



HAL
open science

Introducing High Resolution Mass Spectrometry in the Environmental Metabolic Footprinting metabolomics approach; application on extracted natural herbicide “Myrigalone A”

Hikmat Ghosson, Rahul Suryawanshi, Delphine Raviglione, Marie-Virginie Salvia, Cedric Bertrand

► To cite this version:

Hikmat Ghosson, Rahul Suryawanshi, Delphine Raviglione, Marie-Virginie Salvia, Cedric Bertrand. Introducing High Resolution Mass Spectrometry in the Environmental Metabolic Footprinting metabolomics approach; application on extracted natural herbicide “Myrigalone A”. Natural Products & Biocontrol 2018 (Biocontrol2018), Sep 2018, Perpignan, France. , Biocontrol2018 Abstract Book, P53, pp.90, 2018, Session 5: Evaluation and methods dedicated to the analysis of biocontrol natural products and of their environmental impact. 10.13140/RG.2.2.15025.02407 . hal-02712543

HAL Id: hal-02712543

<https://hal.science/hal-02712543>

Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Introducing High Resolution Mass Spectrometry in the Environmental Metabolic Footprinting metabolomics approach; application on extracted natural herbicide "Myrigalone A"

Hikmat Ghosson^{1,2,*}, Rahul Suryawanshi¹, Delphine Raviglione^{1,2,3}, Marie-Virginie Salvia^{1,2,3}, Cédric Bertrand^{1,2,3,4}

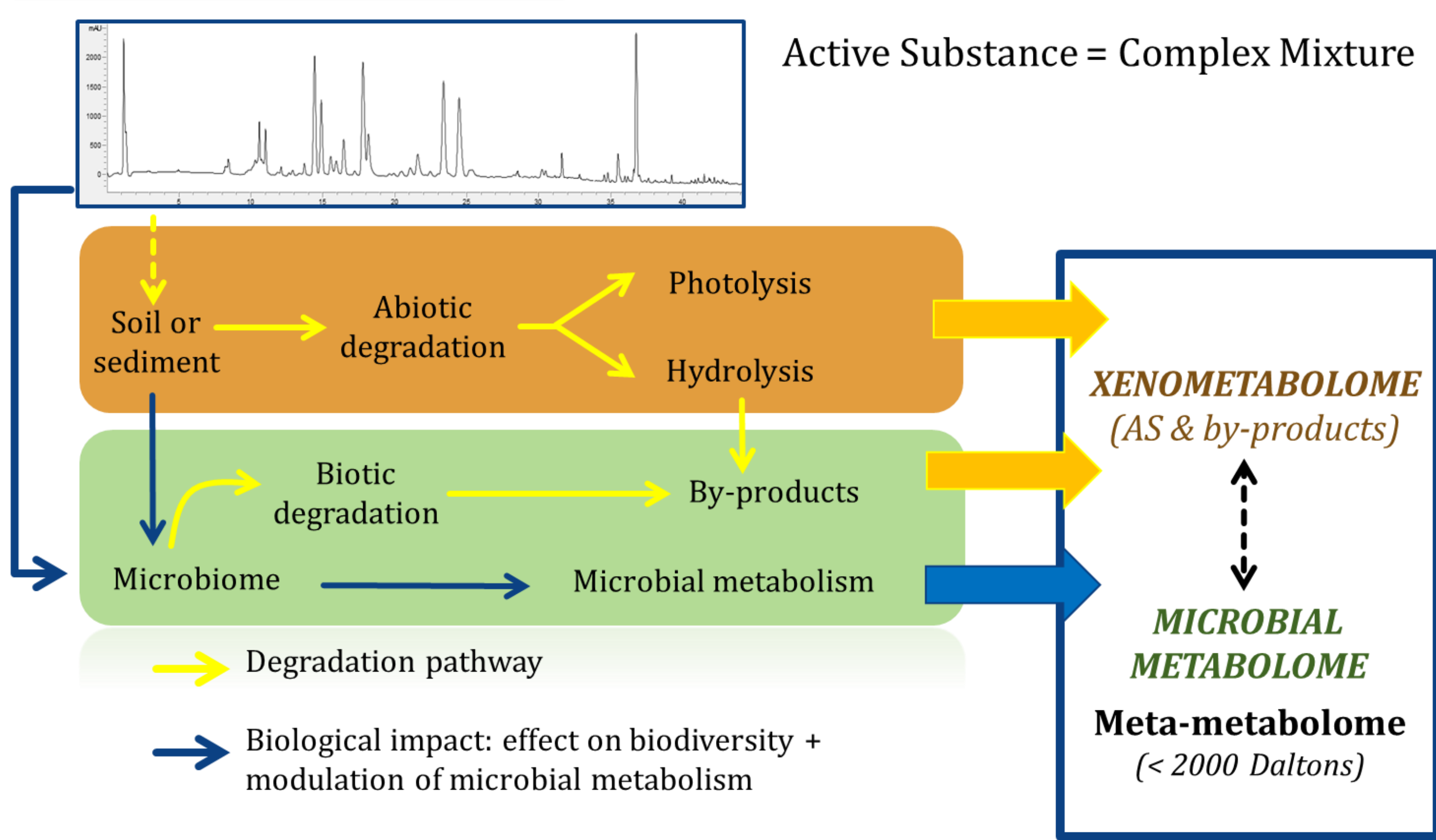
*: hikmat.ghosson@univ-perp.fr

- 1: PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France
 2: UFR Sciences Exactes et Expérimentales, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France
 3: Laboratoire d'Excellence « CORAIL », Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France
 4: S.A.S. AkiNaO, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France

Abstract

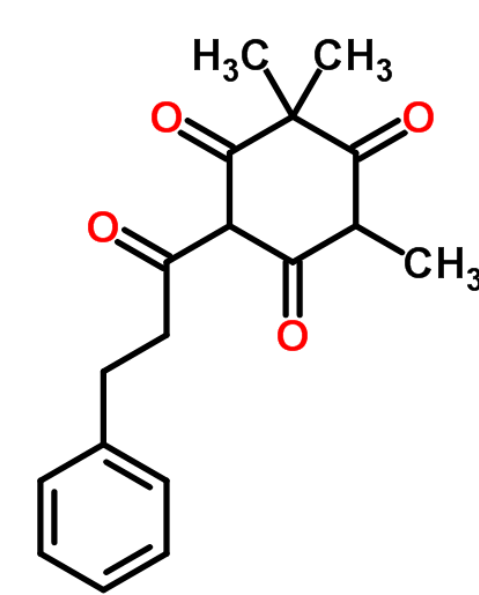
Herbicide consumption is yearly increasing. However, these chemicals are threatening environment, and for instance, soil. Thus, the use of natural bioherbicides is notably recommended, as they are supposed less harmful for the environment and its biodiversity. However, the evaluation of their environmental fate and impact are still not widely studied. This is because of multiple limitations in classical evaluation protocols. Thus, new omics-based methods are being developed in order to adapt suitable protocols to assess the environmental risk of such biocontrol products. Recently, an innovative metabolomics-based approach named "Environmental Metabolic Footprinting" (EMF) was developed as an alternative tool for the evaluation of the environmental fate and impact. The aim of this approach is to determine the "resilience time" as a new integrative evaluation concept. The LC-MS-based EMF was applied successfully on soil^[1] and sediments^[2], it then proved several advantages. Thus, improving this approach was considered in our present work, by introducing High Resolution Mass Spectrometry (HRMS). This improvement allowed higher selectivity by exact mass measuring, providing higher reliability and necessary information for biomarkers discovery. This work was applied to evaluate the environmental impact of a natural complex bioherbicide; the *Myrica gale* extract, containing the "Myrigalone A". The impact of this natural bioherbicide will be compared to the pure synthesized Myrigalone A compound, and also to a reference product "Sulcotrione" as a well-known β -triketone chemical herbicide. Myrigalone A, presenting high affinity to the soil, makes the half-life approach non-applicable. Moreover, the interest of applying EMF in this study is to analyze its by-products and the impact on microbial metabolome.

1. EMF Concept



2. Myrigalone A

- ✓ β -triketone natural herbicide
- ✓ Origin: *Myrica gale*
- ✓ High adsorption on soil ($K_d = 20$ L/Kg)



3. Material and Methods

3.1. Sample preparation

- Four modalities of treatment were applied:
 - ✓ Methanol control « CtrlMeOH »
 - ✓ *Myrica gale* extract-treated soil « MyrN »
 - ✓ Pure Myrigalone A-treated soil « MyrS »
 - ✓ Sulcotrione-treated soil « Sulc » (synthetic herbicide)
- 10 kinetics points: Days 0, 2, 4, 8, 15, 30, 45, 60, 90, 150
- ✓ 5 replicates per modality of treatment by time point (200 samples in total)

Applied doses:
 Myrigalone A: 30 μ g/g
 Sulcotrione: 1.5 μ g/g
 (1x field dose)

3.2. Extraction and LC-MS analysis

- Extraction: Solvent: Methanol + 1% Formic Acid (v/v)
- LC-HRMS: Kinetex 2.6 μ m Polar C18
 QToF MS - m/z 100-1500 - ESI-
- Data treatment: Data pre-treatment: W4M platform
 Multivariate statistics: RStudio



Bruker Maxis AG QToF MS
 HRMS

4. Results

4.1. Targeted raw data treatment

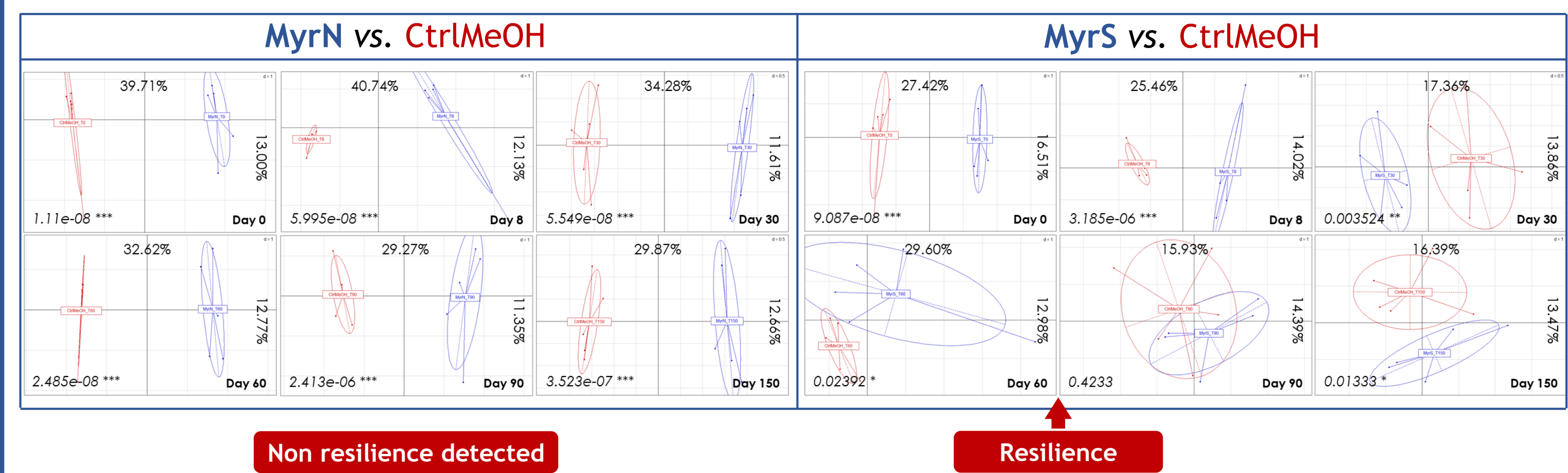
➤ Exploitation of several xenometabolites by exact mass measuring:

Experimental m/z	Potential ion formula	Theoretical m/z	Error (ppm)	Putative metabolite ^{[3],*}
299.1301	[C ₁₈ H ₁₉ O ₄] ⁻	299.1289	4.01	Myrigalone E/B/P/D
297.1142	[C ₁₈ H ₁₇ O ₄] ⁻	297.1132	3.37	n.i.
285.1146	[C ₁₇ H ₁₇ O ₄] ⁻	285.1132	4.91	Demethoxymatteucinol
283.0989	[C ₁₇ H ₁₅ O ₄] ⁻	283.0976	4.59	n.i.
271.0988	[C ₁₆ H ₁₅ O ₄] ⁻	271.0976	4.43	Uvangoletin
269.0831	[C ₁₆ H ₁₃ O ₄] ⁻	269.0819	4.46	n.i.
315.1248	[C ₁₈ H ₁₉ O ₅] ⁻	315.1238	3.17	Myrigalone isomer +O

*: Putative identification was done on the Level 5 of identification confidence according to Schymanski et al.^[4]. n.i.: Not Identified

4.2. Untargeted statistical analysis

- Targeted kinetics monitoring:
 - Comparison of m/z 299.1289 EIC between standard solution, *Myrica gale* extract and different treated soil extracted samples on day 0
- Non-extractible Myrigalone A:
- Application of PCA
- Validation with MANOVA (p-value)



5. Perspectives

- Further data treatment and analysis (endogenous biomarker discovery)
- Improving experimental design for "resilience time" determination
- Further analytical development

References

- [1] Patil C., Calvayrac C., Zhou Y., Romdhane S., Salvia M. V., Cooper J. F., Dayan F., Bertrand C. (2016), Environmental Metabolic Footprinting: A novel application to study the impact of a natural and a synthetic β -triketone herbicide in soil, *Science of The Total Environment*, 566-567, pp. 552-558
 [2] Salvia M. V., Ben Jrad A., Raviglione D., Zhou Y., Bertrand C. (2017), Environmental Metabolic Footprinting (EMF) vs. half-life: a new and integrative proxy for the discrimination between control and pesticides exposed sediments in order to further characterise pesticides' environmental impact, *Environmental Science and Pollution Research*, pp. 1-7
 [3] Popovici J., Comte G., Bagnarol E., Alloisio N., Fournier P., Bellvert F., Bertrand C., Fernandez M. (2010), Differential effects of rare specific flavonoids on compatible and incompatible strains in the *Myrica gale*-Frankia actinorhizal symbiosis, *Applied and Environmental Microbiology*, 76(8), pp. 2451-2460
 [4] Schymanski E., Jeon J., Gulde R., Fenner K., Ruff M., Singer H., Hollender J. (2014), Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence, *Environmental Science & Technology*, 48(4), pp. 2097-2098

Acknowledgments

