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

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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. autofluorophores) [2], untargeted metabolomics can provide a comprehensive and sensitive picture of the molecular response prior physiological/functional responses [3].

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 (OPSIll) and the metabolomics response based on high-resolution mass spectrometry (HRMS).

Workflow

- Biofilm sampling (4 weeks colonization on glass slides)
- Physico-Chemical parameters
- 4-hour exposure (6 concentrations of Terbutylazine)
- Quenching (liquid N2)
- Freeze drying
- Biphasic Extraction (Fast-Prep 5G)
- Liphophilic phase (MTBE)
- Hydrophilic phase (MeOH/H2O)
- Sampling
- PhytoPAM (photosynthetic efficiency)
- Data Processing
- Data Analysis
- Component 1 (23.3%)

Results and Discussion

1) Physico-chemicals parameters

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature [°C]</th>
<th>pH</th>
<th>Conductivity [µS/cm²]</th>
<th>Dissolved oxygen [mg/L]</th>
<th>DOC [ppm]</th>
<th>DP [mg/L]</th>
<th>VDP [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>20</td>
<td>7.5</td>
<td>284</td>
<td>5.5</td>
<td>9.7</td>
<td>9.3</td>
<td>76.6</td>
</tr>
<tr>
<td>May</td>
<td>21.6</td>
<td>7.4</td>
<td>243</td>
<td>6.8</td>
<td>12.4</td>
<td>3.7</td>
<td>55.9</td>
</tr>
</tbody>
</table>

Fig 2: Pond physico-chemical parameters, Dissolved Organic Carbon (DOC), Dissolved Particles (DP), Volatile Dissolved Particles (VOP)

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

Benchmark dose (BMD<sub>16</sub>): 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

- Photophysiological efficiency of periphytic biofilm under Terbutylazine exposure

![Fig 3: Quadratic trend regression of photosynthetic inhibition under Terbutylazine exposure (DOC < 0.05, Dose fitted, Log dose scale, 10 fold, 1-4)](image)

- BMD<sub>16</sub> OPPSIll vary around 10 µg/L
- Similar trends of photosynthetic inhibition between months

3) Metabolomic responses

- PLS-DA scores plot
- PLS-DA scores plot

![Fig 4: PLS-DA plot with exposure data from 0 to 30 µg/L of Terbutylazine herbicide](image)

- BMD<sub>16</sub> for metabolome response over-dose (from hydrophilic fraction, and features observed in positive ionization, data log2 transformed, fold > 0.5 log dose scale, 10 fold, z = 1)

- BMD<sub>16</sub> for metabolite distribution over-dose

![Fig 5: BMD<sub>16</sub> response over-dose](image)

- Discriminant analysis of metabolomic fingerprint using score plot
- Discrimination between control and exposed biofilm
- Similar dose response trends in both month

- Metabolome started to react at low concentration of Terbutylazine (i.e. 0.1 µg/L)
- Reaction of 50% of metabolites BMD<sub>16</sub> 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

These investigations will be prolonged along the year in order provide insight on the influence of initial environmental parameters on sensitivity to chemical stress

Next step

- Further identification of metabolites and pathways that are sensitive to fluctuation of environmental conditions will support biomarkers discovery
- Additional metageneomic analyses will highlight natural taxonomic shift according environmental conditions.

Acknowledgments

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