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

Arthur Medina, Mélissa Eon, Débora Millan-Navarro, Nicolas Mazzella, Nicolas Creusot

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Arthur MEDINA[1], Mélissa EON[1], Débora Millan-Navarro[1], Nicolas MAZZELLA[1], Nicolas CREUSOT[1][2]

[1] INRAE, UR EABX, 50 avenue de Verdun, F-33612 Gazinet Cestas Cedex, France [2] Plateforme Bordeaux Metabolome, F-33140 Villenave d'Ornon, France

Introduction

- In the context of increasing aquatic chemical pollution, the study of microbial communities such as periphytic biofilms (Fig 1) improves the ecological dimension of biomonitoring [1].
- Despite a growing knowledge on biofilms, there is a **paucity of information about the seasonal fluctuation of their sensitivity** to chemical stress [2].
- If classical endpoints often lack of sensitivity and focus only on one component of the biofilm (e.g. autotroph organisms) [2], **untargeted metabolomics** can provide a comprehensive and sensitive picture of the **molecular response prior physiological/functional responses** [3].

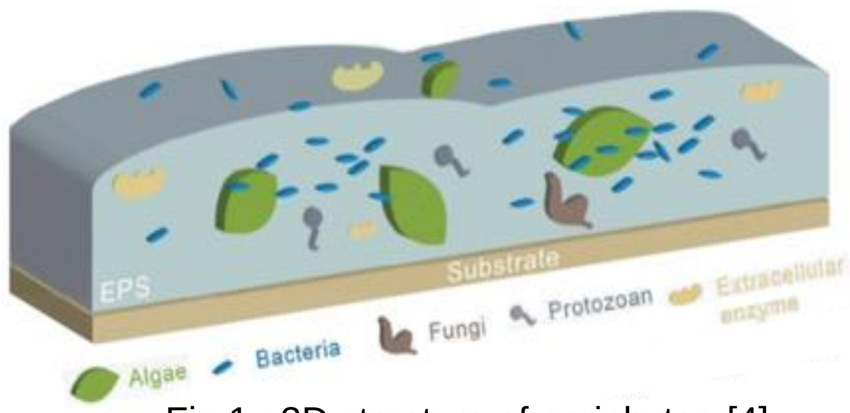
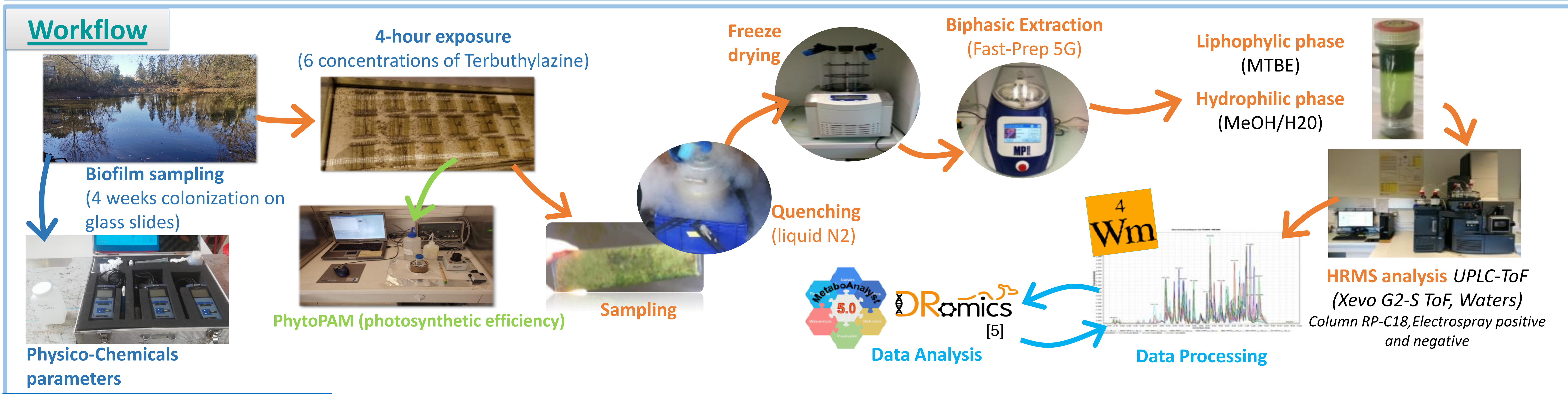


Fig 1 : 3D structure of periphyton [4]

Aim

In this context, the present study aims **to characterize the changes of sensitivity** of freshwater periphyton **over months** through the combined measurement of the **photosynthetic yield** (ΦPSII) and the **metabolomics response** based on high-resolution mass spectrometry (HRMS).

Workflow



Results and Discussion

① Physico-chemicals parameters

Month	Temperature (°C)	pH	Conductivity (µS/cm²)	Disolved oxygene (mg/L)	DOC (ppm)	DP (mg/L)	VDP (%)	Concentration in [NH4] mg/L	Concentration in [Na] mg/L	Concentration in [K] mg/L	Concentration in [Ca] mg/L	Concentration in [Mg] mg/L	Concentration in [NO2] mg/L	Concentration in [NO3] mg/L	Concentration in [PO4] mg/L	Concentration in [SO4] mg/L	Concentration in [Cl] mg/L
April	20	7.5	234	5.5	9.7	9.3	79.6	0.6	10.8	3.6	28.9	2.7	0.0	0.1	0.0	6.3	18.7
May	23.45	7.4	243	6.8	12.4	3.7	91.9	<LQ	11.5	4.0	32.3	2.8	0.3	0.1	<LQ	3.9	20.1

Fig 2 : Pond physico-chemical parameters, Dissolved Organic Carbon (DOC), Dissolved Particules (DP), Volatil Dissolved Particules (VDP)

- Low fluctuations of physico-chemical parameters between months, except for the temperature with an increase of 3.4°C
- Higher concentration of some nutrients with the decrease of water level

Benchmark dose (**BMD_{1sd}**) : statistical reference point using a level of change compare to a control using benchmark response composed by mean control response and z factor of residual standard deviation (sd) [6]

② Photosynthetic responses

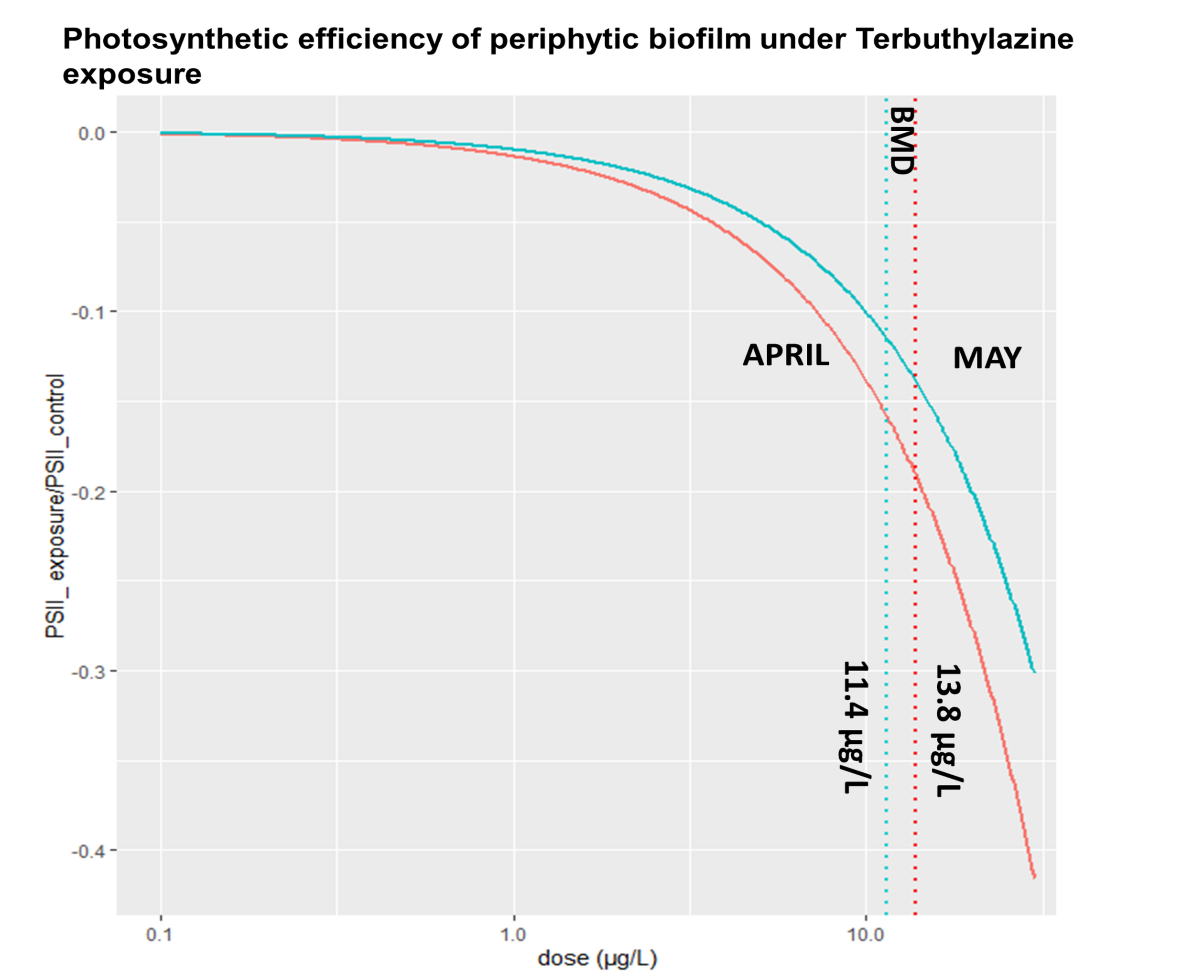


Fig 3 : Quadratic trend regression of photosynthetic inhibition under Terbuthylazine exposure (FDR < 0.05, Dose fitted, Log dose-scale, 10 fold, z =1, Confidence interval bootstrap 1E5 BMD_{1sd} April [8.2,25.3] and BMD_{1sd} May [6.8;17.8] [7])

- **BMD_{1sd}** ΦPSII vary around 10 µg/L
- Similar trends of photosynthetic inhibition between months

③ Metabolomic responses

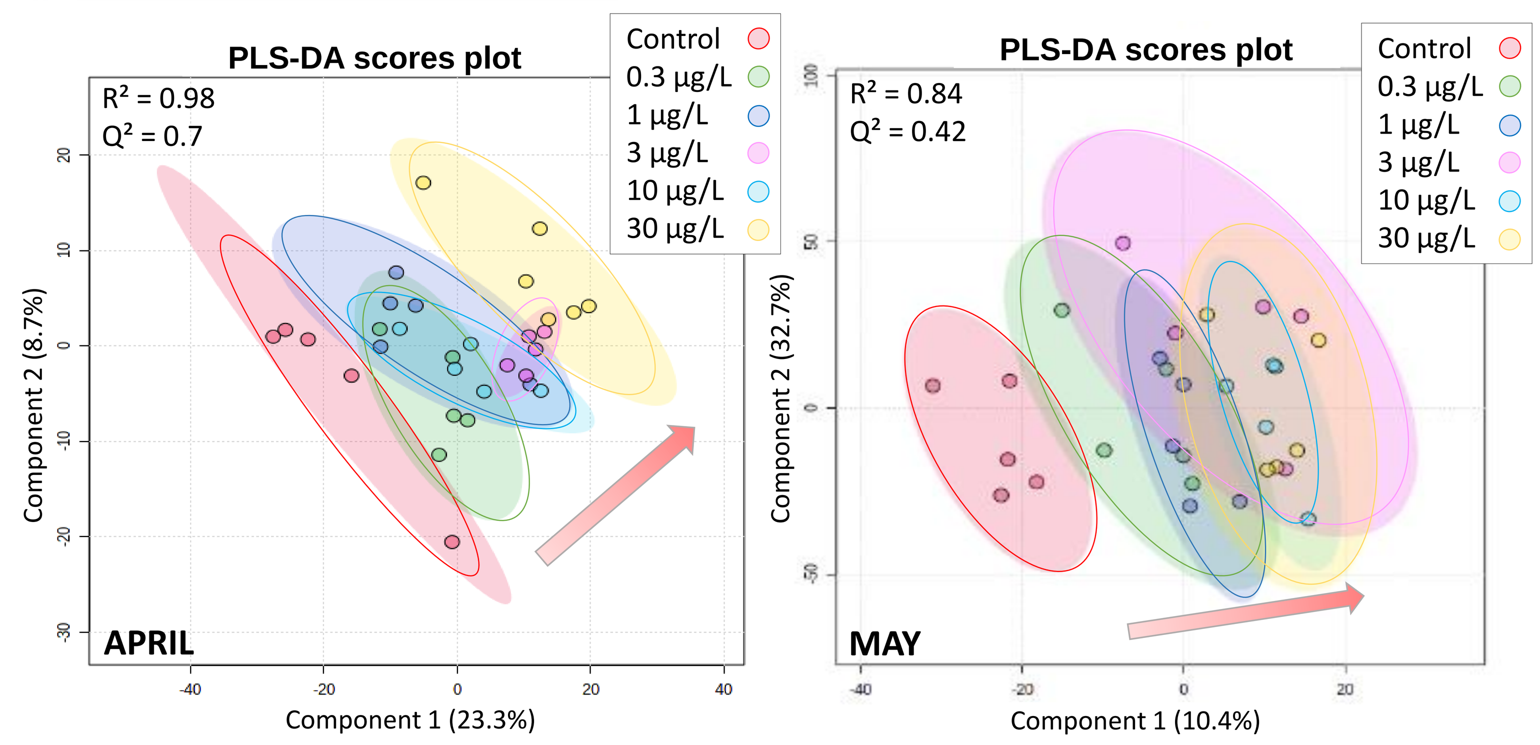


Fig 4 : PLS-DA plot with exposure data from 0 to 30 µg/L of Terbuthylazine herbicide

A) Discriminant analysis of metabolomic fingerprint using score plot

- Discrimination between **control and exposed** biofilm
- **Similar dose response trends** in both month

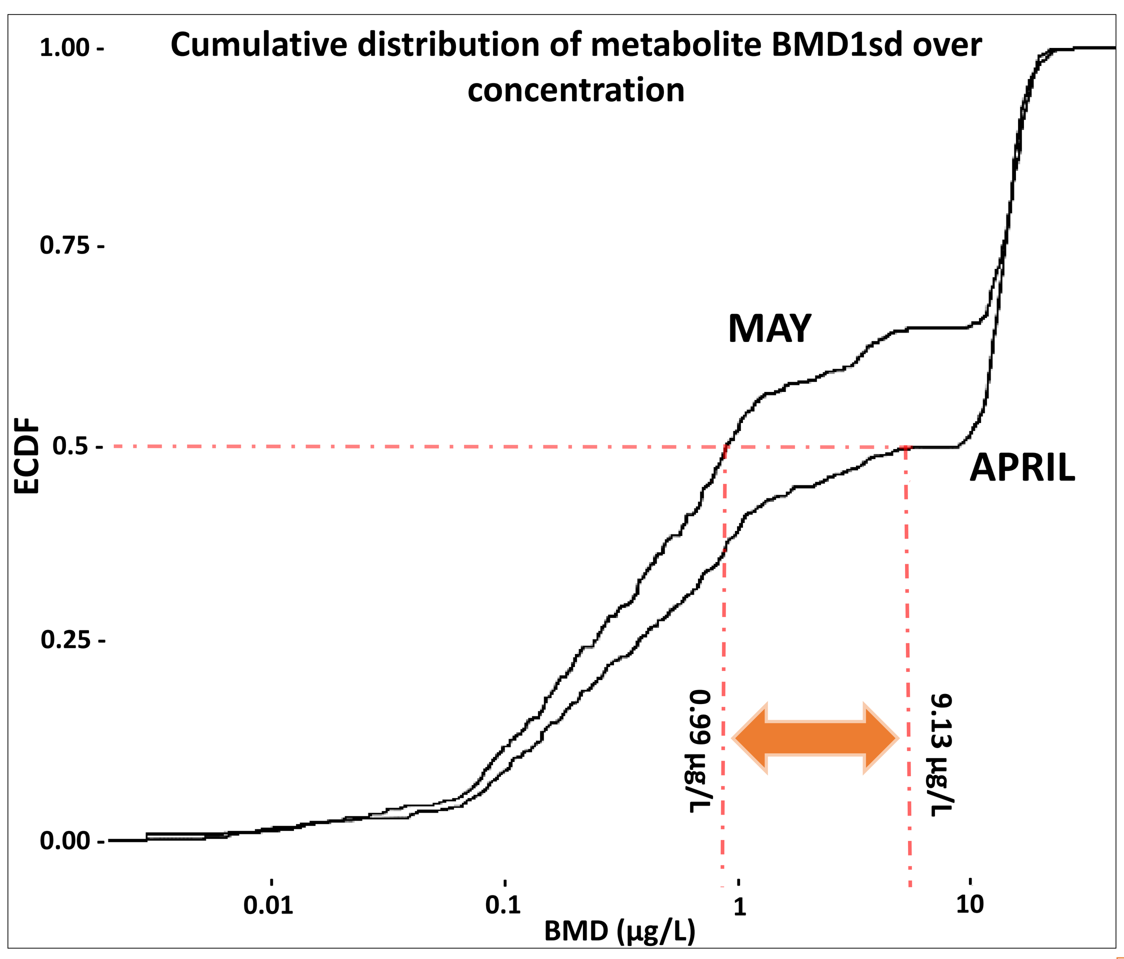


Fig 5 : BMD_{1sd} metabolite distribution over-dose (from hydrophilic fraction, and features observed in positive ionization, data log-2 transformed, FDR < 0.05, Log dose-scale, 10 fold, z =1)

B) Agregated metabolomic responses for April and May using DRomics

- Metabolome started to react at low concentration of Terbuthylazine (i.e. 0.1 µg/L)
- Reaction of 50% of metabolites BMD_{1sd} under 10 µg/L
- **Difference of sensitivity between months** (10-fold change)

Major Outcome

- ✓ **Low fluctuations** of environmental parameters between the two months
- ✓ **Between month sensitivity shift** of metabolomic responses under chemical stress
- ✓ This work highlight **higher sensitivity from metabolomic** at low concentration

Next step

- ✓ **These investigations will be prolonged along the year in order provide insight on the influence of initial environmental parameters on sensitivity to chemical stress**
- ✓ **Further identification of metabolites and pathways** that are **sensitive to fluctuation of environmental conditions will support biomarkers discovery**
- ✓ **Additionnal metagenomic analyses** will highlight natural taxonomic shift according environmental conditions.

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Centre Nouvelle Aquitaine Bordeaux
50 avenue de Verdun, Gazinet
F-33612 Cestas Cedex
Tél. : + 33 (0)5 57 89 08 00
arthur.medina@inrae.fr

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