcriptomic approach dedicated to autotrophs in periphytic biofilms

Chloe Bonnineau*[1], Floriane Larras[2], Stefan Scholz[2], Stéphane Pesce[1], Helena Guasch[3], and Mechthild Schmitt-Jansen[2]

[1]Irstea UR Milieux Aquatiques, Ecologie et Pollutions (UR MALY) – Irstea – 5 rue de la Doua BP 32108 F-69616 Villeurbanne Cedex, France
[2]Helmholtz Centre for Environmental Research - UFZ (GERMANY) (UFZ) – Leipzig, Germany
[3]GRECO, INstitute of Aquatic Ecology, University of Girona – Girona, Spain

## Abstract

In the last decade, omics tools dedicated to microbial communities have been developed to characterize the functional groups of those communities and better understand microbial response to contamination. Most of those tools focused on heterotrophic and fungal communities while omics application dedicated to microalgae and diatoms are mostly restrained to diversity assessment (e.g. based on 18S rRNA or rbcL genes). In this study, the possibility of studying gene expression in autotrophic river biofilm communities was explored and we present the proof of concept for a Functional Gene Array (FGA), based on consensus sequences from genes of key physiological processes. Indeed, microarrays offer a comprehensive tool for system-wide analysis of community functioning based on the simultaneous detection of the activity of thousands of genes. The FGA designed in this study include 83 functional genes chosen to reflect several essential biochemical pathways and specific stress response pathways of microalgae. Probes of these genes were designed from consensus sequences from up to 6 microalgal species (diatoms and chlorophytes). Furthermore, species-specific probes were included resulting in 1554 unique oligonucleotide probes for 83 different genes. RNA extracted from *Chlamydomonas reinhardtii*, *Scenedesmus vacuolatus* and multi-species biofilms was then used to test different hybridisation conditions. Signal intensity was affected by sequence divergence but results showed that a hybridisation temperature of 55°C allowed a good compromise between cross-hybridisation and specificity. Due to the reduced number of probes and available sequence information the designed FGAs is at present particularly useful for ecotoxicological laboratory experiments performed with biofilm communities.

**Keywords:** microarray, microalgae, gene expression, community

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*Speaker

# Importance of the sediment microbial diversity for degradation of pharmaceuticals in rivers

Claudia Coll Mora*†[1], Zhe Li[1], Raven Bier[2], Silke Langenheder[2], and Anna Sobek[1]

[1]Department of Environmental Science and Analytical Chemistry, Stockholm University (ACES-SU) – Svante Arrhenius väg 8, SE-11418 Stockholm, Sweden
[2]Evolutionary Biology Centre (EBC) – Evolutionary Biology Centre, EBC Norbyvägen 14-18 752 36 Uppsala, Sweden

## Abstract

Degradation of pharmaceuticals in aquatic systems is impacted by environmental factors. For instance, the attenuation rate of acetaminophen differed by a factor of 40 between rivers Gr´undlach-Germany and Fyris-Sweden [1]. Although data is limited, previous studies show biodiversity may be linked to the degradation potential of pollutants [2]. Therefore, we performed an experiment based on OECD TG308 to assess the influence of bacterial diversity on pharmaceutical degradation.
Water and sediment were collected from rivers Fyris and Gr´undlach, before and after the discharge of waste water treatment plants (WWTP). The sediments were acclimated with synthetic river water, then spiked with 11 pharmaceuticals at two concentration levels (200$\mu$g/L and 2mg/L). The compounds represent a wide range of biodegradation rates: acetaminophen, acesulfameK, caffeine, carbamazepine, diclofenac, furosemide, ibuprofen, metformin, oxazepam, tramadol and venlafaxine. The 40-day incubation was performed in the dark at 16◦C and enabling an oxygen gradient in the sediment. The water phase was sampled at 10 time points while sediment samples were taken before spiking and at the end of incubation. We are measuring the pharmaceutical concentrations in all samples, and using Illumina-sequencing of bacterial 16SrRNA to analyze the active bacterial community. Subsequently, Rényi's entropy profiles will be calculated.

Our results will show whether microbial communities up- and downstream of a WWTP differ in their potential to degrade pharmaceuticals and whether differences in the composition and diversity can explain the variation in the degradation rates between sampling sites and rivers.
- [1] Li et al.2016.doi:10.1021/acs.est.5b06327 ; [2] Johnson et al.,2015.doi:10.1128/AEM.03286-14

**Keywords:** pharmaceutical degradation, microbial diversity, aquatic systems, OECD308

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*Speaker
†Corresponding author: claudia.coll@aces.su.se

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# Effect of nanoceria biotransformation in activated sludge on the microbiota associated to canola roots

Blanche Collin*[1], Mohamed Barakat[2], Philippe Ortet[2], Wafa Achouak[2], Melanie Auffan[1], Emmanuel Doelsch[3], and Catherine Santaella[2]

[1]Centre européen de recherche et d'enseignement de géosciences de l'environnement (CEREGE) – Institut de Recherche pour le Développement : UMR$_D$161, $AixMarseilleUniversité$ : $UM34, CollègedeFrance, CentreNationaldelaRechercheScientifique$ : $UMR7330 - -AvenueLouisPhilibert - BP80 - 13545Aix - en - Provence, France$

[2]Laboratoire d'Ecologie Microbienne de la Rhizosphère et d'Environnements extrêmes (LEMIRE) – CNRS : UMR7265, CEA, Aix-Marseille Université - AMU – St-Paul-lez-Durance, France

[3]UPR Recyclage et risque (CIRAD) – CIRAD : UPR78 – Aix-en-Provence, Montpellier, Saint-Denis de la Réunion, France

## Abstract

Recent interest in the environmental fate and effects of CeO2 nanomaterials (NMs) has stemmed from its expanded use for a variety of applications (fuel additives, catalytic converters, coating...). The majority of these NMs will end up in wastewater treatment plants (WWTP) where they will partition to sewage sludge during wastewater treatment, and ultimately re-enter the environment through the application of biosolids to agricultural soils. Thus, soil may serve as a primary sink for NMs accumulation in the environment, in which NMs may enter food webs or cause direct toxicity to plants, microbial communities, or other soil organisms. This project aims to study the impact of CeO2 NMs biotransformation on a soil-plant-microbe system using realistic exposure modes. Pristine CeO2-NMs were first aged in a laboratory-scale activated sludge reactor during 5 weeks. The biosolid enriched NMs was then amended to an agricultural soil at environmentally relevant concentration: 1 mg.kg-1 CeO2. Four treatments were performed: control soil, soil amended with 1 mg.kg-1 pristine CeO2-NMs, control biosolid without NMs, biosolid enriched NMs. The selected plant was canola (*Brassica napus*), an oil-producing plant. Bulk Ce L3-edge X-ray absorption spectroscopy was performed in the biosolid before culture in order to evaluate the NMs transformation in the reactor. After the culture, elemental concentrations and distributions were measured in the plant parts by ICP-AES, and laser ablation ICP-MS. Root and rhizosphere bacterial community structure was characterized by sequencing of 16S rRNA gene (Illumina MiSeq) in order to understand the impact of the biotransformation of CeO2-NMs on the microbiota.

**Keywords:** soil, rhizosphere, plant, nanoparticles, bacterial community structure

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*Speaker

# Environmentally relevant copper concentrations affect microbial composition and community functions in marine periphyton biofilms

Natàlia Corcoll*[1], Jianghua Yang[2], Hadrien Kronenberger[1], Thomas Backhaus[1], Xiaowei Zhang[2], and K. Martin Eriksson[3]

[1]Dept. Biological and Environmental Sciences, University of Gothenburg – Sweden
[2]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University – China
[3]Dept. Mechanics and Maritime Sciences, Chalmers University of Technology – Sweden

## Abstract

Copper pollution is common in coastal areas. In particular, the use of copper-based antifouling paints on ship hulls elevates copper concentrations in these environments. This study assesses the effects of dissolved copper on community structure and function of marine periphyton biofilms. Microbial diversity and community structure was studied by 16S and 18S amplicon sequencing, targeting prokaryotic and eukaryotic organisms, respectively. Community function was studied as impacts on algal biomass, photosynthetic pigment profiles and primary production. Additionally, we studied Pollution-Induced Community Tolerance (PICT) using photosynthesis as the endpoint. Periphyton was exposed for 18 days to five copper concentrations, between 0.01 and 10 $\mu$M, in a semi-static test. The amplicon sequencing yielded 7.1 and 5.7 million high quality 16S and 18S reads, respectively. The average numbers of 16S and 18S Operational Taxonomic Units among the samples were 9405 and 1242, respectively. Analysis of Unifrac distances showed that copper significantly changed the eukaryotic community structure at concentrations as low as 0.01 $\mu$M. The prokaryotic community structure was changed at slightly higher concentrations (0.06 $\mu$M). A total of 23 taxa, including species within *Proteobacteria*, *Bacteroidetes, Stramenopiles* and *Hacrobia* classes, were identified as particularly sensitive to copper. Algal biomass, photosynthetic pigments profiles and primary production, were reduced at Cu concentrations of 0.06 $\mu$M and higher. PICT measurements confirmed that copper induced community tolerance in exposed communities. Taken together, these findings indicate that negative impacts from copper might be common in coastal ecosystems.

**Keywords:** amplicon sequencing, 16S rRNA, 18S rRNA, biofilms, copper toxicity

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*Speaker

# Toxicity of nZVI used for nanoremediation towards natural bacterial community from groundwater

Marc Crampon*[1], Catherine Joulian[1], Patrick Ollivier[1], Louis Jolly , and Jennifer Hellal[1]

[1]BRGM – Bureau de Recherches Géologiques et Minières (BRGM) – 3 avenue Claude Guillemin 45100 Orléans, France

## Abstract

Remediation techniques for toxic/persistent contaminants in groundwater are often technologically difficult. Nanoparticles (NP) such as nZVI (Zero-Valent Iron) applicable as *in-situ* reduction or oxidation agents for groundwater treatment give promising results. However, they may also represent an additional contamination. Our study aims to assess the impact of nZVI on the denitrifying activity, the abundance and the structure of a planktonic nitrate-reducing bacterial community.

Pre-culture of the denitrifying bacterial community was carried out from groundwater samples. It was then allocated in reactors containing substrate (sodium acetate), nitrates and a range of nZVI concentrations (from 0 to 300 mg Fe.L-1). Physical (pH, redox potential), chemical (NO3- concentrations) and biological (DNA, RNA) parameters were monitored during one week. The size distribution of nZVI and the mortality of bacteria (Live&Dead observations) were also evaluated. Then the bacterial cultures were transplanted in fresh substrates without nZVI to assess the resilience of the bacterial community.

Denitrification activity was stopped in the presence of nZVI at concentrations higher than 30 mg.L-1, demonstrating the toxic effect of nZVI. Live&Dead observations revealed that the viability of bacteria was lower for lower nZVI concentrations. The presence of NP aggregates was higher for higher nZVI concentrations. They could then result in a lower toxicity for bacteria, even if nZVI concentration is high. DNA and RNA analyses allowed us to observe the impact of nZVI on the activity (*narG* gene), abundance and structure of bacterial community. Finally, resilience was worst for concentrations ranging from 50 to 200 mg.L-1.

**Keywords:** Nanoremediation/nZVI/Toxicity/Bacteria/Groundwater

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*Speaker

Session
FUNCTION
Poster

# The impacts of three herbicides on the fatty acids of the freshwater diatom Gomphonema gracile

Floriane Demailly*[1], Marina Le Guedard[2], Patrice Gonzalez[3], Jean-Jacques Bessoule[4], Nicolas Mazzella[1], François Delmas[1], Agnès Feurtet-Mazel[3], and Soizic Morin[1]

[1]UR EABX – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA (FRANCE) – 50 avenue de Verdun, 33612 Cestas cedex, France

[2]LEB Aquitaine Tansfert – LEB Aquitaine Transfert-ADERA – Bâtiment A3, INRA Bordeaux Aquitaine, 71 avenue Edouard Bourlaux, CS 20032, 33140 Villenave d'Ornon, France, France

[3]EPOC, UMR CNRS 5805, Station Marine d'Arcachon – Université de Bordeaux (Bordeaux, France) – Place du Docteur Bertrand Peyneau , 33120 Arcachon Cedex, France

[4]Laboratoire de Biogenèse Membranaire, UMR CNRS 5200 – Université de Bordeaux (Bordeaux, France) – Bâtiment A3, INRA Bordeaux Aquitaine, 71 avenue Edouard Bourlaux, CS 20032, 33140 Villenave d'Ornon, France

## Abstract

For several years, the intensive use of pesticides caused many problems to the environment, making pesticides major pollutants of aquatic ecosystems (Aydinalp & Porca, 2004). Among biological indicators, biomarkers must be developed in view of their capacity to reveal early toxic effects on organisms of the environment. The aim of this study is to compare the specificities/sensitivities of the fatty acid analysis (composition of satured, unsatured and polyunsatured fatty acids determined by gas chromatography coupled with flame ionization detector) of diatoms, compared to "classical" descriptors analysis (inhibition of photosynthesis, growth, transcriptomic analysis). A model freshwater diatom (*Gomphonema gracile*) was exposed to three herbicides singly, with three different cellular targets, at environmentally relevant and higher concentrations (diuron and S-metolachlor, C1= 1 $\mu$g/L and C2= 10 $\mu$g/L; glyphosate, C1= 5 $\mu$g/L and C2= 50 $\mu$g/L). The diatom cultures were incubated during exponential growth at controlled temperature and light intensity of 17○C and 67 $\mu$mol.m-2.s-1, respectively. After a 1-week exposure, fatty acids compositions of diatoms were determined in parallel with "classical" descriptors. While no or only slight effects of the pesticides were found with the "classical" descriptors, some fatty acid profiles seemed to be specific of pesticide contamination but in-depth fatty acid analyses are still under progress. This study provides a basis for further use of diatom's fatty acid composition as biomarker of toxic contamination in fresh waters. Aydinalp, C., & Porca, M. (2004). The effects of pesticides in water resource. Journal Central European Agriculture. 5 (1), 5-12.

**Keywords:** Microbial ecotoxicology, fatty acids, pesticides, freshwater diatom, Gomphonema gracile

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*Speaker

# Soil particles alter the metabolic profile of Pseudomonas putida grown in the presence of phenanthrene: A vibrational spectroscopy approach

Andrea Fanesi*[1], Asfaw Zegeye[1], Christian Mustin[1], and Aurélie Cebron†[1]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360 – LIEC,
Faculté des Sciences et Technologies, BP 70239, 54506 Vandoeuvre-lès-Nancy, France

### Abstract

In soil, the biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) is strongly influenced by the presence of organic matter and mineral particles. Information regarding how the metabolic profile (i.e biochemical composition) of bacteria is affected by carbon source (simple substrates or PAH) in the presence of soil particles (SPs) is scarce. However, it might be of paramount importance for understanding the bioremediation efficiency of polluted sites.

We hypothesized that properties of SPs specifically alter both the micro-niches of bacteria and the availability of the carbon source, affecting in turn PAHs catabolism and cell metabolic profile. To test this hypothesis, the soil dweller *Pseudomonas Putida* was grown in the absence and presence of different SPs (sand, clay and humic acids) using either phenanthrene or glucose as the sole carbon and energy source. The cell metabolic profiles were identified and differentiated using a combination of vibrational spectroscopy techniques (fast and without sample preparation), such as FT-IR and FT-Raman spectroscopy, and chemometric analysis (PLS-DA).

We showed that the metabolic profile of *P. putida*, (i.e. Raman fingerprints) is influenced by the type of SPs present in the medium, with clay and humic acid affecting it the most. The changes of macromolecules regarded mainly proteins, lipids and nucleic acids. Moreover, in the presence of clay and humic acid a more pronounced discrimination between cells grown on glucose or on phenanthrene was observed, suggesting that specific SPs affect the way cells metabolize carbon. Our results provide new information linking PAHs degradation, bacteria physiology and cell surrounding media.

**Keywords:** PAHs bioremediation, Raman spectroscopy, bacteria, metabolic profiles, soil particles

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*Speaker

†Corresponding author: aurelie.cebron@univ-lorraine.fr

# Availability of mercury to freshwater biofilms: from the gene to the community composition

Severine Le Faucheur[1], Perrine Dranguet[1], Claudia Cosio[1], and Vera Slaveykova*†[1]

[1]University of Geneva – Department F.-A. Forel for Environmental and Aquatic Sciences, Earth and Environment Sciences, Faculty of Sciences, University of Geneva, Uni Carl Vogt, 66 Bvd. Carl Vogt, CH-1211 Geneva, Switzerland

## Abstract

Bioavailability is a key concept allowing to link the changes in the trace metal concentrations and speciation with the intensity of the induced biological effects. However, the link between mercury speciation, bioaccumulation and effects to biofilms is overlooked. This study focusses on the availability of mercury in freshwater biofilms. The amounts of IHg and MeHg accumulated in biofilms were determined. The changes in bacterial, microalgal and fungal communities were followed simultaneously by amplicon sequencing. Biofilms grown in-situ in the Olt River (Romania) reservoirs impacted by Hg-rich effluents from a chlor-alkali platform as well as biofilms exposed to Hg in microcosm were compared. The results revealed a significant correlations between the IHg accumulation in biofilm and the fraction of dissolved Hg non-complexed by natural organic matter. A shift in algal, bacterial and fungal community composition of the biofilms from Olt River and microcosm was observed. However the principal component analysis revealed that in the in-situ grown biofilm in addition to Hg, other contaminants present in the waste water of chlor-alkali production plant, i.e. Na+, Ca2+ and Cl- as well as some water quality variables, i.e. NO3-, SO42-, DOC and Zn contribute to the cominity composition alteration. Nevertheless, an increase in the presence of Hg resistance gene (*merA*) in both in-situ and microcosme exposed biofilms with the increase of the accumulated Hg was found. The outcomes of the results contribute to the development of biofilm-based indicators in support to efforts of mercury monitoring programs in aquatic systems.

**Keywords:** mercury, bioavailability, merA, community composition

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*Speaker

†Corresponding author: Vera.Slaveykova@unige.ch

# Global metabolomic characterizations of Microcystis spp. highlights clonal diversity in natural bloom-forming populations and expands metabolite structural diversity

Séverine Le Manach[1], Charlotte Duval[1], Arul Marie[1], Cécile Bernard[1], and Benjamin Marie*†[1]

[1]Molécules de Communication et Adaptation des Microorganismes (MCAM) – Muséum National d'Histoire Naturelle (MNHN), CNRS : UMR7245, Sorbonne Universités, Sorbonne Universités – France

## Abstract

Cyanobacteria are photosynthetic prokaryotes that are able to synthetize a wild rang of secondary metabolites, with noticeable bioactivity (comprising toxicity and anti-bacterial activity) according to the ribosomal and non-ribosomal processes. *Microcystis* represents one of the most common cyanobacteria taxa constituting intensive blooms that arise in freshwater ecosystems worldwide. With the aim of contributing to better understand the variations in bio-actif metabolite production, which are potentially harmful to other aquatic macro- and micro-organisms, between clones of the same cyanobacterial blooms, we investigate the diversity of several *Microcystis* strains isolated from different freshwater bloom-forming populations from various geographical area. Twenty-four strains of *Microcystis* from the Paris' Muséum Collection of cyanobacteria were compared by an integrated approach combining genotyping and metabolites chemotyping. These characterizations comprise analyses on morphology, Internal Transcript Spacer, microcystin synthesis gene, and global secondary metabolite contents. Metabolomics shotgun analyses by mass spectrometry reveal clear discriminant profiles between strains collected from identical or different localities. A global cluster analysis indicates that microcystin synthesis gene presence explains more of the metabolite diversity distribution, than the species or the sample localities parameters do. A global network generated from MS/MS fractionation patterns of the various metabolites detected in all strains performed with GNPS tool indicates that these *Microcystis* strains produce a wide set of chemically diverse metabolites, comprising only few known potent toxins or protease inhibitors, together with a large set of unknown molecules that still remain to be investigated and characterized at their structure as well as at their potential bioactivity/toxicity/antibacterial levels.

**Keywords:** Cyanobacteria, cyanotoxins, ecotoxicology, secondary metabolites, biodiversity

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*Speaker

†Corresponding author:

# Structural and functional response of river sediment microbial communities to environmental concentrations of copper and arsenic, alone or in mixture

Ayanleh Mahamoud Ahmed*†[1,2], Emilie Lyautey‡[2], Aymeric Dabrin[1], Chloé Bonnineau[1], Bernard Motte[1], Rosy Christophe[1], Josiane Gahou , and Stéphane Pesce§[1]

[1]UR MALY – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture – 5 rue de la Doua, BP32108, 69616 Villeurbanne, France

[2]Centre Alpin de Recherche sur les Réseaux Trophiques et Ecosystèmes Limniques (CARRTEL) – Institut National de la Recherche Agronomique : UMR0042, Université Savoie Mont Blanc : UMRINRA / USMB – Station dH́ydrobiologie Lacustre, Le Bourget-du-Lac 73376, France

## Abstract

While many metals are known to accumulate in sediments, knowledge about the resulting ecotoxicological effects on sediment microbial communities is scarce. The present study aimed at evaluating the structural and functional impact of chronic exposure to environmental concentrations of copper (Cu) and arsenic (As), alone or mixed together, on river sediment microbial communities. Natural uncontaminated surface sediments collected in a French River (Ain) were spiked with As and Cu at a nominal concentration of 40 mg/kg and then were exposed for 21 days in laboratory channels with overlying waters. The response of heterotrophic microbial communities to metals was evaluated both in terms of genetic structure (using ARISA analysis) and functional potential (using exo-enzymatic, metabolic and qPCR analyses). A pollution induced community tolerance (PICT) approach was also performed to assess if the exposure led to an increase in the capacity of microbial communities to tolerate metals. Our results showed rapid and marked effects of Cu alone on the structure and the functions of the exposed communities. Chronic Cu exposure also induced an increase in community tolerance to Cu, as observed by PICT measurement using beta-glucosidase activity. In contrast, the effects of As were mostly undetectable. Under mixture exposure (Cu+As), the effects were similar or higher than those provoked by Cu alone, depending on the measured parameter. Altogether those findings reveal that metals accumulation in sediments can impact exposed microbial communities thus affecting their functional role in aquatic ecosystems.

**Keywords:** Benthic communities, metals, microbial ecotoxicology, PICT, mixture

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*Speaker

†Corresponding author: ayanleh.mahamoud-ahmed@univ-smb.fr

‡Corresponding author: emilie.lyautey@univ-smb.fr

§Corresponding author: stephane.pesce@irstea.fr

# Natural lake sediment microbial community response to exposure to environmental PCB concentrations in microcosms

Ayanleh Mahamoud Ahmed*†[1,2], Stéphane Pesce‡[2], Emmanuel Naffrechoux[3], Nathalie Cottin[3], Chloé Bonnineau[2], Bernadette Volat[2], and Emilie Lyautey§[1]

[1]Centre Alpin de Recherche sur les Réseaux Trophiques et Ecosystèmes Limniques (CARRTEL) – Institut National de la Recherche Agronomique : UMR0042, Université Savoie Mont Blanc – Station d'Hydrobiologie Lacustre, Le Bourget-du-Lac, 73376, France

[2]Milieux aquatiques, écologie et pollutions (UR MALY) – Irstea – 5 rue de la Doua - BP 32108, F-69616 Villeurbanne, France

[3]Laboratoire de Chimie Moléculaire et Environnement (LCME) – Université Savoie Mont Blanc, Université Grenoble Alpes – Campus Scientifique de Savoie-Technolac Le Bourget du Lac Cedex, France

## Abstract

Sediments act as receptor for contaminants that can accumulate over time thus reaching high concentrations. The main objective of this work was to evaluate the impact of chronic exposure to environmental concentrations of PCB on microbial communities from lake sediments. Sediment cores (n = 52) were collected in Lake Annecy (PCB concentrations $<$ 0.9 ng dw-1 $\sum$7PCBi) and exposed to 3 treatments: a reference without PCB contamination, a medium contamination (430 ng dw-1 $\sum$7PCBi) and a high contamination (4300 ng dw-1 $\sum$7PCBi). Sediments were contaminated using a commercial mixture (Aroclor 1254) and were incubated during 14 days at 6$\circ$C with oxygenated overlying water. The resulting effects on communities from depth 0-2 cm, 4-6 cm and 8-10 cm were evaluated independently by assessing microbial enzymatic activities, metabolic potentials, gene abundances and community structure. While PCB exposure modulated bacterial community structure in microbial communities from 0-2 cm depth, PCB exposure did not affect the other descriptors. Microbial communities from 4 to 10 cm depth were not affected by PCB exposure. Our results indicate a limited impact of PCBs on exposed microbial communities suggesting that such exposure would probably not modify their function in lake ecosystems. A low and heterogeneous contamination of the sediment cores could also explain these results. The present work also pointed out methodological challenges to assess PCB ecotoxicological effects on intact sediment columns. If such approaches are necessary to remain representative of the ecosystem, it still raises questions about the contamination procedure and the bioavailability of the contaminants.

**Keywords:** Benthic communities, persistent organic pollutant, microbial ecotoxicology

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*Speaker

†Corresponding author: ayanleh.mahamoud-ahmed@univ-smb.fr

‡Corresponding author: stephane.pesce@irstea.fr

§Corresponding author: emilie.lyautey@univ-smb.fr

# Ecotoxicological impact of leptospermone, a beta-triketone bioherbicide, on fungal community of two arable soils.

Clarisse Mallet*[1], Sana Romdhane[2], Jérémie Beguet[3], Fabrice Martin-Laurent[3], Christophe Calvayrac[2], Cédric Bertrand[4], and Lise Barthelmebs[2]

[1]Microorganismes : génome et environnement (LMGE) – Université d'Auvergne - Clermont-Ferrand I, CNRS : UMR6023, Université Blaise Pascal - Clermont-Ferrand II – Université Blaise Pascal, Campus des Cézeaux, 24, avenue des Landais BP 80026 63 170 AUBIERE, France

[2]Biocapteurs - Analyse- Environnement (BAE) – Université de Perignan Via Domitia – Université de Perpignan Via Domitia, 52 Av Paul Alduy, 66860 Perpignan cedex, France

[3]INRA (UMR Agroécologie) – Institut national de la recherche agronomique (INRA) : UMR1347 – INRA UMR 1347 Agroécologie 17 rue sully BP 86510 21065 Dijon Cedex, France

[4]CRIOBE USR3278, Université de Perpignan Via Domitia – Univ de Perpignan Via Domitia – 58 Avenue Paul Alduy 66860 Perpignan Cedex, France

## Abstract

Ecotoxicological impact of leptospermone, a beta-triketone bioherbicide, was investigated on the abundance and diversity of fungal community which supports important soil ecological functions (i.e. decomposition of organic matter and nutrients recycling) but which is less studied than bacterial community.

A microcosm experiment was carried out on two arable soils (Perpignan and Saint Jean de Fos, France), differing in their physicochemical properties and in history treatment with synthetic beta-triketones. Soil microcosms were treated with 1x or 10x fold recommended dose (FRD) of leptospermone or not (control) and incubated in the dark for 45 days under controlled conditions. Leptospermone dissipation was monitored all along the experiment. Soil DNA was extracted from samples collected at 0, 4 and 45 days and used as template to quantify the abundance of fungi by qPCR targeting 18S rRNA genes and to estimate the diversity of fungal community by pyrosequencing of internal transcribed spacer (ITS) region 2 of the fungal rRNA.

Within the 45 days of incubation, the leptospermone disappeared almost entirely from one of the two soils (i.e., DT50 $<$ 10 days), while 25% remained in the other. In response to leptospermone exposure, the fungal community was significantly modified as compared to control. For both soils, shifts in the fungal diversity was observed in soil microcosms treated with 1x and/or 10x FRD. However, fungal diversity was found to be resilient when leptospermone was dissipated.

**Keywords:** Soil, fungal community, bioherbicide, beta, triketone

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*Speaker

# Assessing biofilm pulse exposure to PSII inhibitors using time-response curves

Soizic Morin*[1], Betty Chaumet[1], and Nicolas Mazzella[1]

[1]Laboratoire Ecosystèmes aquatiques et changements globaux (UR EABX) – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA – 50 avenue de Verdun, 33612 Cestas cedex, France

## Abstract

Diuron-induced photosynthesis inhibition of freshwater biofilms and subsequent EC50s (half maximal effective concentrations) are determined using a 3-hour exposure in average. However, given the known rapid action of diuron, earlier responses can be expected.
In this study, we performed different time-response experiments to confirm the short time lag for diuron to reach its cellular target, and to assess whether time-response curves (i.e. photosynthesis inhibition as a function of time, when diuron exposure is kept constant) can improve the assessment of short-time (pulse) pre-exposure to diuron.

Our results demonstrated that:

i) diuron reaches its cellular target in a very short time-span (

ii) the delay required to reach a 50% photosynthesis inhibition is dependent on test exposure concentrations, and

iii) that time-response curves may be useful to assess previous short time exposure.

In particular, we showed that exposure to diuron leads to maximal achievable inhibition within a minute (exposure to diuron-EC100, i.e. $> 100\mu$g.L-1) to 7:20 minutes (exposure to EC25).
We were also able to demonstrate previous pulsed (3 hours) exposure to increasing concentrations of diuron (0, 5 and 50$\mu$g.L-1) using time-responses curves, providing a new, easy-to-implement, method to assess short-term exposure to PSII inhibitors based on community photosynthesis.

**Keywords:** Biofilm, Freshwater, Diuron, Dose, response curves, Time, response curves

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*Speaker

# Towards easy-handling tools to assess functional effects of contaminants on natural microbial and invertebrate sediment communities

Stéphane Pesce[*,†,1], Sophie Campiche[2], Ayanleh Mahamoud Ahmed[1,3], Carmen Casado-Martinez[2], Chloé Bonnineau[1], Aymeric Dabrin[1], Emilie Lyautey[3], and Benoit J.d. Ferrari[2]

[1]UR Milieux Aquatiques, Ecologie et Pollutions (UR MALY) – Irstea – 5 rue de la Doua BP 32108 F-69616 Villeurbanne Cedex, France

[2]Swiss Centre for Applied Ecotoxicology (Eawag-EPFL) – EPFL-ENAC-IIE-GE, Station 2, CH-1015 Lausanne, Switzerland

[3]Centre Alpin de Recherche sur les Réseaux Trophiques et Ecosystèmes Limniques (CARRTEL) – Institut National de la Recherche Agronomique : UMR0042, Université Savoie Mont Blanc : UMRINRA / USMB – Station dH́ydrobiologie Lacustre, Le Bourget-du-Lac 73376, France

## Abstract

Sediments play a critical role in biogeochemical cycling and in the maintenance of the biodiversity. They are natural sinks for many contaminants which can accumulate over time and impact exposed communities, thereby disturbing the functioning of aquatic ecosystems. However, knowledge about the functional effects of contaminants on benthic communities is scarce and there is a need of ecosystem functioning indicators of ecotoxicological impacts. In the present microcosm study, we assessed the individual and combined effects of Cu and As (40 mg kg dw-1 each, 21 days of exposure) on the capacity of natural micro- and macro-organism communities from an uncontaminated river sediment to consume and decompose particulate organic matter using the bait lamina method (ISO 18311) as well as artificial tablets consisting of cellulose, bran flakes and active coal embedded in an agar matrix. The sediment toxicity was also evaluated using the standardized ostracod toxicity test (ISO 14371:2012). The two tested substrates (i.e. bait lamina and artificial tablets) showed similar results with low effects of As on feeding activity and organic matter breakdown whereas Cu demonstrated a strongest functional effect. When the two metals were combined, a total functional inhibition was observed, whatever the kind of substrate. The ostracod toxicity test also showed high toxicity of Cu-spiked and mixture-spiked sediments and low toxicity of As-spiked sediments. Our results highlight the relevance of artificial organic matter substrates to assess the functional effects of contaminants on sediment microbial and invertebrate communities, opening new perspectives to assess the functional integrity of contaminated sediments.

**Keywords:** Bait Lamina, Functional ecotoxicology, Benthic communities, Mixture, Metals

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[*] Speaker

[†] Corresponding author: stephane.pesce@irstea.fr

sciencesconf.org:ecotoxicomic:170065

# Amplicon pyrosequencing of bacterial and fungal community structure in Norwegian agricultural sandy loam soil - assessment of chemical mixture effects and implications for soil and plant health

Marianne Stenrød*[1] and Erik Lysøe[1]

[1]Norwegian Institute of Bioeconomy Research (NIBIO) – Høgskoleveien 7, NO-1433 Aas, Norway

## Abstract

The main objective of this study was to assess the effects of the single chemicals and mixtures of the fungicide picoxystrobin and the chemical 4-n-nonylphenol frequently occurring in sewage sludge, on soil microbial diversity in an agricultural sandy loam soil. The choice of compounds was based on their individual occurrence and effects in the environment, expected potential for co-occurrence in the field, and expected independent effect mechanisms due to differences in mode of action. Effects of the chemicals were assessed by amplicon pyrosequencing of the bacterial and fungal microbiota on soil samples at 0, 7, 28 and 70 days after chemicals treatment (DAT).

The soil bacterial community was affected by chemicals treatment in a way causing a transient increase in abundance of *Proteobacteria* and decrease in *Acidobacteria* that was reduced through the 70 d incubation period back to a level comparable to the original soil sample. For the fungal community the most apparent effect was found for treatment with the fungicide picoxystrobin at 7 DAT, when *Sordariomycetes* were increased in abundance. The results for the picoxystrobin treatment indicated a slower or lack of recovery, with comparable a-diversity levels at 7 and 70 DAT.

Identification of the main differentially abundant OTUs further indicated effects on abundance of plant pathogenic fungi, and bacteria and fungi of importance for decomposition and nutrient cycling, underlining the need for in-depth studies of the effects of single chemicals and mixtures to assess the potential effects of agrochemicals on soil quality and function.

**Keywords:** pesticides, amplicon pyrosequencing, soil quality

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*Speaker

# Examining the impact of plastic leachates on key marine photosynthetic bacteria

Indrani Sarker[1], Verena Schrameyer[1], Liam Elbourne[1], Ian Paulsen[1], and Sasha Tetu*†[1]

[1]Chemistry and Biomolecular Sciences – Macquarie University, NSW, 2109, Australia

## Abstract

Plastic pollution in the marine realm is an issue of growing concern; one understudied risk related to such pollution is that plastic debris can leach a variety of potentially toxic chemicals. Currently little is known regarding how plastic leachates impact marine organisms particularly primary producers such as photosynthetic marine cyanobacteria. *Prochlorococcus* species are significant players in oceanic primary production and global carbon cycling. Exposure to plastic leachate from common plastic debris items: plastic bags (HDPE) and plastic matting (PVC), impacted *in vitro* growth and primary productivity of *Prochlorococcus marinus* strains. The strains tested, MIT 9312 and NATL2A (model high light and low light-adapted ecotypes), showed distinct differences in their sensitivity to each leachate. Genome-wide transcriptomic analyses revealed a suite of genes were differentially expressed following leachate exposure, including many linked to primary production and stress responses. Understanding how key marine primary producers are affected by plastic leachate is critical to determining potential future impacts of plastic pollution on marine ecosystems as a whole.

**Keywords:** Prochlorococcus, cyanobacteria, plastic pollution, leachate, transcriptomics

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*Speaker

†Corresponding author: sasha.tetu@mq.edu.au

# Isolation of Heavy Metal Resistant Bacteria from Contaminated industrial Effluent in North Algeria

Ouelhadj Akli*[1], Kuschk Peter[2], and Djamel Djenane[3]

[1]Laboratory of food quality and safety – University of Mouloud Mammeri, Tizi ouzou, Algeria
[2]Department of Environmental Biotechnology – UFZ, Leipzig, Germany
[3]Laboratory of food quqlity and safety – University of Mouloud mammeri, Tizi ouzou, Algeria

## Abstract

The resistance of bacteria strains isolated from contaminated industrial effluent at the site of ENIEM, Tizi-Ouzou Algeria to four heavy metals was investigated and the antagonistic effect against six pathogenic bacteria and three fungal strains were analyzed. Eight bacterial strains have been isolated and identified on the basis of the morphological and biochemical characters. On this whole, the metal resistance and the antagonistic activity of five strains have been studied.The MICs of heavy metals were similar for each strain: 10 mg/ml of zinc; 5 mg/ml of aluminium; 3.125 mg/ml of copper and 2.5 mg/ml of chromium, except for *Pseudomonas* sp.1 inhibited to 1.25 mg/ml of chromium. The general order of resistance to the metals was found to be as $\mathrm{Cr} < \mathrm{Cu} < \mathrm{Al} < \mathrm{Zn}$ and the toxic effects of these metals increased with the increase of the concentrations. It can be concluded that all isolates were resistant to Cr but were highly resistant to Zn and Al. The antimicrobial activity was determined in six bacterial strains and three fungal strains, according to the agar duel method. All isolates tested showed an inhibitory effect at least on one of the tested microbial strains, confirming that the isolated bacterial strains are endowed with antimicrobial properties.

**Keywords:** Bacterial resistance, Heavy metals, Chromium, Zinc, Copper, Aluminium, Antimicrobial activity.

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*Speaker

# Herbicide mixture of mesotrione, nicosulfuron and s-metolachlor: biotransformation pathway and toxicity.

Louis Carles[2,1], Muriel Joly[1,2], Frédérique Bonnemoy[1], Martin Leremboure[2], Florence Donnadieu[1], Isabelle Batisson*†[1], and Pascale Besse-Hoggan[2]

[2]Institut de Chimie de Clermont-Ferrand (ICCF) – CNRS : UMR6296, Université Blaise Pascal - Clermont-Ferrand II, Ecole Nationale Supérieure de Chimie de Clermont-Ferrand – 24 Avenue des Landais, 63177 Aubière Cedex, France

[1]Laboratoire Microorganismes : Génome et Environnement, UMR CNRS 6023 (LMGE) – Université Clermont Auvergne – France

## Abstract

To reduce pesticide consumption, the new-generation molecules, currently used in agriculture, are sprayed at lower dosages but often in mixtures to increase their efficiencies. Despite the great environmental concern of unintentional mixture of pesticides coming from agricultural lands, little data is available concerning the effects of these cocktails on the fate and impact of each molecule. We focused our work on a mixture of three herbicides composed of mesotrione ($\beta$-triketone), nicosulfuron (sulfonylurea) and S-metolachlor (chloroacetanilide), commonly applied on maize crops in a short period of time. A *Bacillus megaterium* Mes11 strain [1], isolated from an agricultural soil, was found to be able to biotransform mesotrione and nicosulfuron, alone or in cocktail, but not S-metolachlor. Although the biodegradation pathways were not affected in the case of binary or ternary herbicide mixtures, kinetics of nicosulfuron disappearance as well as those of mesotrione and nicosulfuron metabolite formation were strongly modulated.

The toxicity of the parent compounds and metabolites was evaluated for individual compounds and mixtures with the standardized Microtox® test. Synergistic interactions were evidenced for all the parent compound mixtures, while synergistic, antagonistic or additive toxicity was obtained for metabolite mixtures, depending on the mixture. The results obtained highlight the need to take into account the active ingredient and metabolites all together for the determination of environmental fate and toxicity of pesticide mixtures and thus to improve the Environmental Risk Assessment of pesticide cocktail.

**Keywords:** Pesticide, Mixture, Simultaneous biotransformation, Metabolites, Microbial toxicity

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*Speaker

†Corresponding author: isabelle.batisson@uca.fr

# Toxicity of POCIS passive sampler extracts from a vineyard area in Bordeaux towards two marine microalgae.

Valentin Dupraz*†[1], Dominique Ménard[1], Farida Akcha[1], Hélène Budzinski[2], and Sabine Stachowski-Haberkorn[1]

[1]Laboratoire d'Écotoxicologie (LEX) – Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Université de Nantes, Université de Nantes – rue de l'île d'Yeu, BP 21105, 44311 Nantes, France

[2]Environnements et Paléoenvironnements OCéaniques (EPOC) – Centre National de la Recherche Scientifique - CNRS, École Pratique des Hautes Études [EPHE], Université Sciences et Technologies - Bordeaux I, Observatoire Aquitain des Sciences de l'Univers – Av. des Facultés, 33405 Talence, France

## Abstract

Pesticides used in viticulture end up in aquatic systems downstream of vineyard plots. Concentrations of single substances are often below the maximum residue level imposed by the WFD but few is known about their potential toxicity when present in mixtures. According to their chemical properties, pesticides accumulate over time in POCIS passive samplers deployed in contaminated environments and toxicity of the whole POCIS extracts can be tested after extraction. As phytoplankton plays a key role in the aquatic food web, this study aimed to assess the toxicity of POCIS extracts towards the growth of two marine microalgae, *Tisochrysis lutea* (haptophyte) and *Skeletonema marinoi* (diatom). POCIS were placed in three stations along a contamination gradient in small watercourses close to the vineyards plots over four one-month periods. The toxicity tests consisted in 96-h exposures of microalgae to i) POCIS extracts and ii) single substances expected to be present in the extracts, in sterile 48-well microplates incubated under controlled conditions. Each species was exposed to five dilutions of POCIS extracts and six concentrations of single substances. The toxicity was assessed by calculating the growth rate inhibition. The toxicity tests and analyses to chemically characterize the extracts are currently ongoing. The first results showed a dose-response trend for the two microalgae species, with subtle differences in the toxicity depending on the extracts. The testing of complex mixtures from the environment provides additional information regarding the contamination of aquatic ecosystems and enlights the necessity of focusing on mixtures effects, along with single compound toxicity.

**Keywords:** microalgae, passive samplers, toxicity, mixtures, vineyards

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*Speaker

†Corresponding author:

# Ecotoxicology of Herbicide Exposure on Harmful Estuarine Phytoplankton Under Varying Nutrient Conditions

Stacie Flood*†[1,2], Joann Burkholder[1], and Gregory Cope[3]

[1]Center for Applied Aquatic Ecology – Department of Applied Ecology, North Carolina State University, Raleigh, NC, 27606, United States
[2]National Center for Computational Toxicology – United States Environmental Protection Agency, Research Triangle Park, NC, 27709, United States
[3]Environmental Toxicology Division – Department of Applied Ecology, North Carolina State University, Raleigh, NC, 27695, United States

## Abstract

Anthropogenic inputs of macronutrients (nitrogen and phosphorus) are frequently associated with developing harmful algal blooms (HABs), but little is known about how estuarine phytoplankton assemblages respond to multiple, co-occurring chemical contaminants in chronically disturbed habitats. The available evidence suggests that off-target herbicide exposures can significantly affect phytoplankton assemblage composition, but questions remain about how estuarine phytoplankton respond to environmental stressors and why some strains respond by producing phycotoxins that can kill fish and other aquatic life. The goals of this research were to establish a robust protocol for testing the concentration-related effects of atrazine, a ubiquitous herbicide, on growth and toxin production in potentially harmful estuarine phytoplankton, and then to use that protocol to compare the effects of atrazine exposure both with and without nutrient enrichment. The protocols were developed using the benign estuarine/marine alga, *Dunaliella tertiolecta* (Chlorophyta) and the HAB species tested included the ichthyotoxic haptophyte *Prymnesium parvum* (Haptophyta) and the toxigenic raphidophyte *Chattonella subsalsa* (Raphidophyceae). Species sensitivity in nutrient-replete media followed the general ranking of *D. tertiolecta* > *P.parvum* > *C. subsalsa*, but responses were modulated by salinity, strain origin and exposure duration. Imbalanced nutrient regimes alleviated the growth-inhibiting effects of atrazine for all tested taxa to varying extents. Production of hemolytic substances by the HAB species increased under co-occurring nutrient stress and herbicide exposure, and the greatest toxicity was observed under combined imbalanced nitrogen/phosphorus levels and high atrazine exposures. These findings advance knowledge about how nutrient regimes and herbicides interact to control harmful estuarine phytoplankton population dynamics.

**Keywords:** atrazine, herbicide, phytoplankton, harmful algal blooms, algal toxins

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*Speaker
†Corresponding author: Flood.Stacie@epa.gov

# Simultaneous removal of azo-dyes and hexavalent chromium by a metal and salt tolerant Pseudomonas sp. ZM183 harboring plant growth promoting traits

Sabir Hussain*†[1], Zahid Maqbool[1], Faisal Mahmood[1], Tanvir Shahzad[1], and Muhammad Shahid[2]

[1]Department of Environmental Sciences Engineering, Government College University, Faisalabad – Pakistan

[2]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad – Pakistan

## Abstract

In this study, a metal and salt tolerant dyes decolorizing bacterial strain, *Pseudomonas* sp. ZM183, was isolated from an agricultural soil polluted with textile wastewater containing the dyes, metal ions and salts. This strain had the potential to decolorize different azo dyes in the presence of a mixture of heavy metal ions (Cr2+, Cd2+, Zn2+, Pb2+). The individual and interactive impact of four different variables (*viz.* NaCl content, pH, concentration of yeast extract and level of multi-metal mixture) on decolorization of reactive red-120 by *Pseudomonas* sp. ZM183 was estimated following response surface methodology (RSM). Based on RSM model, optimal decolorization of reactive red-120 by the strain ZM183 was predicted in the medium containing 12.5 g L-1 of NaCl, pH 8.0, 7.5 g L-1 of yeast extract and a multi-metal mixture (Cr: 20 mg L-1; Pb: 40 mg L-1; Cd: 20 mg L-1; Zn: 40 mg L-1). This strain was also found highly efficient in simultaneous removal of hexavalent chromium (25 mg L-1) and different reactive dyes (100 mg L-1). Moreover, the strain ZM183 was also found to harbor the azoreductase gene amplified through PCR using specific primers. In addition to dye decolorization and hexavalent chromium reduction, this strain was also found to have plant growth promoting traits including inorganic phosphate solubilization and indole acetic acid production. Hence, the isolation and characterization of multi-functional *Pseudomonas* sp. ZM183 might serve as a potential bio-resource to develop the strategies for integrated bioremediation and plant growth promotion in agricultural soils contaminated with textile wastewaters.

**Keywords:** Azo, dyes decolorization, multi, metal stress, Pseudomonas sp. ZM183, Plant growth promotion, Azoreductase gene, RSM Modelling

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*Speaker

†Corresponding author: sabirghani@gmail.com

# Cyanotoxins role for cyanobateria in stress conditions

Sandra Kim Tiam*†[1], Katia Comte[1], Caroline Dalle[2], Chakib Djediat[1], Charlotte Duval[1], Kathleen Feilke[3], Muriel Gugger[2], Diana Kirilovsky[3], Sahima Hamlaoui[1], Séverine Le Manach[1], Benjamin Marie[1], Pierre Setif[3], Claude Yéprémian[1], and Cécile Bernard[1]

[1]Molécules de Communication et Adaptation des Micro-organismes, équipe "Cyanobactéries, Cyanotoxines et environnement" (MCAM) – Muséum National d'Histoire Naturelle (MNHN), CNRS : UMR7245 – 12 rue Buffon - RCP 39, 75231 Paris Cedex 05, France

[2]Collection des Cyanobactéries – Institut Pasteur de Paris – 28 rue du Dr Roux, 75724 Paris, France

[3]Institut de Biologie Intégrative de la Cellule (I2BC) – Université Paris-Sud - Paris 11, Commissariat à l'énergie atomique et aux énergies alternatives : DSV/I2BC, Université Paris-Saclay, Centre National de la Recherche Scientifique : UMR9198 – 1 avenue de la Terrasse, 91198 Gif/Yvette cedex, France

## Abstract

Cyanobacteria are photosynthetic prokaryotes having a key role in ecosystem functioning but are also a source of concern for managers since they can form toxic blooms. Environmental risk assessment related to cyanotoxin exposure is particularly complex since the toxin concentrations cannot be predicted based on the cyanobacteria species forming the bloom or on the bloom intensity. Cyanotoxin biologic role characterization could be a key factor for concentration prediction and a better management of environmental risks. Ecological advantages offered or not by toxins in stress condition (high light and high temperature), through their role in photosynthesis and photoprotection, were studied. Five Planktothrix strains have been selected: green (Planktothrix agardhii) or red (Planktothrix rubescens) ecotypes, non-microcystins or microcystins producers and a wild-type green strain and its mutant for toxin production. A 24 hours experiment was realized in laboratory controlled conditions and was composed of two different phases: stress exposure phase (4H) followed by a recovery phase. The cultures were exposed in triplicate to high light intensity (150 $\mu$E/m2/s) or high temperature (33∘C) and controls (20∘C, 6 $\mu$E/m2/s) were run in parallel. Along the experiment, responses of cyanobacteria were evaluated with: i) biophysical measurements (photosynthesis and photoprotective mechanisms), ii) transcriptomic analyses (genes involved in toxin synthesis, photosynthesis, photoprotection, temperature tolerance and oxidative stress regulation), iii) metabolomics analyses (secondary metabolites synthesis), iv) protein analysis (orange carotenoid protein) and v) immunolocalization analysis (microcystins).

**Keywords:** Cyanobacteria, toxins, microcystins, photoprotection, Planktothrix.

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*Speaker

†Corresponding author: sandra.kim-tiam-fook-chong@mnhn.fr

# Light modulated effects of toxicants toward river biofilm photosynthesis: an ecophysio-toxicological approach

Martin Laviale*†[1], Anne Créach[2], Evane Thorel[3], Johann Lavaud[4], and Soizic Morin[3]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – France

[2]Unité de Glycobiologie structurale et fonctionnelle (UGSF) – CNRS : UMR8576, Université Lille I - Sciences et technologies – Bâtiment C9 59655 VILLENEUVE D ASCQ CEDEX, France

[3]Réseaux épuration et qualité des eaux (UR REBX) – Irstea – 50 avenue de Verdun - Gazinet, F-33612 Cestas, France

[4]CNRS/Université Laval UMI 3376 (TAKUVIK) – Canada

## Abstract

Traditional ecotoxicological bioassays using diatoms are often based on growth or photosynthetic activity inhibition under stable and standardized conditions. However, their responses to toxic stress may be modulated by variations in environmental parameters. Light, which is the most important factor for photosynthetic organisms, may modify algal sensitivity to contaminants. Daily light fluctuations, indeed, have consequences on algal photophysiology. The capacity to cope with both toxicants and swift changes in light conditions should thus be taken into consideration. In order to increase the complexity and the realism of ecotoxicological tests, it is necessary to take into account multistress situations.

In this context, short-term experiments (< 24h) were carried out to investigate the combined effects of light and toxicants presenting different modes of action (isoproturon, diuron, copper, norflurazon) on the photophysiology of intact natural biofilms by measurements of chlorophyll fluorescence and pigment composition. Firstly, biofilms were exposed to toxicants under constant light at different irradiance levels (25–300 $\mu$mol m–2 s–1). Secondly, biofilms were exposed using more environmentally realistic conditions (i.e. daily light cycle). The obtained results lead to the design of an ecotoxicological protocol based on the so-called ” light stress recovery experiment ” which is typically used in ecophysiology.

This protocol offers interesting perspectives in environmental risk assessment, providing a more generic and ecologically realistic framework than traditional approaches currently used in ecotoxicology.

**Keywords:** multi, stress, light, toxicants, biofilms, diatoms, photosynthesis

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*Speaker

†Corresponding author: martin.laviale.bio@gmail.com

# Biocidal coatings triggers low diversified Alteromonadaceae dominated biofilm community

Thomas Pollet[1,2], Lyria Berdjeb[3], Cédric Garnier[3], Benjamin Misson[3], Christine Bressy[1], Jean-François Ghiglione[4], and Jean-François Briand*[1]

[1]MAPIEM-EA 4323 – Université de Toulon – France
[2]INRA-UMR BIPAR, Maisons-Alfort, France – Institut national de la recherche agronomique (INRA) – France
[3]PROTEE – Université de Toulon – France
[4]LOMIC – Université Pierre et Marie Curie - Paris 6 – France

## Abstract

Identifying microbial successions are crucial to understand the antifoulants impact on the dynamics of marine biofilm. We investigated the density (flow cytometry) together with the community succession (Metabarcoding) in coastal marine biofilms in Toulon Bay (France), during 75 days of immersion of 2 antifouling coatings (a biocidal named SPC and the other based on physical properties named FRC) and a reference surface (PVC).
Based on seven sampling performed (triplicates), density showed 3.5 times lower value on SPC and FRC compared to PVC. In addition, bacterial succession showed that although pioneer taxa were dissimilar on all the three surfaces, the community on PVC and FRC tend to converge with time whereas biocidal coating tends to diverge.

If Flavobacteriaceae represented critical members of diversified biofilms on PVC, Alteromonadaceae accounted for more than 90% of the community on SPC after 4 days and until 12 days of immersion. Then, bacterial diversity increased progressively, keeping Alteromonadaceae as one of the co-dominant taxa until 75 days of immersion. If pioneer stage on the FRC were also dominated by Alteromonadaceae (around 60%), the rapid increase in diversity reached a higher level than for SPC and promoted the co-dominance of other bacterial families (Rhodobacteraceae, Flavobacteriaceae, ...).
A second Mediterranean site, in the bay of Banyuls exhibited dissimilar metal contamination and hydrodynamics especially), allowed demonstrating a higher efficacy of SPC after 75 days of immersion. Furthermore, SPC exhibited dissimilar diversified communities at the 2 immersion sites. In conclusion, community shaping was mainly driven by biocide but also site.

**Keywords:** Biofilm, biocide, marine microbial ecotoxicology, antifouling

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*Speaker

# Shifting in the structural diversity of archaeal communities with contrasted hydrodynamic regimes in Mediterranean coastal rivers

Brice Reoyo-Prats*[1], Mégane Noyer[1], and Carmen Palacios[1]

[1]Centre de Formation et de Recherche sur les Environnements Méditérranéens (CEFREM) – Université de Perpignan Via Domitia, Centre National de la Recherche Scientifique - CNRS, Université de Perpignan Via Domitia – Perpignan, France

## Abstract

Assessing the impact of anthropogenic contaminants in aquatic environments represents nowadays a fundamental issue. Pollutants concentrations in watercourses mainly depend on land use and hydrometeorological phenomena. These phenomena are particularly contrasted in Mediterranean regions, where regimes of droughts alternate with heavy rainfalls. We have demonstrated that typical storm events in these regions are at the origin of multipollution episodes in Mediterranean coastal rivers. In this study, we examine the impact of these sudden changes in contaminants on microorganisms attached to suspended matter of the Têt River, a major coastal watercourse in the South-East of France. Microbial biofilms are the first to interact with contaminants, degrading or transforming them. This has been mainly studied in bacterial and algal biofilms. Here, we focus in the archaeal component of biofilms attached to particles. Water quality measurements, including the analyses of more than 350 micropollutant molecules, were performed from upstream to foreshore waters in two complete drought-flood-drought hydrological cycles. A high frequency sampling during heavy rain periods at two contrasted stations was also carried out. The diversity of Archaea was studied using Illumina targeted metagenomics analysis on the 16S rRNA gene. Their communities were mainly composed of two phyla, Thaumarchaeota and Euryarchaeota, and sometimes a third phyla, Crenarchaeota. However, a great quantity of sequences did not match anything in the databases. Variations in phylogenetic composition could be directly linked to changes in pollutants levels, particularly during heavy rainfalls. These results indicate the importance of the archaeal compartment in aquatic ecosystems reactivity to pollutants.

**Keywords:** Microbial ecotoxicology, multipollution, aquatic ecosystems, Mediterranean, archaeal communities, structural diversity.

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*Speaker

# Combined effect of a realistic pesticide mixture and climate change-related stressors on river biofilms.

Ferran Romero*[1], Vicenç Acuña[1], Anna Freixa†[1], Julio C. López-Doval[1], Maria Casellas[1], Natalia Mingorance[1], and Sergi Sabater[1,2]

[1]Catalan Institute for Water Research (ICRA) – Spain
[2]Institute of Aquatic Ecology - University of Girona (IEA - UdG) – Spain

## Abstract

Chemical stressors such as agricultural pesticides are known to significantly impact community composition and functioning in stream ecosystems. Moreover, agricultural streams are often subjected to climate change-related stressors, such as warming and flow reduction. Since these stressors are often investigated separately, uncertainty remains over the combined impacts that they may pose to freshwater communities. An additional knowledge gap results from the comparison between the responses on a temporal scale, and to which extent the interactions observed at short term remain when the stressors combination is applied at long-term (e.g. one month). Using river biofilms and microfauna (micrometazoans and ciliophora) as target communities, we evaluated the individual and combined effects of a realistic pesticide mixture and two climate change-related stressors (i.e. flow reduction and warming). The experimental procedure followed a full-factorial design using 24 artificial indoor channels. The biofilm response was evaluated both at short and long-term. Biofilm and microfauna analyses covered a wide range of physiological and structural variables, from molecular approaches to ecosystem metabolism measurements. Overall, our results show that climate change-related stressors drove the overall effects and shaped the response of the river biofilm and the microfaunal community to agricultural pesticides. We conclude that the response of a freshwater community to the combined action of chemical and climate change-related stressors cannot be predicted straightforwardly based on the individual effects.

**Keywords:** multiple stressors, river biofilm, microfauna, pesticides, climate change.

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*Corresponding author:
†Speaker

# Responses of two strains of the marine microalga Tetraselmis suecica to binary mixtures of two PSII inhibitors

Rossana Sussarellu*[1], Valentin Dupraz[1], Dominique Ménard[1], Hélène Budzinski[2], and Sabine Stachowski-Haberkorn[1]

[1]Laboratoire d'Ecotoxicologie, Institut Français de Recherche pour l'Exploitation de la Mer (LEX, IFREMER) – Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) – Rue de l'Ile d'Yeu - BP 21105 - 44311 Nantes Cedex 03, France

[2]CNRS, UMR 5805, EPOC, Laboratoire de Physico Toxico Chimie de l'environnement (UMR EPOC, LPTC) – Université de Bordeaux (Bordeaux, France) – 351 Cours de la Libération, 33405 Talence, France

## Abstract

Irgarol (triazine) and diuron (phenylurea) are two biocides commonly used in copper-based antifouling paints. They both act as photosystem II (PSII) inhibitors due to their binding action on the D1 protein in PSII. They are thus particularly toxic to photosynthetic organisms such as microalgae. This study aimed to describe the interactions between the two compounds in binary mixtures towards two strains of *Tetraselmis suecica*: a wild strain (TW) and a mutant strain (TM) resistant to diuron.

The toxicity tests consisted in 96-h exposures to contaminants in 48-well microplates. Each strain was exposed to each substance at 6 concentrations. The binary mixtures were achieved following a fixed-ratio ray design with 5 mixture ratios. The toxicity was assessed by calculating EC50 values based on the 96-h growth rate inhibition. The results were analysed using Concentration Addition (CA) reference model, and Helwett and Volund models for synergy and antagony.

The EC50 obtained were: 4.2 ± 0.13 $\mu$g.L-1 diuron and 0.65 ± 0.027 $\mu$g.L-1 irgarol for TW, when EC50 for TM were 20-fold higher for diuron and almost twice for irgarol. For binary mixtures, a slight but significant concentration-dependent synergy was demonstrated in both strains. To further investigate the mechanisms responsible for such effects, transcriptome analyses (RNAseq HiSeq 3000, paired-end 2x150 bp) were performed from cDNA librairies of both strains in control condition and in condition with 50 % growth rate inhibition after exposure to a mixture. The analyses are currently ongoing.

**Keywords:** microalgae, PSII inhibitors, resistance, toxicity, binary mixtures, transcriptomic

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*Speaker

# Quick screening of isolates relevant in microbial ecotoxicology by tandem mass spectrometry

Béatrice Alpha-Bazin*†[1], Karim Hayoun[1], Gerard Steinmetz[1], Pauline Petit[2], Corinne Rivasseau[2], Olivier Pible[1], and Jean Armengaud[1]

[1]Laboratory Innovative technologies for Detection and Diagnostics (LI2D) – CEA - Direction de la Recherche Fondamentale - Institute Frederic Joliot – CEA Marcoule, 30207 Bagnols sur Ceze, France

[2]Laboratoire de Physiologie Cellulaire Végétale (LPCV) – CEA - Direction de la Recherche Fondamentale - Biosciences Biotechnology Institute of Grenoble, Centre National de la Recherche Scientifique - CNRS, Institut National de la Recherche Agronomique - INRA, Université Grenoble Alpes – CENG 17 rue des Martyrs, 38054 Grenoble, France

Session
RISK ASSESEMENT
Poster

## Abstract

We developed a new strategy to quickly identify microorganisms based on tandem mass spectrometry and characterize their main metabolic potential. Following plate isolation, a single colony may be used for extracting bacterial proteins. These are then proteolyzed with trypsin, and the resulting peptides are sequenced by high-resolution tandem mass spectrometry. A home-developed bioinformatics pipeline allows from a simple 60 min measurement identifying the microorganisms present in the sample either at the genus, species or subspecies level, certifying if the isolate is pure or present in mixture with some contaminants, and obtaining its functional characterization based on the identified proteins. Dereplication of isolates, taxonomical identification or functional focus on the most interesting microorganisms are quickly achieved. We applied this methodology for exploring bacteria present in the cooling pool for spent fuel in a nuclear reactor. We used different culture media to isolate bacteria. The quick screening approach will be illustrated with an overview of the numerous identified bacteria. This methodology is applicable to a large variety of samples and thus has a great potential in microbial ecotoxicology.

**Keywords:** tandem mass spectrometry, taxonomical identification, screening

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*Speaker

†Corresponding author: beatrice.alpha-bazin@cea.fr

# Development of a method to test soil protists in ecotoxicology and its application to an herbicide. Euglypha rotunda (Rhizaria) proposed as model organism

Nathalie Amacker*†[1,2], Edward Mitchell[1,3], and Nathalie Chèvre[2]

[1]Laboratory of Soil Biodiversity, Université de Neuchâtel (UniNE) – Emile-Argand 11 CH-2000 Neuchâtel, Switzerland
[2]Institut des dynamiques de la surface terrestre, Université de Lausanne (IDYST, UNIL) – UNIL Mouline CH-1015 Lausanne, Switzerland
[3]Jardin botanique de Neuchâtel – CH-2000 Neuchâtel, Switzerland

## Abstract

Many ecotoxicological tests have been elaborated the last decades to address the effect of toxic compounds in environment with a special focus on metazoans. The protists (micro-eucaryotes) that constitute the major part of eukaryotic diversity and drive ecosystems functions (such as biogeochemical cycles) are clearly under-represented. To our knowledge not a single model organism for soil ecotoxicology exists among the Rhizaria, a highly diverse group or terrestrial and aquatic protists.

We aim to fill this gap by developing a robust ecotoxicity test for the common soil testate amoeba *Euglypha rotunda*. In particular, we aim in finding out i) the variables that influence the growth of the amoeba in the laboratory, and ii) the environmental conditions allowing reproducible growth of the organisms, so that the effects of chemical on that growth can be highlighted. In a second time, we investigated the effects of an herbicide, *S*-metolachlor on the growth of the amoeba and of the bacteria *Escherichia coli* (co-inoculated as carbon source). The *S*-metolachlor is a chloroacetamide that affects the production of very-long-chain-fatty acids (VLCFAs). The protozoan growth is expected to be influenced as VLCFAs are important players of cell division that is the most common reproduction strategy of testate amoebae.

Conditions supporting reproducibility could be defined and the effect of *S*-metolachlor evaluated. The protozoan growth was reduced when exposed at around 15 $\mu$g/L of *S*-metolachlor but not at higher concentration. The bacteria were not affected at all.

**Keywords:** testate amoebae, E. coli, small trophic chain, S, metolachlor

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*Speaker

†Corresponding author: nathalie.amacker@gmail.com

# Environmental surveillance of marine oil releases using an autonomous ecogenomic sensor – development of a surface plasmon resonance based biosensor for Oleispira antarctica

Andrea Bagi*†[1], Knapik Kamila[1], Mari Mæland Nilsen[1], Adriana Krolicka[1], Catherine Boccadoro[1], and Thierry Baussant[1]

[1]International Research Institute of Stavanger – Environment Department, Mekjarvik 12, Randaberg, N-4070, Norway

## Abstract

Environmental changes and pollution monitoring from expansion of oil and gas exploration activities pose a practical challenge in remote marine environments, e.g. pristine Arctic region. Timely delivery of critical information for mitigating environmental impacts in such areas are necessary but manpower and access to infrastructure are limited. There is a need for automatized low-manpower monitoring and rapid communication of critical data. This poster presentation will introduce the GENOMAPE project lead by the International Research Institute of Stavanger (IRIS), Norway. The central technology is the ESP (Environmental Sample Processor, MBARI), a cutting-edge autonomous genosensor that uses molecular detection of microorganisms from water samples. Here, nucleic acid (primarily rRNA) signals from hydrocarbon degrading microorganisms will be used to infer occurrence of small to medium oil releases. Our aim is to develop molecular assays targeting obligate hydrocarbon-degrading bacteria using phylogenetic genes in a manner compatible with the analytical modules of the ESP. Surface plasmon resonance (SPR), a highly sensitive, label-free and rapid optical sensing technology is one analytical module that will be integrated in ESP in the future. We are working on adapting a portable SPR unit for the detection of bacterial rRNA, using morpholino probes immobilized gold surface. Currently, a single probe targeting 16S rRNA of *Oleispira antarctica* and synthetic oligonucleotides are used for method development. The poster will present the vision and workplan of GENOMAPE and describe in detail the ongoing SPR assay development.

**Keywords:** surface plasmon resonance, hydrocarbon degrading bacteria, marine environmental monitoring

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*Speaker

†Corresponding author: Andrea.Bagi@iris.no

# Impact of pharmaceutical industry effluents on microorganism biodiversity inside river biofilms

Cédric Bernarde[1], Anne Goubet[1], Pierric Jeannin*†[2], and Jean-Jacques Pernelle[1]

[1]UR HBAN, Equipe EPURE – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA – 1, rue Pierre-Gilles de Gennes CS 10030 92761 Antony Cedex, France

[2]SANOFI – SANOFI Recherche – route d'Avignon 30390 ARAMON, France

## Abstract

The continental water quality evaluation is currently achieved with normalized indexes based on observations, like the French "indice biologique global normalisé (IBGN)" or the "indice biologique diatomique (IBD)". These complex processes need the intervention of experimented specialists for taxonomic assignment. The development of next-generation sequencing (NGS) would open a new way for a tool based on molecular inventory of the microbiota of rivers' biofilms. Such a tool could efficiently support the evaluation of water quality by a regular monitoring biofilm population and the differences that could be identified between the rivers upstream and downstream a discharge point.

Sanofi initiate collaboration with IRSTEA to propose a tool allowing estimating quickly the impact of effluents of wastewater treatment plants (WWTP) on the receiving environment. This project will allow defining supports for biofilm development, choosing primer sets in order to describe microbiological diversity with the most exhaustive manner. We will also define the number of sample and the immersion duration. The project covers also the installation of the "pipeline" for the analysis and data filtration.

We present here some details of the project and very first results.

**Keywords:** biofilm, impact, next, generation sequencing

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*Speaker

†Corresponding author:

# Development of whole-cell bacterial sensors for lanthanides bioavailability

Charlotte Berthelot[*1], Asfaw Zegeye[1], and Patrick Billard[1]

[1]LIEC – Université de Lorraine, CNRS : UMR7360, Université de Lorraine – France

## Abstract

Lanthanides (Ln) are economically important metals with wide applications in technology and industry. With their increasing release into the environment, there is a need for the development of new tools for the monitoring of Ln bioavailability. Here we describe the design of bacterial bioreporters and their use in liquid- or solid-phase bioassays to measure the presence of Ln in a variety samples. Bioreporters were constructed by coupling the Ln-responsive methanol dehydrogenase genes *xoxF* and *mxaF* from *Methylobacterium extorquens* (Vu et al., 2016) and two alcohol dehydrogenase counterparts from *Pseudomonas putida* (Wehrmann et al., submitted) to the *luxCDABE* reporter genes. In both strains, the expression of the two dehydrogenase genes were inversely regulated in response to available Ln concentrations. This transcriptional response was specifically triggered by light Ln (La, Ce, Pr, Nd) in *M. extorquens*, while *P. putida* bioreporters additionally responded to medium Ln (Sm, Gd). In optimized assay conditions, as low as 1 nM Lncould be detected within 4 hours of incubation. As a proof-of-principle application, the bioassays were tested on contaminated soil samples as well as with Ln-containing solids such as Nd-magnets. Our results indicated that the bioreporter are sensitive enough to detect Ln in these samples, thus making them potential a ecotoxicological tool for Ln monitoring. Vu HN, Subuyuj GA, Vijayakumar S, Good NM, Martinez-Gomez NC, Skovran E. 2016. Lanthanide-Dependent Regulation of Methanol Oxidation Systems in *Methylobacterium extorquens* AM1 and Their Contribution to Methanol Growth. J Bacteriol 198: 1250-1259

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**Keywords:** lanthanides, bioreporter, Ln monitoring

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[*] Speaker

# Exposures of soil microbial communities to realistic antibiotics concentrations: key parameters for environmental risk assessment

Olivier Crouzet*[1], Anaïs Goulas[1], Claire-Sophie Haudin[1], Pierre Benoit[1], Alain Hartmann[2], Géraldine Depret[2], Agnès Richaume-Jolion[3], Thomas Pommier[3], and Sylvie Nazaret[3]

[1]UMR 1402 ECOSYS – Institut National de la Recherche Agronomique - INRA, AgroParisTech, Université Paris-Saclay – 78026 Versailles cedex, France

[2]Agroécologie – Agrosup, Institut national de la recherche agronomique (INRA) : UMR1347, Université de Bourgogne – BP 86510, F-21000 Dijon, France

[3]Ecologie microbienne (EM) – CNRS : UMR5557, Ecole Nationale Vétérinaire de Lyon, INRA : UR1193, Université Claude Bernard - Lyon I – La Doua, 69622 Villeurbanne, France

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## Abstract

While the occurrence and persistence of antibiotics in environment are recognized as major environmental and human health issues, their ecotoxicological impacts on soil microbial communities are still overlooked for environmental risk assessment. Most available studies highlight effects on soil microbial communities at antibiotic concentrations or under exposure pathways that are far from realistic conditions. To investigate the relationships between SMX (sulfamethoxazole) availability and impact on microbial nitrogen-cycling communities and antibiotic resistance genes (ARGs), a dose-effect approach was performed, with sludge compost or farmyard manure amended soil microcosms. The organic wastes were contaminated before mixing in soils, to obtain SMX concentration surrounding those usually measured in amended agricultural soils. Microbial descriptors were monitored during 84 days following amendments. The total and available fractions of SMX were quantified by UPLC-MS/MS. At these low but environmentally realistic concentrations, only nitrification was impaired by SMX, in compost-amended soils and little consistent effect was observed on denitrification. However, SMX availability was similar in both compost- or manure-amended soils, thus not explain the short-term effects. A temporal decrease of SMX availability contributed to the nitrification recovery, in compost-amended soils. The dynamics of ARGs were partly explained by SMX gradient, but the duration of the experiment and the type of organic waste influenced the ARGs responses to SMX. Although it is necessary to define normal operating ranges of these descriptors, for further interpretations of their suitability as indicators in risk assessment, nitrification and abundances of ARGs are sensitive descriptors of AB effects on soil microbial functioning.

**Keywords:** soil microbial communities, antibiotics, functional endpoint, resistance genes

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*Speaker

# Can biodegradation of cis-1,2-dichloroethene and vinyl chloride by bacterial consortiums of a contaminated aquifer be directly linked to functional gene abundance?

Catherine Joulian[1], Camille Bourdier[1], Jérémie Denonfoux[2], Gwenaël Imfeld[3], Stéphanie Ferreira[2], Stéphane Vuilleumier[4], Louis Hermon[1,4], and Jennifer Hellal*[1]

[1]Environmental Biogeochemistry and Water Quality Unit (D3E/BGE) – Bureau de Recherches Géologiques et Minières (BRGM) – France
[2]Genoscreen – Institut Pasteur de Lille – 1 rue du Professeur Calmette, Lille 59000, France
[3]Laboratoire d'HYdrologie et de GEochimie de Strasbourg (LHYGES) – Ecole Nationale du Génie de l'Eau et de l'Environnement – UMR7517 CNRS EOST/UdS, 1, rue Blessig 67084 Strasbourg, France
[4]Génétique moléculaire, génomique, microbiologie (GMGM) – université de Strasbourg, CNRS : UMR7156 – 28 Rue Goethe 67083 Strasbourg, France

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## Abstract

The extensive use of aliphatic chlorinated hydrocarbons (*e.g.* tetrachloroethene and its degradation products) has resulted in their accumulation and persistence in aquifers worldwide, representing a serious risk for human health and the environment. Polluted sites are generally diagnosed and monitored using classical physico-chemical assessment of the plume. In addition, the evaluation of molecular biomarkers reflecting a biodegradation potential may enhance the evaluation of *in situ* attenuation and assist site diagnosis. The aim of this study was to evaluate whether the biodegradation of *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) by the bacterial consortiums of a contaminated aquifer could be directly linked to the abundance of functional genes involved in their degradation (*tceA*, *vcrA* & *bvcA*). This approach may help interpret the abundance patterns of functional genes on-site. Groundwater samples contaminated with cDCE and VC were incubated under i) oxic conditions to evaluate the potential of oxidative pathways, ii) anoxic conditions with addition of H2 to evaluate reductive dechlorination, and iii) anoxic conditions with addition of N2 and 10 mM Fe(III), since Fe(II) was present in the aquifer. Degradation was most effective in the Fe(III) condition, with significant reduction of DCE and formation of VC after approximately 3 months, following Fe(III) and sulfate reduction. The *vcrA* gene was present during the entire experiment and its abundance did not vary significantly. In contrast, *tceA* gene abundance was only detected during the active phase of DCE and VC degradation, suggesting that it may be a good biomarker of DCE and VC degradation.

**Keywords:** vinyl chloride, dichloroethene, biomarkers, reductive dechlorination, functional genes

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[*] Speaker

# Hospital effluents, not un exclusive source of contaminant spread in sub-saharian urban rivers

Amandine Laffite*[1], Vera Slaveykova[1], and John Poté†[1]

[1] Forel Departement for Environmental Sciences – Bd Carl Vogt 66, CH-1211 Geneva, Switzerland

## Abstract

The contamination of freshwater resources with anthropogenic pollutant is particularly alarming in developing countries where most rivers and lakes are receiving urban and hospital wastewater without any prior treatment. Cumulative with the knowledge gaps in antibiotic prescription and the free access to last resort antibiotics, under-developing countries may represent a broth culture to antibiotic resistance emergence, maintenance and dissemination. In this study, we investigate the contamination of sediment and the resistance profile of extending-spectra $\beta$-lactamase *E. coli* (ESBLECs) isolated from 4 urban river subjected to hospital outlet pipes (HOP) discharge in order to determine the effect of HOP discharge on urban river quality and the pattern of antibiotic resistance dissemination. ARGs (*blaTEM, blaCTX-M, blaSHV* and *aadA)* and selected bacterial species (i.e *E.coli*, *Enterococcus*, Pseudomonas *spp.*) where quantified in sediments using quantitative PCR (qPCR), toxic metals content were quantified by ICP-MS, and ESBLECs isolates where subjected to pulse-field electrophoresis to assess their clonality and characterized for their $\beta$-lactams resistance. The results highlight the great concentration of toxic metals (i.e. Cr, Cu, Zn, Cd, Pb and Hg) in sediments and the high content of FIB and ARGs copy number in all sampling sites. The analysis of ESBLECs profile showed the high diversity of clone dissemination and a high resistance profile against $\beta$-lactams. These findings indicates the human and environmental potential risk link to exposure to these contaminants and the need of developing strategies to limit the spread of these emerging contaminant.

**Keywords:** hospital effluents, emerging contaminant, antibiotic resistance, metal resistance

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*Speaker

†Corresponding author: John.Pote@unige.ch

# Integrating functional responses of autotrophic microorganisms to chemicals in ecological risk assessment

Floriane Larras*†[1], Elise Billoir[2], Stefan Lips[1], and Mechthild Schmitt-Jansen[1]

[1]Helmholtz Centre for Environmental Research, Department Bioanalytical Ecotoxicology (UFZ) – Leipzig, Germany

[2]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Metz, France

## Abstract

Microorganisms (e.g. bacteria, fungi and algae) are implied in various ecosystem functions such as biogeochemical cycles or pollutants degradation. That means that they are crucial for ecosystem functioning, here considered as the overall performance of a system. In the environment, organisms are exposed to anthropogenic pressures which are known to potentially induce structural and functional changes. Many studies report causal links between chemicals exposure and functional responses, but little is known about the involved biochemical pathways supporting specific functions. Moreover, most of *a priori* ecological risk assessment (ERA) tools are based on structural endpoints and do not necessarily ensure the protection of functional endpoints. The recent raise of OMICs approaches (e.g. transcriptomics and metabolomics) opens the perspective in ecotoxicology to explore pathways involved in ecological functions. Based on OMICs approaches, we identified functional responses of microorganisms across different biological organization levels to improve linkage of pathways, responsive to chemical exposure, and ecosystem functions. Such results present a great potential for the development of an integrative ERA tool based on microorganisms functions sensitivity at different biological levels. The challenge remains how to include these data in ERA strategies. As a first step, we developed an approach based on multi-level sensitivity distributions. We exemplify a first version of this tool, using the transcriptomic and metabolomic responses of *Scenedesmus vacuolatus* to triclosan. As a perspective, the tool will be transposed and adapted to benthic microbial communities in order to release an integrative ERA tool considering ecological systems.

**Keywords:** Microorganisms, Functions, OMICs, Ecological Risk Assessment

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*Speaker

†Corresponding author: floriane.larras@ufz.de

# Lignin degradation pathway in Scedosporium species.

Wilfried Poirier*†[1], Amandine Gastebois[1], Patrick Vandeputte[1], Jean-Philippe Bouchara[1,2], and Sandrine Giraud[1]

[1]Groupe dÉtude des Interactions Hôte-Parasite (GEIHP) – Université d'Angers : EA3142 – UFR Sciences pharmaceutiques et ingénierie de la santé 16, Bd Daviers 49045 Angers cedex 01, France
[2]Laboratory of Parasitology-Mycology – Centre Hospitalier Universitaire – Angers, France

## Abstract

Scedosporium are usually soil saprophytes. Among the ten species recognized within the genus, three have been reported regularly causing infections in human, i.e. Scedosporium apiospermum, Scedosporium aurantiacum and Scedosporium boydii. Environmental studies were conducted to get a better knowledge on the ecology of these opportunistic molds. Scedosporium species are abundant in human-made environments and have been associated with nutrient-rich substrates, in relation with specific traits of the fungi: ability to survive at low oxygen pressure, to tolerate high salt concentration and to degrade hydrocarbons. Nevertheless, the natural habitat of these fungi is still unknown. Recent experiments performed in our laboratory highlighted the Scedosporium capacity to grow on media containing the different components of lignocellulose as sole carbon source, suggesting lignocellulolytic properties.

Here we attempted to define the metabolic pathways involved in the degradation of lignocellulose in Scedosporium species, focusing on S. apiospermum and S. aurantiacum since genomic data were available only for these two species. A literature search was performed in order to identify enzymes involved in lignin degradation in other fungi and conserved sequences which were then used to screen Scedosporium genomic data by tBLASTn (Basic Local Alignment Search Tool) searches. 15 candidates were identified. qPCR experiments demonstrated that 8 of them exhibited an important increase in their expression level in media containing lignocellulose components. Moreover, similar results were obtained in presence of 4-hydroxybenzoate, suggesting the involvement of this pathway in adaptation of these fungi to polluted environments.

**Keywords:** Scedosporium, human, impacted areas, polluted environments, lignin degradation, 4, hydroxybenzoate.

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[*] Speaker
[†] Corresponding author: wilfried.poirier94@gmail.com

# Impact of repeated treatments of the pesticides chlorpyrifos or tebuconazole on soil microorganisms using alternative methods

Veronika Storck[1], Nadine Rouard[1], David Bru[1], Jeremie Beguet[1], Lucio Botteri[2], Ayme Spor[1], Marion Devers[1], and Fabrice Martin-Laurent*[1]

[1]Agroécologie – Institut National de la Recherche Agronomique : UMR1347 – INRA - 17 rue Sully - BP 86510 - 21000 Dijon, France
[2]AEIFORIA srl (AEIFORIA) – Fidenza, Italy

## Abstract

Pesticides can harm non-target organisms such as soil microorganisms involved in important ecosystem functions. In the European Union, during pesticide authorization process conducted by European Food Safety Authority (EFSA), potential risks on soil microorganisms are solely evaluated by estimating its impact on mineralization of nitrogen which is a too general test to detect pesticide ecotoxicity towards microorganisms. In consequence, pesticide risk assessment conclusions on soil microorganisms edited by EFSA can differ from academic researches carried out with more advanced techniques allowing to monitor the abundance, diversity and activity of microbial community. This is the case for the insecticide chlorpyrifos (CHL) and the fungicide tebuconazole (TCZ) studied for their microbial ecotoxicological impact using new experimental design and advanced techniques.
A new experimental design consisting in repeated exposure of soil microcosms to CHL or TCZ was used to evaluate (i) pesticide biodegradation, (ii) the impact on purification functions, and (iii) on the diversity of soil bacteria.

In response to repeated CHL treatments, the capability of soil microorganisms to degrade it increased. On the contrary, soil microorganisms kept a low ability to degrade TCZ. Soil purification functions were slightly (CHL) or not (TCZ) affected by pesticide treatments. However, the soil bacterial diversity changed in response to repeated exposure to both pesticides. *Nitrospira* (reducing effects) and *Lysobacter* (enhancing effects) were the two genera the most importantly modified in their abundance.
This study provides new insight in the fate and ecotoxicological impact of CHL and TCZ on soil microorganisms.

**Keywords:** pesticide, microorganisms, microbial ecotoxicology

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*Speaker

# Predictive assessment of the impact of biocidal substances on soil microbial respiration and nitrogen transformation

Anne Straczek*†1 and Béatrice Chion1

[1]French Agency for Food, Environment and Occupational Health  Safety. Regulated products assessment Department, – ANSES – 14 Rue Pierre et Marie Curie, 94 701 Maisons-Alfort Cedex, France

### Abstract

The purpose of the Biocidal Products Regulation (BPR) is to improve the free movement of biocidal products within the Union while ensuring a high level of protection of human, animal health and the environment. The approach is based on, first the approval of the active substance (inclusion in the Union list) and afterwards the authorisation of products that contain such active substances.

For the environment, the risk is mainly assessed for the aquatic and the terrestrial compartments. For each compartment, the predicted environmental concentration (PEC) is compared to the predicted no effect concentration (PNEC). The PNEC is derived from the lowest endpoint for the compartment taken into account and the appropriate assessment factor. Soil ecotoxicity endpoints can be provided for earthworms, plants and soil microorganisms. Ecotoxicity data for soil microorganisms are often coming from two OECD Tests Guidelines: Carbon (OECD 217) and Nitrogen (OECD 216) Transformation Tests.

These tests guidelines are also used for the risk assessment of the Plant Protection Products. Nevertheless, different requirements and interpretation of the results are applied for each regulation. These differences deal with the threshold of toxicity, the number of tested concentrations to derive the endpoints, the duration of the tests... These adaptations are either directly proposed in the tests guidelines, or have been included in the Guidance on the BPR (volume IV Environment, Part B).

This poster presents the OECD 216 and 217 guidelines and their adaptations for the BPR. Moreover, the case of the assessment of microbial biocidal substance is discussed.

**Keywords:** biocide product, soil, respiration, nitrogen transformation, OECD Guidelines for the Testing of Chemicals

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[*] Speaker

[†] Corresponding author: Anne.STRACZEK@anses.fr

# A pilot experimental installation to evaluate the efficiency of a pharmaceutical industry treatment plant and the resulting decrease in effluents toxicity to aquatic microbial biofilms

Vincent Tardy*†[1], Chloe Bonnineau[1], Agnès Bouchez[2], Christophe Rosy[1], Bernard Motte[1], Pierric Jeannin[3], and Stéphane Pesce[1]

[1]Irstea UR Milieux Aquatiques, Ecologie et Pollutions (UR MALY) – Irstea – 5 rue de la Doua BP 32108 F-69616 Villeurbanne Cedex, France
[2]UMR CARRTEL – Institut National de la Recherche Agronomique - INRA (FRANCE) – 75 av. de Corzent, 74203 Thonon-les-Bains, France
[3]SANOFI – SANOFI Recherche – route d'Avignon 30390 ARAMON, France

## Abstract

Pharmaceutical wastewaters are usually characterized by a complex mixture of active substances and metabolites with variable characteristics and composition throughout the year. Accordingly, assessing the efficiency of pharmaceutical industry treatment plants and the resulting decrease in effluent ecotoxicity and ecological risk throughout the treatment process remains very challenging.

Microbial biofilms have been proven to be early warning natural assemblages to detect acute and long-term effects produced by toxic substances, including pharmaceuticals. Being composed of both autotrophic and heterotrophic microorganisms exhibiting a large range of sensitivity to many toxicants, biofilms are thus relevant models to assess ecotoxicological risks of effluents by studying effects on microbial structure, diversity and functions.

In this context, in collaboration with the pharmaceutical firm SANOFI, we installed a pilot system, connected to the wastewater treatment plant of a SANOFI production factory, to evaluate, throughout the treatment steps, the decrease in chronic and acute toxicity of pharmaceutical production effluents for natural biofilms. The system consists of five artificial outdoor channels, each sub-divided into three compartments to ensure replication, continuously filled with wastewater collected after secondary, tertiary and quaternary treatments, respectively, as well as with stream water collected at the immediate upstream and downstream from the discharge. The chronic and acute toxicity of effluents on natural biofilms will be evaluated by combining structural and functional analysis as well as a pollution community tolerance (PICT) approach using pharmaceutical mixtures directly extracted from passive samplers (Polar Organic Chemical Integrative Samplers, POCIS) immersed for several weeks in the different channels.

**Keywords:** Ecotoxicological risk, Microbial diversity, Microbial functions, PICT

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[*] Speaker
[†] Corresponding author:

# Development of a new functional marker for $\beta$-triketone herbicides exposure in agricultural soils

Clémence Thiour Mauprivez*[1,2], Fabrice Martin-Laurent[2], Christophe Calvayrac[1], and Lise Barthelmebs†[1]

[1]Biocapteurs-Analyse-Environnement (BAE-LBBM) – Université de Perpignan Via Domitia : USR3579 – F-66860, Perpignan, France

[2]Agroécologie – Institut National de la Recherche Agronomique : UMRINRA 1347, Université Bourgogne Franche-Comté, Institut National Supérieur des Sciences Agronomiques, de lÁlimentation et de lÉnvironnement, Institut National Supérieur des Sciences Agronomiques, de l'Alimentation et de l'Environnement – 17 rue Sully - BP 86510 - 21000 Dijon, France

## Abstract

The $\beta$-triketone herbicides are maize selective herbicides that have been largely applied in replacement of atrazine, banned in Europe in 2004. Their mode of action lays on the inhibition of the p-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme of the carotenoid biosynthesis.

In recent studies, we showed that within the soil bacterial community, many microorganisms possess a functional HPPD enzyme involved in tyrosine metabolism. These "non-target organisms" harbor the target of the $\beta$-triketone herbicides and consequently may be affected in response to its exposure. From this point of view, the bacterial community harboring the *hppd* gene might be a relevant marker to assess the ecotoxicological impact of $\beta$-triketone herbicides on soil bacterial functional diversity.

Within this context, the objective of our work is to check for the interest of *hppd* bacterial community as a marker of exposition and/or of impact sensitive to $\beta$-triketone herbicides. This will require the development of a molecular tool box to assess the abundance, activity and diversity of the *hppd* bacterial community in various arable soils exposed to $\beta$-triketone herbicides. The abundance and the activity of the *hppd* bacterial community will be monitored from the nucleic acids extracted directly from soils, and the diversity of the *hppd* community will be evaluated by a metagenomic study based on the high-throughput sequencing of *hppd* amplicons.Our results will lead to the selection of a set of characteristic *hppd* sequences, allowing the development of *hppd* DNA chips to assess the ecotoxicological impact of $\beta$-triketone herbicides on soil microorganisms.

**Keywords:** $\beta$ triketone herbicides, impact, ecotoxicology, marker, soil

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RISK ASSESSEMENT
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*Speaker

†Corresponding author: barthelm@univ-perp.fr

# Biodiversity of an opportunistic pathogen in the water cycle

Florian Vautrin*[1]

[1]Laboratoire d'Ecologie Microbienne (LEM) – Université Claude Bernard - Lyon I (UCBL) – 16 rue Raphael Dubois, 69100 Villeurbanne, France

## Abstract

Rising urbanization waterproofs cities. To avoid flooding, many infrastructures have been set up to manage pluvial water flows, whose infiltration basins. Rainwater loads on dust and pollutants during its passage in the atmosphere and on the ground. These particles rich in pollutants such as PAH, PCB, pesticides... end up on the surface of the infiltration basin to form the so-called "urban sediments". This represents a new ecological niche for microorganisms, such as *Nocardia*, which are able to degrade these compounds and use it as carbon or nitrogen sources for its growth.
*Nocardia* is an actinomycete opportunistic pathogen witch causes nocardiosis, a granulomatous and suppurative infection and would affect about 1 million persons each year around the world. The target patients are mainly immunocompromised.

The aim of this study was to assess the impact of human activity on the enrichment and biodiversity of the pathogenic actinobacteria in anthropised environment such as infiltration basins.
The high rate of pollution in anthropised environment lead to a modification of the bacterial communities in enhancing the proliferation of some bacterial species, because of their ability to integrate pollutants in their metabolism. Urban sediments constitute a new ecological niche for some pathogenic species, and so concentrate these bacteria and increase the risk of their dissemination in the environment.

**Keywords:** Infiltration basin, biodiversity, bacterial community, opportunistic pathogen, urban pollution

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*Speaker

# Identication, isolation and purification of two toxic Cyanobacteria of Al-Mactaa lake (Mostaganem, west Algeria)

Aicha Zellal*†[1] and Sidi Mohammed El-Amine Abi-Yad[1]

[1]Laboratory of Aquaculture Bioremediation (AquaBior) – Department of Biotechnology (I.G.M.O campus). Faculty of Natural Sciences and Life, University of Oran, BP 1524, EL M'Naouer - 31000 Oran., Algeria

## Abstract

The frequent dominance of eutrophic freshwaters by cyanobacteria (blue-green algae) is of increasing concern because these organisms can produce several types of potent toxins (cyanotoxins). This paper presents the first data on the identication, isolation and purification of some cyanobacteria, collected from a eutrophic lac (Al-Mactaa, Mostaganem, west Algeria) from July to August 2014 and isolated strain cultivated under laboratory conditions. Images of the cyanobacteria specimens were subsequently analyzed and matched with cyanobacteria in authoritative references and literatures on cyanobacteria taxonomy. The result of the taxonomical analysis showed many different species of cyanobacteria like: Anabaena sp, phormidium-chalybeum, Spirulina sp, Oscillatoria sp, Calothrix sp, Microcystis sp, Chroococcus turgidus, Merismopedia sphagnicola, Aphanocapsa sp. Culture samples of cyanobacteria on the solid medium BG11 and the successive subcultures helped purify two toxic strains of cyanobacteria (Aphanocapsa sp and Anabaena sp), cells were harvested by centrifugation, frozen, freeze-dried and kept at -20◦C until further use.

**Keywords:** Cyanobacteria, Eutrophic lake, Aphanocapsa sp., Anabaena sp., West Algeria

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*Speaker

†Corresponding author: zellalaicha@yahoo.fr

# Interaction between dark-septate-endophytes and ectomycorrhizal fungi in the context of trace-element contaminated sites

Charlotte Berthelot[1], Julie Foulon[2], Thierry Béguiristain[1], Michel Chalot[2], Corinne Leyval[1], and Damien Blaudez*†[1]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – CNRS : UMR7360, Université de Lorraine – Faculté des Sciences et Technologies, Université de Lorraine F-54506 VANDOEUVRE-LES-NANCY, France

[2]Laboratoire Chrono-environnement (LCE) – Centre National de la Recherche Scientifique : UMR6249, Université de Franche-Comté – UFR Sciences et Techniques - 16, route de Gray - 25030 Besançon Cedex, France

## Abstract

Dark septate endophytes (DSE) are ascomycetous fungi that colonize living plant without causing apparent negative effects. They are characterized by melanized hyphae and microsclerotia within plant roots. High tolerance of DSE to metal pollution and their relatively high abundance in contaminated habitats suggest that DSE might have an important function for host survival in these conditions. Therefore, in the context of phytoremediation assisted by symbiotic fungi, we studied this group of fungi into more details. We first isolated seven DSE strains from poplar roots from metal-polluted sites. Fungal isolates were identified as members of the *Phialophora*, *Phialocephala*, *Cadophora* and *Leptodontidium* genera. A screening of the strains was performed to select the best promising symbiont. Their were characterized for their plant-growth-promoting abilities through different tests such as production of indol-3-acetic acid, release of volatile organic compounds or production of antifungal compounds. For the most promising strains, an inoculation experiment was performed to monitor the effect of the fungi on the growth of poplar and birch on metal-contaminated soils. Moreover, we also investigated the interaction between DSE and endomycorrhizal fungi through a dual-inoculation of host plant. Finally the use of DSE in the phytomanangement of metal-contaminated soils will be discussed.

**Keywords:** Phytomanagement, endophyte, trace, elements, plant growth promoting abilities

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[*] Speaker

[†] Corresponding author: damien.blaudez@univ-lorraine.fr

# Impacts of elevated ozone and temperature on wheat rhizosphere microbial communities

Frédérique Changey[1] , Matthieu Bagard[1], and Thomas Lerch[1*]

[1]Institut d'Ecologie et des Sciences Environnementales de Paris (IEES-Paris) – CNRS : UMR7618 – Université Paris-Est Créteil Val de Marne (UPEC) Faculté de sciences et technologie Bâtiment P 61, avenue du Général de Gaulle 94010 Créteil Cedex, France

## Abstract

Ozone (O3) has increased significantly in recent decades as the result of anthropogenic activities. This gas is responsible for reductions in crop yields and plant biomass production of forest and grassland ecosystems. Rhizosphere microorganisms are in close relation with plant roots. They feed organic compounds released by roots and, as a feed-back, they are involved in improvement of plant fitness. Little is known on the impact of elevated O3 on rhizosphere microbial communities. Our objectives were to access O3 and temperature impacts on wheat physiology, soil parameters and rhizosphere microbial communities. The experiment consisted on a 3 week fumigation with or without O3 (70ppb) of two different varieties of wheat (Premio and Soissons) at two temperatures (20◦C and 30◦C). Samples without plant were used as controls. The effect of O3 was measured on plant physiological parameters (biomass, CO2 assimilation...), on soil parameters (pH and DOC). Microbial communities' structure, abundance and functions were evaluated by T-RFLP, qPCR and MicroRespTM techniques. The results show a significant effect of ozone on plants (in particular by reducing their root biomass) in interaction with temperature (Premio was less resistant to O3 at 30◦C). Shifts in the microbial communities' structure were also observed under elevated ozone conditions, with a reduction of bacteria and increase of fungi. These changes were largely shaped by plant physiology and soil properties including pH and DOC. This study provides new insight into our understanding of the influence of O3 and temperature on the interaction between plants and soil microbial communities.

**Keywords:** Ozone, Temperature, Rhizosphere, microbial communities

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[*]Speaker

# Interactive impacts of silver and phosphorus on autotrophic biofilm elemental and biochemical quality for a macroinvertebrate consumer

Michaël Danger*[1], Alexandre Bec[2], Clément Crenier[1], Vincent Felten[1], Jessica Ferriol[3], Aridane Gonzalez[4], Fanny Perrière[2], Loïc Ten-Hage[3], and Joséphine Leflaive[3]

[1]Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) – Université de Lorraine, Centre National de la Recherche Scientifique : UMR7360 – 15 Avenue du Charmois, 54500 Vandoeuvre-lès-Nancy, France

[2]Laboratoire Microorganismes: Génome et Environnements, UMR CNRS 6023 (LMGE) – Université Clermont Auvergne – France

[3]Laboratoire Ecologie Fonctionnelle et Environnement (EcoLab) – CNRS : UMR5245, Observatoire Midi-Pyrénées, PRES Université de Toulouse, Université Paul Sabatier (UPS) - Toulouse III, Institut National Polytechnique de Toulouse - INPT – 118 Route de Narbonne 31062 Toulouse, France

[4]Institut Universitaire Européen de la Mer (IUEM) – CNRS : FR2195, Université de Bretagne Occidentale (UBO) – Technopôle Brest-Iroise, Place Copernic, 29280 Plouzané, France

## Abstract

Autotrophic biofilms are fundamental biological compartments of many aquatic ecosystems, representing notably a major resource for many invertebrate consumers. To date, most studies have tried to understand the impacts of stressors on microbial communities or on functional processes taking place into the biofilm mat. Far less studies investigated the indirect effects of stressors on upper trophic levels through alterations of the quality of biofilms. We investigated, through a laboratory study, the single and combined effects of phosphorus (P) availability and silver contamination on the elemental (C:N:P ratios) and biochemical (fatty acid profiles) compositions of a diatom-dominated biofilm. We hypothesized that 1) P would enhance the elemental quality while 2) P and silver, through the replacement of diatoms by more tolerant primary producer species, would reduce the biochemical quality of biofilms for their consumers. The quality of biofilms for consumers was assessed for a common crustacean species, *Gammarus fossarum*, by measuring organisms survival and growth rates. Results mainly showed that species replacement induced by both stressors affected biofilm fatty acid compositions, and that P immobilization permitted to achieve low C:P biofilms, whatever the level of silver contamination. Gammarids growth and survival were not significantly impacted by the ingestion of silver-contaminated resource. On the contrary, we found a significant positive relationship between the biofilm P-content and gammarids growth, while biofilm fatty acid contents were unrelated to this parameter. This study underlines the large indirect consequences stressors could play on basal resources quality for consumers, and, in turn, on the whole food web.

**Keywords:** Diatoms, fatty acids, ecological stoichiometry, Gammarus fossarum

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*Speaker

# Carbon nanotubes shape the bacterial communities associated with the diatom Nitzschia linearis

Yehia El-Temsah , Eric Pinelli[1], Laurent Verneuil[1], Jérôme Silvestre[1], Florence Mouchet[1], Laury Gauthier[1], and Maïalen Barret[*,†,1]

[1]Laboratoire Ecologie Fonctionnelle et Environnement (EcoLab) – CNRS : UMR5245, Observatoire Midi-Pyrénées, PRES Université de Toulouse, Université Paul Sabatier (UPS) - Toulouse III, Institut National Polytechnique de Toulouse - INPT – 118 Route de Narbonne 31062 Toulouse, France

## Abstract

Carbon nanotubes (CNTs) are nanomaterials with a wide range of current and developing applications. Their increasing use raises the question of their ecotoxicological impact. In aquatic ecosystems, diatoms account for more than 25% of primary productivity. Diatoms are intimately associated with bacterial communities in benthic biofilms. Diatoms-Bacteria interactions condition the carbon and nutrient cycling. Here, the impact of CNTs on a model ecosystem was assessed. This ecosystem was sampled in the Garonne river, simplified by cultural methods, and was finally constituted by the diatom *Nitzschia linearis* and a bacterial consortium. This model ecosystem was exposed to CNTs at 0, 0.1, 1 and 10 mg/L. After 6 days, cells were harvested either by filtration at 0.45 $\mu$m (to collect all bacteria and diatoms), or by filtration at 5$\mu$m (diatoms/"attached" bacteria) followed by filtration at 0.45 $\mu$m (planktonic bacteria). DNA was extracted from these filters to measure the abundance of bacteria (qPCR) and to determine the structure of bacterial communities (high-throughput sequencing). A negative effect of CNTs on the abundance of bacteria was evidenced at 50mg/L. Only the planktonic fraction was significantly affected. Besides, CNTs exposure modified the structure of bacterial communities in this planktonic fraction from 1 mg/L. While species affiliated to Bacteroidetes phylum were dominant in planktonic community of control incubations, they were strongly lower at 1 and 10 mg/L. On the contrary, the Proteobacteria phylum increased. These results demonstrate that CNTs impact the bacterial communities at relatively low concentration, with potential disruption of their functions in aquatic ecosystems.

**Keywords:** Diatoms, bacteria, nanoparticle, 16S rRNA, metabarcoding

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[*] Speaker

[†] Corresponding author: maialen.barret@ensat.fr

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# Potential use of a biopolymer extracted from marine waste as antifungal agent

Hassiba Laribi-Habchi*†1 and Ibtissem Galizia1

1Functional analysis laboratory of chemical processes – University of Blida 1, Algiers, Algeria

## Abstract

For a long time, agriculture across the world was dependent on pesticides. Today, it is influenced by a movement that favours practices that are more sustainable and environmentally safe. To meet these new demands, producers wishing to exploit natural resources and make them profitable must turn to new agronomic practices that combine culture performance and protection at a low environmental cost. In this context, the development of biological polymer able to protect our natural resources is a strategy that is attracting more attention. .Natural and healthy for the environment since it comes from the shells of shellfish, polymer of chitosan is recognized in several fields and areas of activity, especially for its antifungal properties. Currently used extensively in agriculture, it is possible to think that chitosan can also be used in forestry to meet several needs. This study helps to highlight the antifungal properties of chitosan at different concentrations against *Aspergillus Niger*. The results show a highly significant inhibition at dose of 1 g / l.

**Keywords:** Chitosan, Organic polymer, Environment

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*Speaker

†Corresponding author: larhassiba@yahoo.fr

# Gastrointestinal microbial community changes in Atlantic cod (Gadus morhua) exposed to crude oil

Daniela Pampanin*[1,2], Andrea Bagi†[1,2], Even Riiser[3], Hilde Molland[2], Bastiaan Star[3], Thomas Haverkamp[3], and Magne Sydnes[2]

[1]International Research Institute of Stavanger (IRIS-Environment) – N-4070, Stavanger, Norway
[2]Department of Mathematics and Natural Sciences – University of Stavanger, N-4036, Stavanger, Norway
[3]Centre for Ecological and Evolutionary Synthesis – Department of Biosciences, University of Oslo, PO Box 1066, Blindern, N-0316 Oslo,, Norway

## Abstract

Expansion of offshore oil exploration increases the risk of marine species being exposed to oil pollution in currently pristine areas. The adverse effects of oil exposure through toxic properties of polycyclic aromatic hydrocarbons have been well studied in Atlantic cod (*Gadus morhua*). Nevertheless, the fate of conjugated metabolites in the intestinal tract and their effect on the diversity of intestinal microbial community in fish is less understood. Here, we investigated the intestinal microbial community composition of Atlantic cod after 28 days of exposure to crude oil (concentration range 0.0-0.1 mg/L). Various evidence for altered microbial communities was found in fish exposed to high (0.1 mg/L) and medium (0.05 mg/L) levels of oil concentration. First, altered banding patterns were observed on denaturing gradient gel electrophoresis for samples pooled from each treatment group. Secondly, based on 16S rRNA sequences, higher levels of oil exposure were associated with a loss of overall diversity of the gut microbial communities. Furthermore, 8 operational taxonomic units (OTUs) were found to have significantly different relative abundances in samples from fishes exposed to high and medium oil concentrations when compared to samples from fishes exposed to low oil concentration or no oil. Among these, only one OTU, a Deferribacterales, had increased relative abundance in samples from fish exposed to high oil concentration. The results presented here contribute to a better understanding of the effects of oil contamination on the gut microbial community changes in fish and highlight the importance of further studies into the area of host-microbiome interactions.

**Keywords:** gut microbiota, Atlantic cod, oil contamination

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*Speaker

†Corresponding author: Andrea.Bagi@iris.no

# Participant list

| Name | Affiliation | Country | e-mails |
|---|---|---|---|
| Souhila Alouache | ENSSMAL | Algeria | alouache.enssmal@gmail.com |
| Beatrice Alpha-Bazin | Li2D | France | beatrice.alpha-bazin@cea.fr |
| Nathalie Amacker | Unine | Switzerland | nathalie.amacker@gmail.com |
| Pornanong Aramwit | Chulalongkorn Univ. | Thailand | aramwit@gmail.com |
| Maria Argudo | Girona Univ. | Spain | maria.argudo@udg.edu |
| Jean Armengaud | Li2D | France | jean.armengaud@cea.fr |
| Charlotte Arnal | Veolia | France | charlotte.arnal@veolia.com |
| Muhammad Arslan | UFZ | Germany | arsilan324@gmail.com |
| Joan Artigas | LMGE | France | joan.artigas_alejo@uca.fr |
| Andrea Bagi | IRIS | Norway | andrea.bagi@iris.no |
| Louise Barberis | LEHNA | France | louise.barberis@univ-lyon1.fr |
| Maïalen Barret | Ecolab | France | maialen.barret@ensat.fr |
| Diana Barros | CBMA | Portugal | dianamcbarros@gmail.com |
| Lise Barthelmebs | BAE | France | barthelm@univ-perp.fr |
| Isabelle Batisson | LMGE | France | isabelle.batisson@uca.fr |
| Patrick Baudy | Koblenz-Landau Univ | Germany | baudy@uni-landau.de |
| Christine Bazin | INSAVALOR | France | christine.bazin@provademse.com |
| Fabrice Bedin | APAVE | France | fabrice.bedin@apave.com |
| Aissa Belouatek | Abdelhamid Ibn Badis Univ. | Algeria | abelouatek@gmail.com |
| Annette Berard | EMMAH | France | annette.berard@inra.fr |
| Emmanuelle Bergeron | Ecologie Microbienne | France | emmanuelle.bergeron@univ-lyon1.fr |
| Cecile Bernard | MCAM | France | cbernard@mnhn.fr |
| Charlotte Berthelot | LIEC | France | charlotte.berthelot@univ-lorraine.fr |
| Patrick Billard | LIEC | France | patrick.billard@univ-lorraine.fr |
| Elise Billoir | LIEC | France | elise.billoir@univ-lorraine.fr |
| Julien Blaise | Eurofins | France | JulienBlaise@eurofins.com |
| Damien Blaudez | LIEC | France | damien.blaudez@univ-lorraine.fr |
| Anne-Laure Blieux | Welience | France | anne-laure.blieux@welience.com |
| Berta Bonet | GEES - Uob | UK | b.bonet@bham.ac.uk |
| Chloe Bonnineau | IRSTEA | France | chloe.bonnineau@irstea.fr |
| Philippe Bouchard | LMGE | France | philippe.bouchard@uca.fr |
| Agnes Bouchez | CARRTEL | France | agnes.bouchez@inra.fr |
| Emilie Bourgeois | Ecologie Microbienne | France | emilie.bourgeois@sfr.fr |
| Kristian Koefoed Brandt | University Of Copenhagen | Denmark | kkb@plen.ku.dk |
| Jean-François Briand | MAPIEM | France | briand@univ-tln.fr |
| Cecile Capdeville | Ecolab | France | cecile.capdeville1@univ-tlse3.fr |
| Louis Carles | Clermont Auvergne Univ. | France | louis.carles64@gmail.com |
| Fernanda Cassio | CBMA | Portugal | fcassio@bio.uminho.pt |
| Aurelie Cebron | LIEC | France | aurelie.cebron@univ-lorraine.fr |
| Virginie Chapon | CEA | France | virginie.chapon@cea.fr |
| Betty Chaumet | IRSTEA | France | betty.chaumet@irstea.fr |
| Giulia Cheloni | UNIGE | Switzerland | giulia.cheloni@unige.ch |
| Clement Coclet | PROTEE | France | clement.coclet@univ-tln.fr |
| Blanche Collin | CEREGE | France | collin@cerege.fr |
| Nathalie Coquille | IFREMER | France | nathalie.coquille@gmail.com |
| Natalia Corcoll | Gothenburg Univ. | Sweden | natalia.corcoll@gu.se |
| Benoit Cournoyer | Ecologie Microbienne | France | benoit.cournoyer@univ-lyon1.fr |
| Sophie Courtois | Suez | France | sophie.courtois@suez.com |
| Marc Crampon | BRGM | France | m.crampon@brgm.fr |
| Cristiana Cravo-Laureau | EEM | France | cristiana.cravo-laureau@univ-pau.fr |
| Aurélien Croze | Lyon Univ. | France | aurelien.croze@hotmail.fr |
| Olivier Crouzet | ECOSYS | France | olivier.crouzet@inra.fr |
| Armelle Crouzet | Agroécologie | France | armellecrouzet@gmail.com |
| Michael Danger | LIEC | France | michael.danger@univ-lorraine.fr |
| Marie-Laure Delignette-Muller | LBBE | France | ml.delignette@gmail.com |

| Name | Affiliation | Country | e-mails |
|---|---|---|---|
| Floriane Demailly | IRSTEA | France | floriane.demailly@irstea.fr |
| Clement Descarpentries | UCA | France | clement.descarpentries@gmail.com |
| Werner Desiante | Eawag | Switzerland | werner.desiante@eawag.ch |
| Anna Dupont | | | annaadupont@gmail.com |
| Valentin Dupraz | IFREMER | France | valentin.dupraz@ifremer.fr |
| Robert Duran | Pau Univ. | France | robert.duran@univ-pau.fr |
| Marie-Jose Durand | GEPEA | France | marie-jose.durand-thouand@univ-nantes.fr |
| Elizabeth Edwards | Toronto Univ. | Canada | elizabeth.edwards@utoronto.ca |
| Emilie Egea | FCS Rovaltain | France | e.egea@fcsrovaltain.org |
| Talaat El Sebai | NRC | Egypt | talaatelsebai@gmail.com |
| Martin Eriksson | Chalmers Univ. | Sweden | martin.eriksson@chalmers.se |
| Sonja Fagervold | Obs Banyuls | France | fagervold@obs-banyuls.fr |
| Andrea Fanesi | LIEC | France | andrea.fanesi@univ-lorraine.fr |
| Sabine Favre-Bonte | Ecologie Microbienne | France | sabine.favre-bonte@univ-lyon1.fr |
| Stacie Flood | Raleigh Univ. | USA | flood.stacie@epa.gov |
| Laurence Fraissinet-Tachet | Ecologie Microbienne | France | fraissin@univ-lyon1.fr |
| Anna Freixa | ICRA | Spain | afreixa@icra.cat |
| Nicolas Gallois | CEA | France | nicolas.gallois@cea.fr |
| Marion Garacci | ECOLAB | France | marion.garacci@etu.ensat.fr |
| Jeanne Garric | IRSTEA | France | jeanne.garric@irstea.fr |
| Philippe Garrigues | CNRS | France | philippe.garrigues@u-bordeaux.fr |
| Jean-François Ghiglione | MAPIEM | France | ghiglione@obs-banyuls.fr |
| Nicolas Grosjean | LIEC | France | nicolas.grosjean4@gmail.com |
| Abdulsamie Hanano | AECS | Syria | ashanano@aec.org.sy |
| Alain Hartmann | Agroécologie | France | alain.hartmann@inra.fr |
| Jennifer Hellal | BRGM | France | j.hellal@brgm.fr |
| Pascaline Herbelin | EDF R&D | France | pascaline.herbelin@edf.fr |
| Louis Hermon | BRGM | France | louis.hermon@etu.unistra.fr |
| Marina Hery | HSM | France | marina.hery@umontpellier.fr |
| Kevin Hidalgo | INRA | France | kevin.hidalgo@gmx.com |
| Anna Hua | GEPEA | France | anna.hua@univ-nantes.fr |
| Sabir Hussain | Government College Univ. | Pakistan | sabirghani@gmail.com |
| Pauline Jacob | Veolia | France | pauline.jacob@veolia.com |
| Pierric Jeannin | SANOFI | France | pierric.jeannin@sanofi.com |
| Dimitrios Karpouzas | Thessaly Univ. | Greece | dkarpouzas@bio.uth.gr |
| Ahmad Khodr | L'ORÉAL | France | akhodr@rd.loreal.com |
| Sandra Kim Tiam | MCAM | France | sandra.kim-tiam-fook-chong@mnhn.fr |
| Apostolos Koutsaftis | ADAMA Deutschland Gmbh | Germany | apostolos.koutsaftis@adama.com |
| Amandine Laffite | UNIGE | Switzerland | amandine.laffite@unige.ch |
| Hassiba Laribi-Habchi | Blida Univ. | Algeria | larhassiba@yahoo.fr |
| Elia Laroche | Montpellier Univ. | France | elia.laroche@umontpellier.fr |
| Floriane Larras | UFZ | Germany | floriane.larras@ufz.de |
| Martin Laviale | LIEC | France | martin.laviale.bio@gmail.com |
| Marie Le Jean | LIEC | France | marie.lejean@univ-lorraine.fr |
| Florian Lemmel | LIEC | France | florian.lemmel@univ-lorraine.fr |
| Thomas Lerch | IEES-Paris | France | thomas.lerch@u-pec.fr |
| Corinne Leyval | LIEC | France | corinne.leyval@univ-lorraine.fr |
| Emilie Lyautey | CARRTEL | France | emilie.lyautey@univ-smb.fr |
| Ayanleh Mahamoud-Ahmed | IRSTEA | France | ayanleh.mahamoud-ahmed@univ-smb.fr |
| Mohamed Maldani | Moulay Ismail Univ. | Morocco | maldani.mohamed@hotmail.com |
| Clarisse Mallet | LMGE | France | clarisse.mallet@uca.fr |
| Benjamin Marie | MCAM | France | benjamin.marie@mnhn.fr |
| Samantha Marshall | Syngenta | UK | samantha.marshall@syngenta.com |
| Fabrice Martin-Laurent | Agroécologie | France | fabrice.martin@inra.fr |
| Jean Martins | IGE | France | jean.martins@univ-grenoble-alpes.fr |
| Shiny Martis B | INSA | France | shiny.mb@gmail.com |
| Michael-Thomas Marx | BAYER | Germany | michaelthomas.marx@bayer.com |

| Name | Affiliation | Country | e-mails |
|---|---|---|---|
| Leïla Meistertzheim | LOMIC | France | dussud@obs-banyuls.fr |
| Caroline Michel | BRGM | France | c.michel@brgm.fr |
| Jean-Michel Monier | ENOVEO | France | jm.monier@enoveo.com |
| Soizic Morin | IRSTEA | France | soizic.morin@irstea.fr |
| Christian Mougin | INRA | France | christian.mougin@inra.fr |
| Sylvie Nazaret | Ecologie Microbienne | France | sylvie.nazaret@univ-lyon1.fr |
| Julie Neury-Ormanni | IRSTEA | France | julie.neury-ormanni@irstea.fr |
| Oluwatosin Oladipo | North-West Univ. | South Africa | tosin1oladipo@gmail.com |
| Rahma Omrani | Monastir Univ. | Tunisia | rahmaomrani-114@hotmail.fr |
| Akli Ouelhadj | Mouloud Mammeri Univ. | Algeria | ouelhadj_akli@hotmail.com |
| Rosa Padilla | Ecologie Microbienne | France | ropagui@hotmail.com |
| Frederic Palais | Solvay | Belgium | frederic.palais@solvay.com |
| Daniela Pampanin | IRIS-Environment | Norway | daniela.pampanin@iris.no |
| Claudia Pascoal | CBMA | Portugal | cpascoal@bio.uminho.pt |
| Olivier Perceval | AFB | France | olivier.perceval@afbiodiversite.fr |
| Stephane Pesce | Irstea | France | stephane.pesce@irstea.fr |
| Ines Petric | RBI | Croatia | ipetric@irb.hr |
| Wilfried Poirier | GEIHP | France | wilfried.poirier94@gmail.com |
| Thomas Pollet | INRA | France | thomas.pollet@vet-alfort.fr |
| Lorenzo Proia | ESA - ULB | Belgium | proialorenzo@hotmail.it |
| Edoardo Puglisi | UCSC | Italy | edoardo.puglisi@unicatt.it |
| Catherine Rafin | UCEIV | France | rafin@univ-littoral.fr |
| Rick Relyea | RPI | USA | relyer@rpi.edu |
| Brice Reoyo-Prats | CEFREM | France | brice.reoyo@univ-perp.fr |
| Cecile Revellin | Agroécologie | France | cecile.revellin@inra.fr |
| Agnes Richaume | Ecologie Microbienne | France | agnes.richaume@univ-lyon1.fr |
| Jennifer Rocca | Duke Univ | USA | jennifer.rocca@duke.edu |
| Francesco Romaniello | UCSC | Italy | francesco.romaniello@unicatt.it |
| Florent Rossi | LMGE | France | florent.rossi@univ-bpclermont.fr |
| Sergi Sabater | IRCA | Spain | pilar.eijo@udg.edu |
| Wilfried Sanchez | FCS Rovaltain | France | w.sanchez@fcsrovaltain.org |
| Catherine Santaella | LEMIRE | France | catherine.santaella@cea.fr |
| Mechthild Schmitt-Jansen | UFZ | Germany | mechthild.schmitt@ufz.de |
| Dorina Seitaj | Frontiers | Switzerland | dorina.seitaj@frontiersin.org |
| Claire Sergeant | CENBG | France | sergeant@cenbg.in2p3.fr |
| Marie Simonin | Duke University | USA | marie.simonin@duke.edu |
| Vera Slaveykova | Geneva Univ. | Switzerland | vera.slaveykova@unige.ch |
| Federica Spina | Turin Univ. | Italy | federica.spina@unito.it |
| Giulia Spini | UCSC | Italy | giulia.spini@unicatt.it, |
| Sabine Stachowski-Haberkorn | IFREMER | France | sabine.stachowski.haberkorn@ifremer.fr |
| Marianne Stenrød | NIBIO | Norway | marianne.stenrod@nibio.no |
| Anne Straczek | ANSES | France | anne.straczek@anses.fr |
| Jürg Oliver Straub | Hoffmann-La Roche | Switzerland | juerg.straub@roche.com |
| Rossana Sussarellu | IFREMER | France | rossana.sussarellu@ifremer.fr |
| Cyril Sweetlove | L'ORÉAL | France | csweetlove@rd.loreal.com |
| Vincent Tardy | Irstea | France | vincent.tardy@irstea.fr |
| Sasha Tetu | Macquarie Univ. | Australia | sasha.tetu@mq.edu.au |
| Clemence Thiour-Mauprivez | BAE-LBBM | France | thiourmauprivezclemence@gmail.com |
| Ahmed Tlili | Eawag | Suisse | ahmed.tlili@eawag.ch |
| Edward Topp | Agriculture And Agri-Food | Canada | ed.topp@agr.gc.ca |
| Florian Vautrin | Ecologie Microbienne | France | florianvautrin@gmail.com |
| Quentin Vincent | LSE-LIEC | France | quentin.vincent@univ-lorraine.fr |
| Stephane Vuilleumier | Strasbourg Univ. | France | eh_torabi@ut.ac.ir |
| Lukas Yvo Wick | UFZ | Germany | lukas.wick@ufz.de |
| Regina-Michaela Wittich | Frontiers | Switzerland | r.wittich@csic.es |
| Aicha Zellal | Aquabior | Algeria | zellalaicha@yahoo.fr |
| Jochen Zubrod | Koblenz-Landau Univ. | Germany | zubrod@uni-landau.de |