



7th Biofilm Workshop

20th-22nd June 2022

Organized by:

Soizic Morin
Nicolas Creusot
Mélissa Eon
Juliette Rosebery

INRAE

UR1454
ÉCOSYSTÈMES AQUATIQUES
& CHANGEMENTS GLOBAUX [EABX]

Abstract book

INRAE

ECOTOX



Observatoire Aquitain
OSU
des Sciences de l'Univers



université
de **BORDEAUX**

eau
GRAND SUD-OUEST
AGENCE DE L'EAU ADOUR-GARONNE

Oral communications

- Microorganisms from wastewater alter microbial composition and function in stream biofilms, Ahmed Tlili [et al.] **3**
- Metabolomics insight in the response and tolerance of periphytic biofilms to wastewater effluent, Nicolas Creusot [et al.] **4**
- A Metabolomic Approach to Determine the Effects of Cobalt on River Biofilms, Simon Colas [et al.] **5**
- Metabolomic and photosynthesis responses of freshwater periphyton to natural vs synthetic fungicides, Ilan Viemont-Lefevre [et al.] **6**
- Determining *in situ* periphyton quality responses to nutrient and pesticides via a fatty-acids approach, Anders K Nilsson [et al.] **7**
- The influence of photosynthetic bioaerosols on the dynamics of natural freshwater reservoirs - a model study using meta-metabolomics approach, Cyril Jousse [et al.] **8**
- Geosmin production in freshwater biofilms: a multi-scale study to disentangle driving factors in a global change scenario, Carmen Espinosa [et al.] **9**
- Effects of the interaction between nutrient concentration and DIN:SRP ratio on geosmin production by freshwater biofilms, Carmen Espinosa [et al.] **10**
- The metabolites of light: untargeted metabolomic approaches bring new clues to understand light-driven adaptation of intertidal mudflat biofilm, Caroline Doose [et al.] **11**
- Allelopathic interaction within aquatic phototrophic biofilms - a case study with filamentous green algae and diatoms, Joey Allen [et al.] **12**

Posters

- Metabolomic insight in the responses of stream biofilms to the herbicide diuron, Nicolas Creusot [et al.] **13**
- Characterization of the metabolomic response of freshwater biofilms to urban wastewater effluents, Mélissa Eon [et al.] **14**
- Potential role of aquatic macrophytes in the regulation of cyanobacterial blooms: Allelopathic approach, Samuel Maubert [et al.] **15**
- Periphyton communities under multiple stress - Detecting community shifts via DNA-Metabarcoding, Sophie Oster [et al.] **16**
- Linking glyphosate exposure to pollution induced community tolerance in periphyton - a microcosm study, Landry Ughetto [et al.] **17**

Microorganisms from wastewater alter microbial composition and function in stream biofilms

Ahmed Tlili¹, Louis Carles¹, Simon Wullschleger¹, Adriano Joss¹, Rik Eggen¹, Kristin Schirmer¹, Nele Schuwirth¹, Cristian Stamm¹

¹Eawag, Swiss Federal Institute of Aquatic Science and Technology - Dübendorf, Switzerland

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

Keywords: periphyton, prokaryotes, eukaryotes, DNA metabarcoding, pollution, induced community tolerance

Metabolomics insight in the response and tolerance of periphytic biofilms to wastewater effluent

Nicolas Creusot^{†1}, Louis Carles³, Mélissa Eon¹, Simon Wullschleger⁴, Soizic Morin¹, Christian Stamm⁴, Ahmed Tlili³

¹INRAE, UR EABX, 50 avenue de Verdun Gazinet, F-33612 Cestas, France

²Plateforme Bordeaux Metabolome, F-33140 Villenave d'Ornon, France

³Eawag, Environmental Toxicology department, 133 Uberlandstrasse, 8600 Dubendorf, Switzerland

⁴Eawag, Environmental Chemistry department, 133 Uberlandstrasse, 8600 Dubendorf, Switzerland

†Corresponding author: nicolas.creusot@inrae.fr

Abstract: Recent evidences highlighted the effect of urban effluent on the structure/functions of downstream periphytic communities associated to increased tolerance to chemical stress. Nevertheless, the molecular mechanisms of these responses remain poorly understood while usual descriptors provide a partial picture of the phenotype of the communities. In this context, this study aims to gain knowledge about the molecular/biochemical responses of periphyton under urban stress through the implementation of untargeted metabolomics approach. To do so, following exposure in indoor channels connected to an urban effluent, the metabolomic responses of the biofilms were characterized in parallel of structural and functional responses. First, no marked effects were noted on the photosynthesis, respiration, primary/secondary production, as well as on biomass whereas exposure to the raw effluent led to tolerance acquisition to further chemical stress. Then, the metabolomic profiles showed discrepancies between all the conditions demonstrating a clear effect of the effluent on the molecular phenotype of the biofilms. Moreover, the clear separation between raw and ultrafiltrated conditions highlighted the potential contribution of the microbes from the effluent in the response. Such pattern paralleled with the shift in microbial diversity. The influence of the microbes was further confirmed through HCA showing that control and ultrafiltrated conditions were clustered together. Further trend analysis revealed up/down regulation on several pathways. Finally, statistical comparison between the metabolomic profiles of raw vs ultrafiltrated conditions highlighted that only 11% down- and 13% up-regulated signals contributed to the tolerance of the biofilm. Overall, this study shows that the metabolomic response is more sensitive than usual descriptors through its ability to discriminate all the experimental conditions. Moreover, it shows that urban microbes contribute to these various molecular/biochemical responses. Further investigations are ongoing to confirm the identity of the candidate metabolites and pathways and discriminate their link to urban microbes vs chemicals.

A Metabolomic Approach to Determine the Effects of Cobalt on River Biofilms

Simon Colas^{† 1}, Benjamin Marie², Mathieu Milhe-Poutingon¹, Patrick Baldoni-Andrey³, Amiel Boullemant⁴, Claude Fortin⁵, Séverine Le Faucheur^{† 1}

¹ Université de Pau et des Pays de l'Adour, E2S-UPPA, CNRS, IPREM, Pau, France - Université de Pau et des Pays de l'Adour, E2S-UPPA, CNRS, IPREM, Pau, France

² UMR 7245 CNRS/MNHN Molécules de Communication et Adaptations des Micro-organismes, Muséum National d'Histoire Naturelle, Paris, France - UMR 7245 CNRS, France

³ TotalEnergies, Pole d'Etudes et de Recherche de Lacq, France - TotalEnergies, Pole d'Etudes et de Recherche de Lacq, France

⁴ Rio Tinto Closure, France - Rio Tinto Closure, France

⁵ Institut National de la Recherche Scientifique - Eau Terre Environnement, Québec, Canada

†Corresponding authors: simon.colas@univ-pau.fr; severine.le-faucheur@univ-pau.fr

Abstract: In the context of an energy transition, the mining of cobalt (Co) has been intensive over the past decade. As a consequence, an increase of its concentrations is observed in several aquatic environments, including rivers. The objective of this study is thus to examine Co effects on biofilms using untargeted metabolomics. For this purpose, mature biofilms colonized on glass slides were exposed to increasing Co concentrations (0, 10⁻⁷, 10⁻⁶, 5.10⁻⁶, 10⁻⁵ mol.L⁻¹) for 7 days in triplicate in outdoor microcosms (15 L) filled with water from the river Gave de Pau. Each day, water was sampled whereas biofilms were collected after 1, 3 and 7 days of exposure in order to analyze for total and intracellular Co, chlorophyll-a content, community structure and metabolomic. Accumulated Co was mainly intracellular, and very good correlations between log₁₀ concentrations of dissolved Co and intracellular Co concentrations were found at each exposure time (R²adj: 0.91, 0.95, 0.97 for days 1, 3 and 7, respectively). In total, 2117 metabolites were measured, and 159 were annotated. An important "microcosm" effect was noticed when comparing metabolites before and after exposure to microcosm conditions. An increase in lysodiacylglyceryltrimethyl homoserines (LDGTS), sphingosines and a decrease in flavonoids were observed in response to the increasing Co concentrations. An increase of LDGTS and sphingosines was also observed as a function of exposure time, as well as a decrease of lactones. Further biofilm analyses will determine the role of the biofilm composition on this modification of the metabolite composition.

Keywords: Biofilm, Cobalt, Accumulation, Metabolomic

Metabolomic and photosynthesis responses of freshwater periphyton to natural vs synthetic fungicides

Ilan Viemont-Lefevre¹, Mélissa Eon¹, Arthur Medina¹, Nicolas Creusot^{† 1}

¹ EABX - INRAE, France

[†] Corresponding author: nicolas.creusot@inrae.fr

Abstract: Agricultural activity leads to the release of thousands of compounds such as fungicides to aquatic ecosystems that might be harmful for exposed organisms such as periphytic microbial communities that play key role in ecosystem functions. Although previous studies have shown adverse effect of fungicides on the structure and functions of periphytic biofilms or their component, there is still a paucity of knowledge on short term molecular/biochemical mechanisms involved in such long term impairment on ecosystem function(s). This is strongly needed to support the discovery of biomarkers allowing the early and sensitive detection of the alteration of ecosystem function. To tackle this challenge, untargeted meta-metabolomics is an approach of choice since it provides a comprehensive picture of the biochemical activity and the phenotype (i.e. molecular phenotype through the identification of metabolites and their pathways) of a community, as a result of interactions with the environment. In this context, the present study aims to highlight the link between short-term response at the molecular level (metabolomics) and long term response at the function level (photosynthesis, microbial activity) of periphytic biofilms to the model fungicides copper (Cu, as natural fungicide) and tebuconazole (TBZ, as synthetic fungicide). To this end, short-term exposure (4h) to serial dilution of Cu and TBZ were first performed under controlled conditions (T°C 18°C, L/d 12:12). Following these exposure, photosynthetic yield using Phyto-PAM (as function) and metabolomics analysis were implemented. The results showed effect of copper on both photosynthetic activity and metabolic pattern whereas only metabolomics responded to tebuconazole. According this first experiment, two concentrations have been selected in order to further implement one month exposure to determine the kinetic of responses and their synchronism. This investigation will provide additional evidence about long-term effect on microbial activity.

Keywords: Biofilm, metabolomic, photosynthesis, copper, tebuconazole

Determining *in situ* periphyton quality responses to nutrient and pesticides via a fatty-acids approach

Anders K Nilsson¹, Andreas Håkansson², Maria Kahlert³, Thomas Backhaus⁴, Karin S.I. Johansson⁴, [Natàlia Corcoll](#)^{† 5}

¹ Section for Ophthalmology, Department of Clinical Neuroscience, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden

² Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

³ Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Sweden

⁴ Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

⁵ Gothenburg Global Biodiversity Centre, Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

† Corresponding author: natalia.corcoll@gu.se

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

Keywords: pesticides, periphyton, ecotoxicity, fatty acids

The influence of photosynthetic bioaerosols on the dynamics of natural freshwater reservoirs – a model study using meta-metabolomics approach

Cyril Jousse^{† 1,2,4}, Anne-Hélène Le Jeune³, Hermine Billard³, Céline Judon¹, Marie Lagrée², Guillaume Voyard¹, Fanny Noirmain³, Jean-Luc Baray⁴, Delphine Latour³, Pierre Amato^{1,4}

¹ Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut de Chimie de Clermont-Ferrand (ICCF), France

² Université Clermont Auvergne, INRAE, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont (PFEM), France

³ Université Clermont Auvergne, CNRS, Laboratoire Microorganismes : Génome et Environnement (LMGE), France

⁴ Université Clermont Auvergne, CNRS, Observatoire de Physique du Globe de Clermont-Ferrand (OPGC), France

†Corresponding author: cyril.jousse@uca.fr

Abstract: The atmosphere is a reservoir of a highly diverse microbial diversity, coming from many ground ecosystems. This compartment, considered as habitat for microflora, is the place of metabolic activities and the way to connect distant environments. Among bioaerosols, photosynthetic microorganisms (PSMO) are also involved in the colonization of various, and sometime fragile, landscapes. Thus, we hypothesized that their balance could be modified by such deposition. In our project we tried to i) observe PSMO contribution in wet depositions (rain and snowfalls) and ii) evaluate the effect of PSMO intakes on aquatic microbial dynamics. To do this, we harvested around 50 atmospheric samples (before and during COVID crisis). On each sample, physicochemical analyses have been performed as well as flow cytometry to evaluate the diversity and try to sort PSMOs. On few samples, 16S-18S amplifications permit to observe the phylogenetic diversity. In a second step, we performed an *in vitro* experiment with a consortium made of one non-axenic culture of microalgae (isolated from atmospheric samples) and one non-axenic culture of cyanobacteria *Microcystis aeruginosa*. At three time points, 4 samples have been harvested to evaluate the evolution of the system (using flow cytometry and ionic chromatography) and 12 samples have been submitted to metabolomics experiment (LC/HRMS profiling). After multivariate analyses (O/PLS-DA and ASCA), 56 features have been selected to identify the corresponding metabolites (amino and organic acids, carbohydrates and cyanotoxins). In another way, we try to merge metabolomics and cytometry data packages using multiblock methodology (MB-PLS). Our final goal is to highlight the combination of metabolic adaptations from each microbial player. Our project raised several questions about i) the ability to infer / validate the origin of the deposited microflora, and ii) the process to collect, store and analyze atmospheric microbes. This is also a first step towards meta-metabolomics experiments, in order to reveal subtle metabolic evolution in consortia - as the siege of silent struggles.

Geosmin production in freshwater biofilms: a multi-scale study to disentangle driving factors in a global change scenario.

Carmen Espinosa^{1,2}, Meritxell Abril², Laia Llenas², Marc Ordeix³, Lidia Vendrell², Lorenzo Proia²

¹ Aigües de Vic, Spain

² BETA Tech Center, TECNIO Network, University of Vic-Central University of Catalonia, Spain

³ Center for the Study of Mediterranean Rivers, University of Vic - Central University of Catalonia, Spain

Abstract: Geosmin is a microbially-produced metabolite responsible for earthy and musty odor in surface waters, causing important issues to drinking waters facilities because detected by humans at very low concentrations. Cyanobacteria are the most important geosmin-producing microorganisms and its lifestyle include planktonic and benthic species growing within biofilm communities. This talk presents the results of different studies aiming to disentangle the drivers of geosmin production within biofilm communities. A microcosms experiment was focused on the effects of nutrient concentrations and nitrogen to phosphorus ratio (N:P) on geosmin production and release to the water, whereas a mesocosm approach, in experimental flumes, was used to understand the role of water flow and light availability in the biofilm intracellular geosmin production. The main hypotheses and conclusions reached with controlled experiments have been contrasted against the results obtained through the field monitoring carried out during 3 years at 5 sampling sites along the Ter River catchment (NE Spain). All these studies identified the cyanobacteria *Oscillatoria* sp as the dominant geosmin producer in the Ter River. The microcosm experiment confirmed that geosmin production and release is favored by decreased N:P, especially under higher nutrient concentrations. This result was confirmed by the field monitoring in which the concentration of geosmin in water proportionally increased while the N:P was gradually decreasing. The mesocosm experiments evidenced that lower water flows are optimal to favor the growth of *Oscillatoria* sp. in river biofilms triggering the intracellular production of geosmin, especially under lower light conditions. The long-term field monitoring results agreed with these conclusions and highlighted that geosmin episodes in the Ter River are influenced by the hydrological regime being the year with the lowest water flow when the highest geosmin concentrations were detected. Overall, these studies identified the set of co-occurring events needed to trigger geosmin episodes in fluvial ecosystems.

Keywords: Geosmin, biofilms, nutrients, flow, global change.

Effects of the interaction between nutrient concentration and DIN:SRP ratio on geosmin production by freshwater biofilms

Carmen Espinosa^{1,2}, Meritxell Abril¹, Laia Llenas¹, Marc Ordeix³, Lidia Vendrell¹, Lorenzo Proia¹

¹ BETA Tech Center, TECNIO Network, University of Vic-Central University of Catalonia, Crtra de Roda, 70, 08500 Vic, Spain

² Aigües de Vic, C/ de la Riera, 6 - 08500 Vic, Spain

³ Center for the Study of Mediterranean Rivers, University of Vic - Central University of Catalonia, Passeig del Ter, 08560, Manlleu, Spain

Abstract: The global increase of cyanobacterial blooms occurrence has been associated with the presence of compounds that generate earthy and musty odor in freshwater systems, among which geosmin stands out. The lack of information on the factors associated to geosmin production by benthic organisms has driven the development of this study, whose main goal is to determine the effects of nutrient concentration and DIN:SRP ratio on geosmin formation and release.

The experiment presented in this talk was performed in 18 microcosms under controlled conditions for 21 days, using a natural biofilm suspension from Ter river (NE, Spain) to promote biofilm settlement. Six treatments were set crossing three DIN:SRP ratios (A = 4:1, B = 16:1 and C = 64:1) with two nutrient concentrations (Low and High). During the experiment, various structural and functional attributes of the biofilm were evaluated. After 7 days of experiment, geosmin was detected in biofilm, being higher under high nutrient concentration and low DIN:SRP ratio conditions. In this treatment, geosmin in biofilm reached its maximum concentration at day 16 (3.8 ± 0.9 ng/mg), decreasing at the end of the experiment (21d) due to cyanobacteria detachment and geosmin release into the water (136 ± 6 ng/L). Overall, this experimental study showed that high nutrient concentration and low DIN:SRP ratio favored the *Oscillatoria* genus development within biofilm communities, generating the optimal conditions for geosmin production. The interaction between these two factors was demonstrated to be a potential driver of benthic geosmin production and release, and should be monitored and controlled in rivers exploited for drinking water purposes.

Keywords: Geosmin, biofilms, nutrient concentration, DIN:SRP ratio, microcosms.

The metabolites of light: untargeted metabolomic approaches bring new clues to understand light-driven adaptation of intertidal mudflat biofilm.

Caroline Doose¹, Cédric Hubas^{† 1}

¹ Biologie des Organismes et Ecosystèmes Aquatiques – Muséum National d’Histoire Naturelle (MNHN), France

[†]Corresponding author: cedric.hubas@mnhn.fr

Abstract: The microphytobenthos (MPB) inhabiting marine sediments is a microbial community of primary producers playing a key role in shallow coastal environments and estuarine ecosystem functioning. The diatoms, brown microalgae usually dominating the MPB, can phase their behavior and metabolism to the strong intertidal light variability through non-photochemical quenching (NPQ) by dissipating the excess of light energy physiologically through the xanthophyll cycle, or by vertically migrating in the sediment.

These light-adaptation mechanisms trigger a lot of scientific interest with numerous studies in literature, but the biological processes and metabolic mechanisms involved in light-driven vertical migration remain largely unknown. Untargeted metabolomic methods could bring new research avenues to better understand their mechanisms and possible implications in others cellular metabolic pathways. In this work we exposed a migratory biofilm of mudflat for 30 min to a light gradient of photosynthetically active radiation (PAR) from 50 to 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. To explore the global metabolic responses to the irradiances and identify compounds playing significant roles in the MPB light adaptation, we applied an untargeted metabolomic analysis method previously adapted for MPB in our laboratory. After bioinformatic treatment and filtering, 35 compounds were found in CHCl_3 fractions and 72 in MeOH fractions. The samples exposed to HL (500, 750 and 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) were essentially characterized by alka(e)nes. These molecules could protect MPB organisms from the excess of light by two mechanisms depending on the taxa. In microalgae and various algal genera, n-alkanes biosynthesis is presumed to be linked to photosynthetic membranes where the decarbonylation is catalyzed by the recently described photoenzyme fatty acid photodecarboxylase (FAP). In cyanobacteria, the presence of these hydrocarbons in the thylakoid membranes could regulate the redox balance under the oxidative stress induced by the overwhelmed photosynthesis.

Keywords: microphytobenthos, mudflat biofilms, light, adaptation, untargeted metabolomic analysis

Allelopathic interaction within aquatic phototrophic biofilms – a case study with filamentous green algae and diatoms

Joey Allen¹, Joséphine Leflaive²

¹ Ecosystèmes, biodiversité, évolution [Rennes] – Centre National de la Recherche Scientifique : UMR6553, Observatoire des Sciences de l'Univers de Rennes, Institut Ecologie et Environnement, Université de Rennes 1, France

² Laboratoire Ecologie Fonctionnelle et Environnement (EcoLab) – CNRS : UMR5245, Université Paul Sabatier (UPS) - Toulouse III – 118 Route de Narbonne 31062 Toulouse, France

Abstract: Phototrophic biofilms are hotspots of microbial activity in many aquatic ecosystems. This leads to an intense competition between the organisms that promotes species producing allelopathic compounds to exclude competitors. Therefore, many species isolated from biofilms produce one or several allelopathic compounds. However, a question that remains is the consequences of the production of allelopathic compounds for biofilm functioning. We investigated allelopathic interactions between *Uronema confervicolum*, a filamentous green alga isolated from river biofilms and diatoms isolated from the same environment. Algal biomass extracts and culture filtrates have been used to identify potential effects on diatom growth and adhesion properties and to identify the involved allelopathic compounds. The effects of environmental factors (nutrient limitation, light and gas supply) and growth phase on the production and release of the different allelopathic compounds have also been studied. Finally, we performed an in-depth study of the effect of anti-adhesion allelochemicals on diatoms. Two polyunsaturated fatty acids have been identified as allelopathic compounds affecting growth and photosynthesis while other unidentified compounds were able to inhibit the adhesion of diatoms. The regulation of allelopathic compounds productions did not follow classical plant defence theories, with a production enhanced by light but also by nutrients concentration. We identified a strong inhibition of diatom extracellular polymeric substances matrix by allelopathic compounds. Based on these results, we will discuss the implications of allelopathy for biofilm functions and the way to further understand allelopathy effects on aquatic ecosystems.

Keywords: Allelopathy, filamentous green algae, diatoms, fatty acids

Metabolomic insight in the responses of stream biofilms to the herbicide diuron

Nicolas Creusot^{1,2}, Betty Chaumet¹, Mélissa Eon¹, Nicolas Mazzella¹, Soizic Morin¹

¹ INRAE, UR EABX, 50 avenue de Verdun Gazinet, F-33612 Cestas, France

² Plateforme Bordeaux Metabolome, F-33140 Villenave d'Ornon, France

Abstract: Regarding the increasing aquatic pollution, there is a need to establish the causality link between the exposure to chemicals and the effect(s) at the ecosystem level. To tackle this challenge, metabolomic allows the simultaneous characterization of all exposures and induced effects on cellular biomolecules (i.e. molecular phenotype). Also, periphytic biofilms are increasingly used because of their relevance to investigate the impact of multiple environmental stressors at the community level (function and structure).

This study aims to investigate the metabolomics response of periphytic communities to a model herbicide in parallel of more usual functional (photosynthesis) and structural descriptors. To this end, stream biofilms colonized at a reference site were exposed to the diuron - a model compound for photosynthesis inhibition- at the laboratory under different controlled conditions. The metabolomics responses were assessed through an untargeted approach by using an UPLC-ToF system

The results revealed the down regulation of two plant-specific fatty acids, the docosapentaenoic acid and the eicosapentaenoic acid in parallel of the inhibition of the photosynthetic activity. These are two omega-3 fatty acids that play a major in the sustainability of the trophic chain and so eco-systemic services since they are difficult to produce by higher trophic level (i.e. high metabolic cost). Overall, this study highlighted the relevance of untargeted metabolomic approach to improve knowledge about biochemica alteration associated to impairment of ecological function towards the discovery of ecosystem biomarkers.

Characterization of the metabolomic response of freshwater biofilms to urban wastewater effluents

Mélissa Eon¹, Manon Karolkowski¹, Adeline Arini², Soizic Morin¹, Nicolas Creusot¹

¹ Ecosystèmes aquatiques et changements globaux - Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement : UR1454, France

² UMR EPOC - Université de Bordeaux, France

Abstract: Urban wastewater treatment plants (WWTPs) release large amounts of pollutants (e.g. chemicals, microbes) into aquatic environments that may impact exposed organisms. Among these organisms, aquatic biofilms, as a complex assemblage of microorganisms with a short life cycle (e.g. microalgae, bacteria) and by their key role in aquatic ecosystems (e.g. primary production), are relevant and increasingly used to investigate the impact of chemical contamination at the community level. Despite increasing knowledge on the impact of chemical stress on these communities, the underlying (molecular/biochemical) mechanisms remain poorly described while usual descriptors provide a partial picture of the phenotype. To tackle this issue, untargeted metabolomics approach is relevant through the simultaneous characterization of chemical exposure and the global response of the whole biofilm. In this context, this study focuses on the characterization of the potential impact of urban WWTPs on aquatic periphytic microbial communities by implementing an untargeted metabolomic approach. To do so, biofilm colonization/exposure was carried out during 4 weeks at upstream and downstream sites from three WWTPs along the main tributary of Arcachon Bay (Belin Béliet: upstream, Salles: intermediate; Mios: downstream). First, multivariate analyses (PCA and HCA) showed discrepancies in the metabolomics profile between the three WWTPs and between upstream and downstream site of each WWTP. This difference is more marked at the downstream site (Mios), likely because of increasing disturbances along the tributary (e.g. detection of pesticides only on the downstream site). Moreover, the strong discrimination between upstream and downstream sites at Mios suggested that this WWTP is a source of pollutants. At this site, further univariate analysis combined to pathways analysis allowed preliminary annotation (i.e. based only on MS1) that highlighted a potential impact on the respiration and photosynthesis pathways. Overall, this study confirms the relevance of untargeted metabolomics to highlight potential impact of urban discharges on aquatic microbial communities.

Keywords: Metabolomic, Biofilm

Potential role of aquatic macrophytes in the regulation of cyanobacterial blooms: Allelopathic approach

Samuel Maubert¹, Juliette Rosebery¹, Mélissa Eon¹, Sylvia Moreira¹, Nicolas Creusot¹

¹ Ecosystèmes aquatiques et changements globaux – Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement : UR1454, France

Abstract: In a context of global change, the Aquitaine lake of Parentis-Biscarrosse (France) is subject to a double issue: on the one hand, the proliferation of two species of invasive aquatic macrophytes (*E. densa* and *L. major*) and, on the other hand, the upsurge of cyanobacteria blooms. Invasive macrophytes have a high growth rate and a very efficient vegetative reproduction. Recently, modern chemical approaches have shown that this reproductive success could be explained by the secretion of allelopathic compounds which affect the environmental communities. Moreover, studies have also shown that cyanobacteria would be the most sensitive algal group to this kind of interactions.

The objective of this study is therefore to characterize by a non-targeted metabolomic approach, the effect of allelopathic compounds produced by *E. densa* and *L. major* on the toxigenic cyanobacteria *M. aeruginosa*. For this purpose, an experiment was carried out by putting in contact this strain of cyanobacteria and extracts of *E. densa* and *L. major*. The exposure took place in microcosms (glass balloons), during 96 hours and in a thermoregulated chamber. In a first step, measurements of chlorophyll concentration and photosynthetic yield were performed every day using a PhytoPAM. In a second step, the allelopathic relationships between our organisms were investigated through non-targeted metabolomic analyses. The results obtained indicate an inhibition of the photosynthetic yield of *M. aeruginosa* for macrophyte extract concentrations of 10g/L. The first results of the metabolomic analyses show a significant discrimination of our conditions, which suggests that there is a sensitivity of the *M. aeruginosa* metabolome to macrophyte extracts. Currently, the metabolomic analyses are still in progress.

Keywords: Macrophytes, Cyanobacteria, Allelopathy, Metabolomic, Regulation

Periphyton communities under multiple stress – Detecting community shifts via DNA-Metabarcoding

Sophie Oster¹, Mugilvannan Sivagnanam¹, Verena C. Schreiner¹, Sabine Filker², Mirco Bundschuh^{1,3}

¹ Institute of Environmental Sciences, University of Koblenz-Landau, Germany

² Department of Molecular Ecology, University of Kaiserslautern, Germany

³ Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Sweden

Abstract: Assessing the effects of multiple stressors in surface waters is currently an important topic in ecotoxicology research. Multiple stress may include combinations of chemicals or excessive input of nutrients, hydromorphological degradation, temperature increase or salinisation. Due to the increasing effect complexity associated with the occurrence of multiple stressors, e. g. as a result of global climate change, a better understanding of the interactive effects of stressors on communities and their responses is required. In freshwater systems, periphyton communities contribute to primary production, represent an important food source for grazing animals and host a large biodiversity including bacteria and algae. Thus, chemical stressors, such as antibiotics or herbicides, can directly interfere with these communities. Species turnover in the periphyton community composition (horizontal interaction) could in turn indirectly affect the food webs depending on primary production and grazers (vertical interaction). The aim of our study, therefore, was to investigate the combined effects of chemical stress and nutrient enrichment on the community composition of freshwater periphyton. Using artificial indoor streams as microcosms, we set up experiments by colonising natural periphyton on ceramic tiles and exposed them to four different concentrations of the antibiotic ciprofloxacin and the herbicide propyzamide (i.e., chemical stress; 0, 1, 10, 100 and 1000 µg/L), as well as nutrient poor or nutrient rich media (i.e., nutrient stress; modified Borgmann and KUHL, respectively). As periphyton communities underlie seasonal changes, we performed two experiments during winter and two during summer. Via DNA-Metabarcoding, we evaluated the species composition of microeukaryotes and prokaryotes within chronically exposed periphyton (14 days). The data is currently being analysed and the results will be the focus of this poster.

Keywords: Biofilms Multiple Stress Metabarcoding

Linking glyphosate exposure to pollution induced community tolerance in periphyton – a microcosm study

Landry Ughetto¹, Sarah Chéron^{2,3}, Martin Laviale^{2,3}, Chloé Bonneau¹

1 INRAE, UR RiverLy, Centre de Lyon-Villeurbanne - INRAE, France

2 Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC) - Centre National de la Recherche Scientifique, Université de Lorraine - Site Charmois : 15 Avenue du Charmois, F-54500 VANDOEUVRE-LES-NANCY ; Site Aiguillettes : Faculté des Sciences et Technologies, F-54506 VANDOEUVRE-LES-NANCY ; Site Bridoux : Campus Bridoux - Rue du Général Delestraint, F-57070 METZ ; Site Thionville : IUT de Thionville-Yutz - Espace Cormontaigne, F-57970 THIONVILLE-YUTZ, France

3 Zone Atelier du Bassin de la Moselle [LTSER France] (ZAM) - Centre National de la Recherche Scientifique, Université de Lorraine - F-57000 Metz, France

Abstract: Glyphosate is the herbicide most frequently detected in freshwaters at concentrations up to several $\mu\text{g/L}$ (Carles et al. 2019) and can negatively affect aquatic ecosystems. In particular, microbial structure of periphyton can be modified by exposure to environmental concentrations of glyphosate ($\sim 10 \mu\text{g/L}$, Pesce et al. 2009). In addition to these structural changes, chronic exposure to a pesticide is likely to induce an increase in microbial tolerance to this pesticide, following the PICT (Pollution induced community tolerance) theory. Thus, preliminary studies have shown a positive correlation between microbial tolerance to glyphosate and glyphosate concentrations in the river La Cleurie (France). Nevertheless, other contaminants could also have influenced glyphosate tolerance. In this context, our study aims to demonstrate the causal relationship between glyphosate exposure and microbial tolerance to glyphosate in controlled conditions. To do so, periphyton sampled from upstream of La Cleurie was exposed to a gradient of glyphosate (0,1 to 150 $\mu\text{g/L}$) in artificial channels. After 2, 4 & 6 weeks of exposure, periphyton tolerance to glyphosate and to AMPA, the main degradation product of glyphosate, was determined. After the first 2 weeks of exposure, preliminary results show a lower microbial tolerance to glyphosate in periphyton exposed to low chronic concentrations (0,1; 0.5 $\mu\text{g/L}$) than in control but very few effects in periphyton exposed to higher concentrations. The analysis of microbial responses after 4 & 6 weeks of exposure would complete these first results and contribute to better describe the dynamic of the acquisition of tolerance to glyphosate in periphyton.

Keywords: Microbial ecotoxicology, biofilm, PICT, herbicide, AMPA, photosynthesis