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Multiomic approach to characterize the link between the structural biodiversity and the microbial activity along the natural evolution of periphytic biofilm

MICROBIOMIQ

BIOSEFAIR Metaprogram

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Aquatic periphytic microbial communities are complex assemblages of microorganisms (algae, bacteria, fungi, cyanobacteria, protozoa) that form biofilms more or less attached to submerged surfaces (inert or living) in most aquatic ecosystems. These communities play a major role in ecosystem functioning and in maintaining ecosystem services (*e.g.,* primary production, biogeochemical cycles).

In the context of global change, one of the major challenges for environmental and human health is to better understand how these communities respond to multiple pressures in terms of acclimation, adaptation, and resilience, in order to preserve the associated ecosystem functions and services. In particular, in the face of chemical contamination of these ecosystems, a current challenge is to determine how “natural” fluctuations in structural biodiversity and microbial functioning, linked to environmental conditions, modulate these communities’ response to chemical stress.

New so-called “omics” analytical methods and their combination open new perspectives in microbial eco(toxico)logy to address these knowledge gaps.

MICROBIOMIQ aimed to better understand the link between microbial activity (i.e., the metabolome), structural biodiversity (species and gene diversity through metagenomics), and sensitivity to chemical stress in periphytic biofilms, in connection with “natural” changes in the composition and functioning of these communities driven by environmental conditions. By implementing an approach combining metagenomics and meta-metabolomics on these biofilms, the project focused on the modulation of photosynthesis, a key function performed by these communities and strongly involved in biogeochemical cycles and primary production.

More specifically, MICROBIOMIQ aimed to answer the following questions:

Q1. What is the relationship between temporal changes in biodiversity, microbial activity, and photosynthetic function within aquatic periphytic communities under natural conditions?
Q2. What are the consequences of these natural changes on the sensitivity of these communities to chemical stress?
Q3. What is the relationship between structural and functional changes in the modulation of photosynthetic activity under chemical pressure in controlled conditions?

To achieve this, MICROBIOMIQ combined *in situ* investigations (longitudinal monitoring over 15 months) and laboratory experiments (monthly acute exposures) based on a multidisciplinary approach integrating metagenomics, meta-metabolomics, measurements of photosynthetic activity, and physicochemical characterization of the environments. The combination of these methods made it possible to assess in an innovative and precise way the interrelationships between changes in environmental factors, taxonomic diversity, microbial activity, and photosynthetic function, as well as their sensitivity to chemical pressure under natural and controlled conditions, supported by bioinformatic approaches.

The project’s results improve our understanding of the link between natural changes in microbial biodiversity and the functioning of aquatic periphytic communities, as well as their sensitivity to chemical pressure. This new knowledge is an essential prerequisite for establishing models capable of predicting, in the context of global change, the evolution of ecosystem functions and associated services based on biodiversity descriptors (eDNA) and/or metabolomic descriptors. All the metadata collected in this project may subsequently support the development of management tools within water quality biomonitoring approaches.

Methodology

To answer Q1 to Q3 questions, two tasks were implemented.

Task 1 aimed to address Q1 and Q2. It consisted of a longitudinal monitoring study conducted in the Gazinet pond over 15 months (12 months effectively sampled between March 2022 and August 2023). The taxonomic structure, microbial activity, and photosynthesis of periphyton (one measurement per month) were monitored alongside water physicochemical parameters and meteorological conditions.

Each month, a portion of the collected periphyton was exposed to a model herbicide (terbuthylazine) for 4 hours in order to determine the community's sensitivity at the molecular/biochemical level (metabolomics) and at the physiology/function level (photosynthetic yield).

Task 2 aimed to address Q3. A 4-week exposure experiment (July 2024) was conducted in artificial channels using periphyton collected from the same pond (June 2023). Taxonomic structure, microbial activity, and periphyton photosynthetic activity were measured at the initial time point and then after 24 hours, 72 hours, 1, 2, 3, and 4 weeks, alongside monitoring of water physicochemistry.

Results

About **task1**, a bioinformatic pipeline has been established for the combined analysis of metabolomic and metagenomic data (QIIME II, Phyloseq, Vegan, MixOmics, HillR), largely based on the approach of Shaffer et al. (2022). Application of this pipeline to our datasets revealed a strong abundance of the phylum Proteobacteria throughout the year. On the metabolite level, the class of amino acids and peptides (NPC_pathway) was the most abundant.

Alpha (within-sample) and beta (between-sample) diversity based on Hill metrics (q0: taxon richness = presence/absence; q1: Shannon entropy = diversity of typical taxa; q2: Inverse Simpson = diversity of dominant taxa) were then determined (Chao et al., 2014). Despite variations observed throughout the year in alpha diversity for both taxa and metabolites, only the diversity of typical and dominant taxa showed a significant decrease over the course of the study (Figure 1).

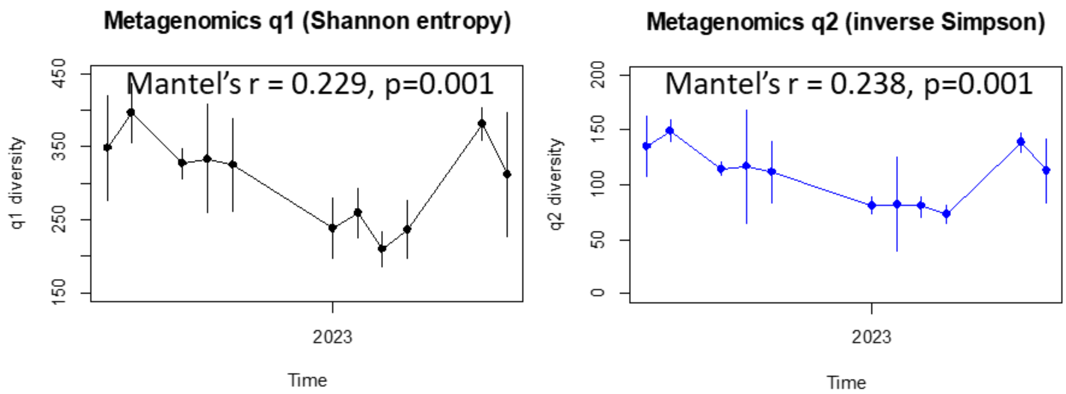

Figure 1. Annual fluctuation of alpha diversity (Hill indices q1 and q2))

No correlation was found between taxonomic diversity and metabolic diversity, indicating a certain asynchrony between structural biodiversity and functional biodiversity (Q1). However, taxonomic alpha diversity was correlated with temperature variations: the diversity of all taxa (presence/absence) decreased with temperature, whereas the diversity of typical and dominant taxa increased with temperature.

Regarding beta diversity, seasonal variations with occasional drifts were observed for taxonomic beta diversity, whereas microbial activity showed longer-term variations (Figure 2).

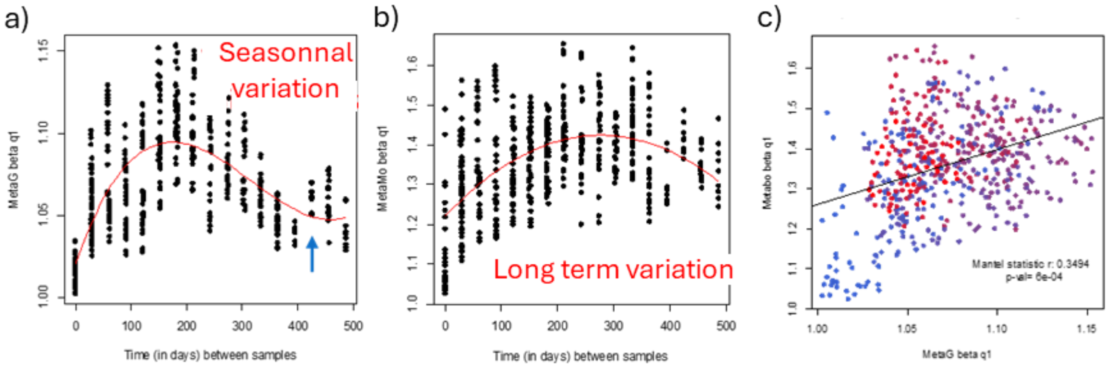

Figure 2. Variation in beta diversity (Hill q1, typical taxa) for taxa (a) and metabolites (b), and their correlation (c)

In contrast to alpha diversity, taxonomic and metabolite beta diversity showed weak but significant correlations (Figure 2c), even after correction for temporal autocorrelation, highlighting the importance of taxon abundance in shaping the periphyton meta-metabolome.

Implementation of a DIABLO multi-block approach revealed a predominant role of temperature in taxonomic structuring, whereas microbial activity appeared to be influenced by a greater number of factors, particularly nutrients (SO_4^{2-}, NO_3^-) (Q1).

Alongside these dynamics in community structure and functioning, a change in sensitivity was observed at the metabolome level, whereas the sensitivity of photosynthetic function remained relatively stable (Medina et al., 2024; Figure 3) (Q2).

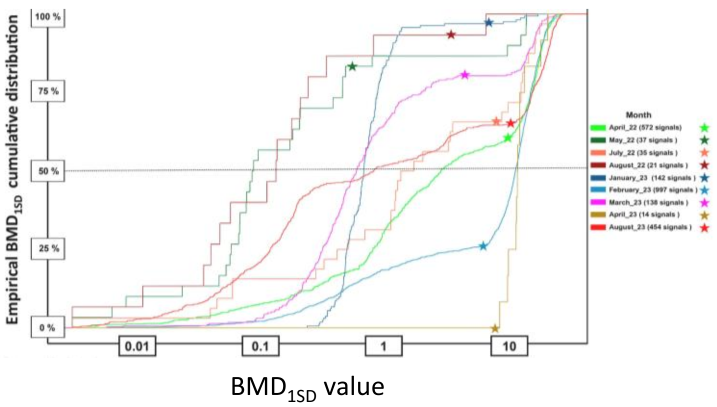

Figure 3. Comparison of sensitivity (BMD1SD) of the meta-metabolome (aggregated response presented as an empirical cumulative distribution) and photosynthesis (stars) between April 2022 and August 2023 (Medina et al., 2024).

These investigations revealed strong heterogeneity in the metabolomic response to terbuthylazine in terms of the number of metabolites, response profiles, and metabolite classes, suggesting that toxicity pathways may vary over time (data not shown, Medina et al 2024). In comparison, the small yet significant variations in photosynthetic sensitivity raise broader questions regarding the interpretability of molecular/biochemical status assessments in terms of actual physiological and functional consequences for the community. As it stands, these results suggest that similar physiological responses may arise from diverse molecular pathways.

These findings led us to further investigate which internal factors (annual dynamics of basal metabolism and physiological status) and external factors (environmental parameters) may contribute to this fluctuation in sensitivity. To this end, a DIABLO multi-block analysis was conducted (Figure 4, from Medina et al., in preparation).

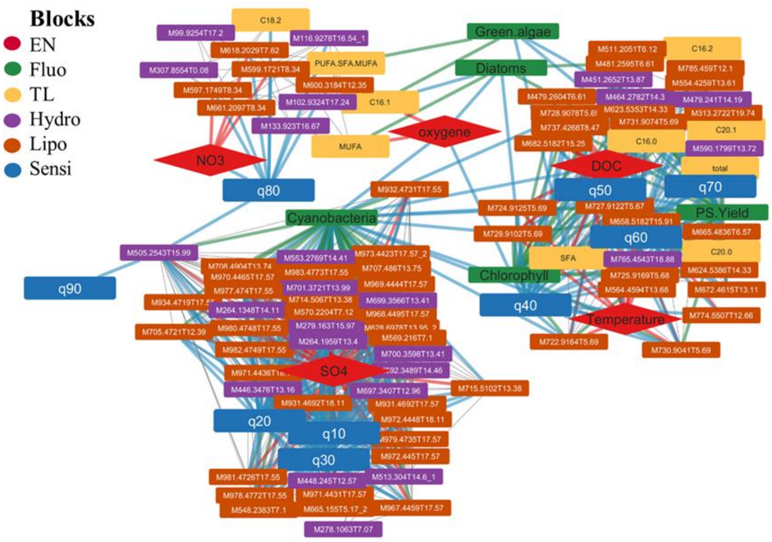

Figure 4. Correlation network (multi-block sPLS-DA, DIABLO analysis) of the basal dynamics of the periphyton meta-metabolome in relation to environmental variations. The network was constructed using the variables that contributed most within each of the seven blocks, with correlations greater than 0.7 (Pearson linear correlation coefficient). The correlation edges were colored according to the blocks they connect: Hydro/Lipo/TL (black), EN (red), fluo (green), sensi (blue).

This analysis confirmed the influence of certain environmental factors on metabolome dynamics. It notably showed that metabolome sensitivity could be described at three levels — high (q10–q30), medium (q40–q70), and low (q80–q90) — each closely associated with one or more physicochemical parameters (SO_4^{2-}, temperature and DOC, NO_3^-). These results highlight the need to pursue further research to refine our understanding of basal dynamics and micropollutant responses in periphyton communities influenced by multiple environmental factors.

Task 2 was implemented in July 2023. However, bacterial contamination occurred around the third week of exposure, limiting full exploitation of the acquired data (confirmed by metagenomic analyses). The results are currently being analyzed to determine whether this contamination can be considered an additional stress factor or whether analyses should be restricted to the first 14 days of exposure.

Overall, the MICROBIOMIQ project enabled the acquisition of substantial knowledge to better understand fluctuations in the structural and functional biodiversity of periphyton in response to their environment, particularly chemical stress. These investigations highlighted an asynchrony between taxonomic and metabolic diversity, reflecting phenomena of functional redundancy (structure changes but functioning remains stable) and phenotypic plasticity (functioning changes but structure remains stable). The differences observed between meta-metabolome and photosynthesis responses are consistent with this interpretation.

Valorisation

Publications

Arthur Medina, Melissa Eon, Nicolas Mazzella, Chloé Bonnineau, Débora Millan-Navarro, Aurelie Moreira, Soizic Morin, Nicolas Creusot. Sensitivity shift of the meta-metabolome and photosynthesis to the chemical stress in periphyton between months along one year and a half period: Case study of a terbuthylazine exposure,Science of The Total Environment, Volume 957, 2024, 177681, ISSN 0048-9697. https://doi.org/10.1016/j.scitotenv.2024.177681.

Communications orales :

Arthur Medina, Mélissa Eon, Débora Millan-Navarro, Nicolas Mazzella, Nicolas Creusot (2024). *Variation de sensibilité du métabolome et du rendement photosynthétique de biofilms periphytiques exposés à un herbicide modèle.* Presented at : 8ème colloque du réseau INRAE, La Rochelle, France (2024-11-13 - 2024-10-15), https://hal.inrae.fr/hal-04734503

Medina Arthur, Eon Melissa, Débora Millan-Navarro, Mazzela-Di-Bosco Nicolas, Creusot Nicolas. Seasonal fluctuation of metabolomic and photosynthetic yield response of in situ freshwater biofilms exposed to a model herbicide. *SETAC Europe 33 Annual Meeting*, SETAC EUROPE, Apr 2023, DUBLIN, Ireland. ⟨hal-04129911⟩

Posters

Nicolas Creusot, Arthur Medina, Alidou Sana, Mélissa Eon, Zoé Delporte, Josep Valls Fonayet, Chloé Bonnineau, Erwan Guichoux, Soizic Morin, Olivier Lepais (2024). *Microbiome-Metabolome association approach to unravel the relationship between taxonomic and functional biodiversity in freshwater periphyton facing global change.* Presented at : 20th annuel conference of the Metabolomics Society, Osaka (JP), Japon (2024-06-16 - 2024-06-20), https://hal.inrae.fr/hal-04734680

Arthur Medina, Mélissa Eon, Débora Millan-Navarro, Nicolas Mazzella, Nicolas Creusot. Annual variation of the meta-metabolome and photosynthesis sensitivities and their relationship in freshwater microbial communities exposed to a model herbicide. *16èmes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique*, Jun 2024, Saint-malo, France. ⟨hal-04768319⟩

Arthur Medina, Mélissa Eon, Nicolas Creusot. Metabolomics insight on the photosynthesis impairment under chemical stress in freshwater biofilms in agriculture context. *L'école automnale du RFMF*, Oct 2023, Sainte-Foy-lès-Lyon, France. ⟨hal-04768456⟩