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

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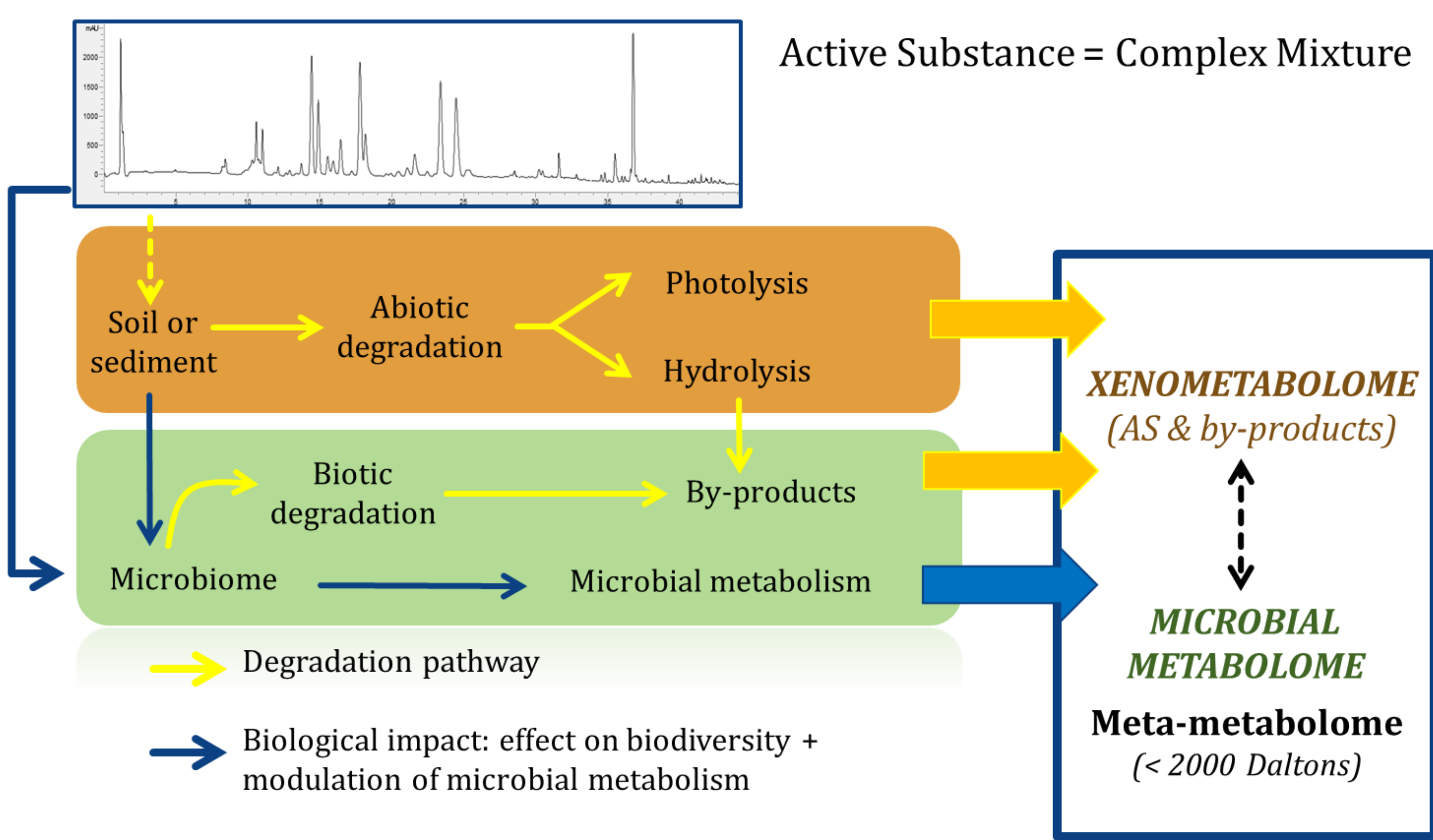
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Abstract

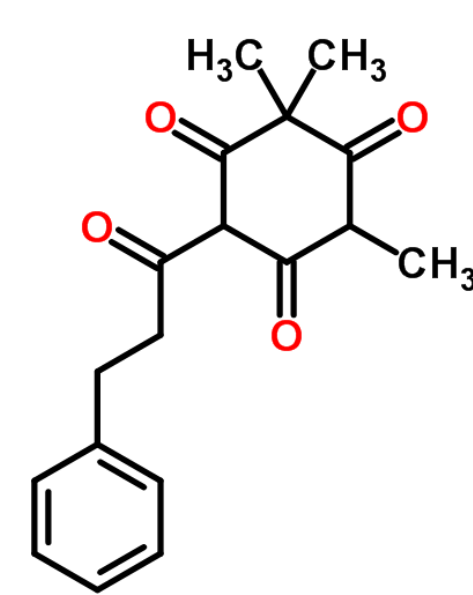
Herbicide consumption is yearly increasing. However, these chemicals are threatening environment, and for instance, soil. Thus, the use of natural bioherbicides is notably recommended, as they are supposed less harmful for the environment and its biodiversity. However, the evaluation of their environmental 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)

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



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

HRMS: Bruker Maxis AG QToF MS

4. Results

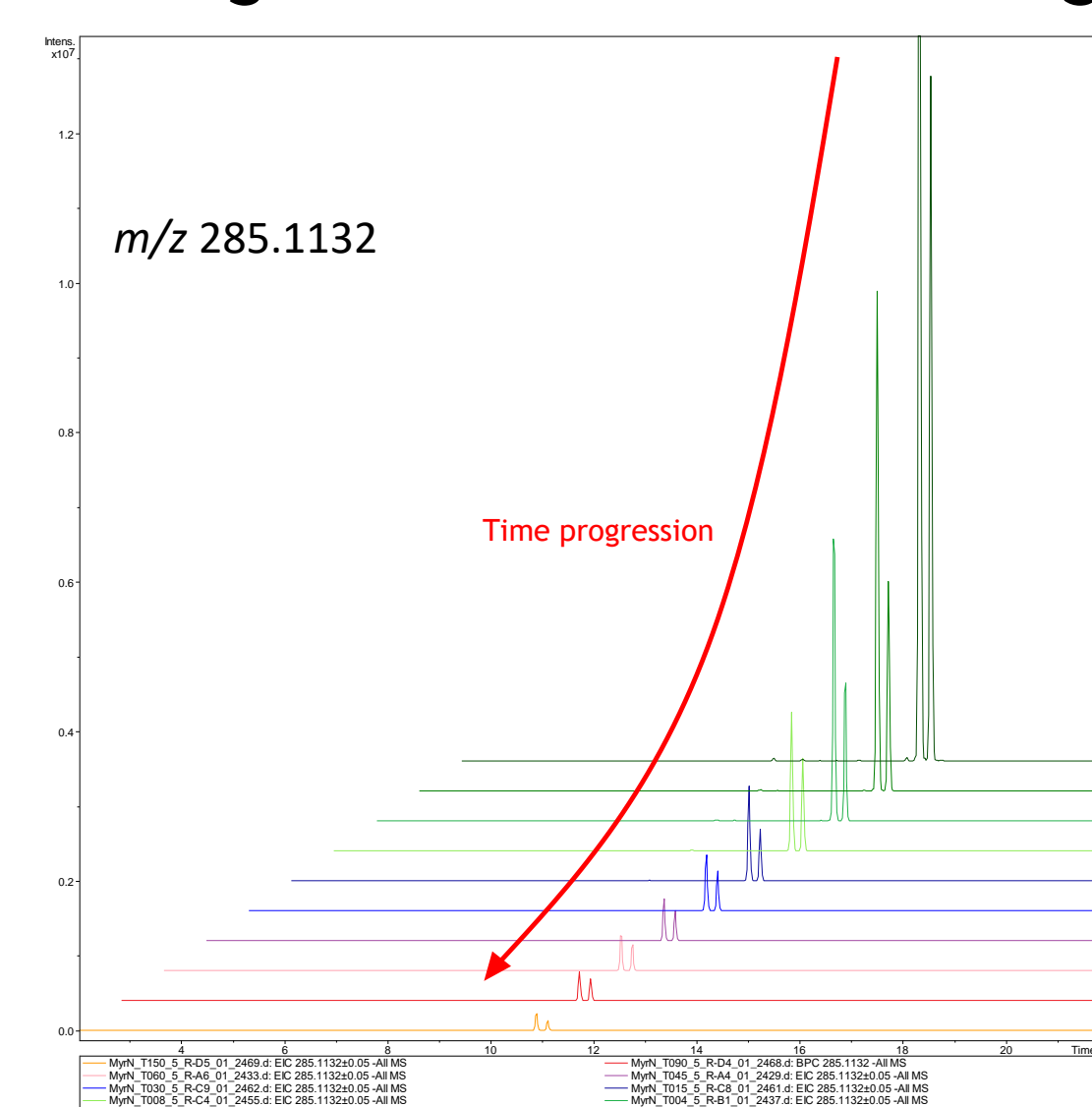
4.1. Targeted raw data treatment

- Exploitation of several xenometabolites by exact mass measuring:

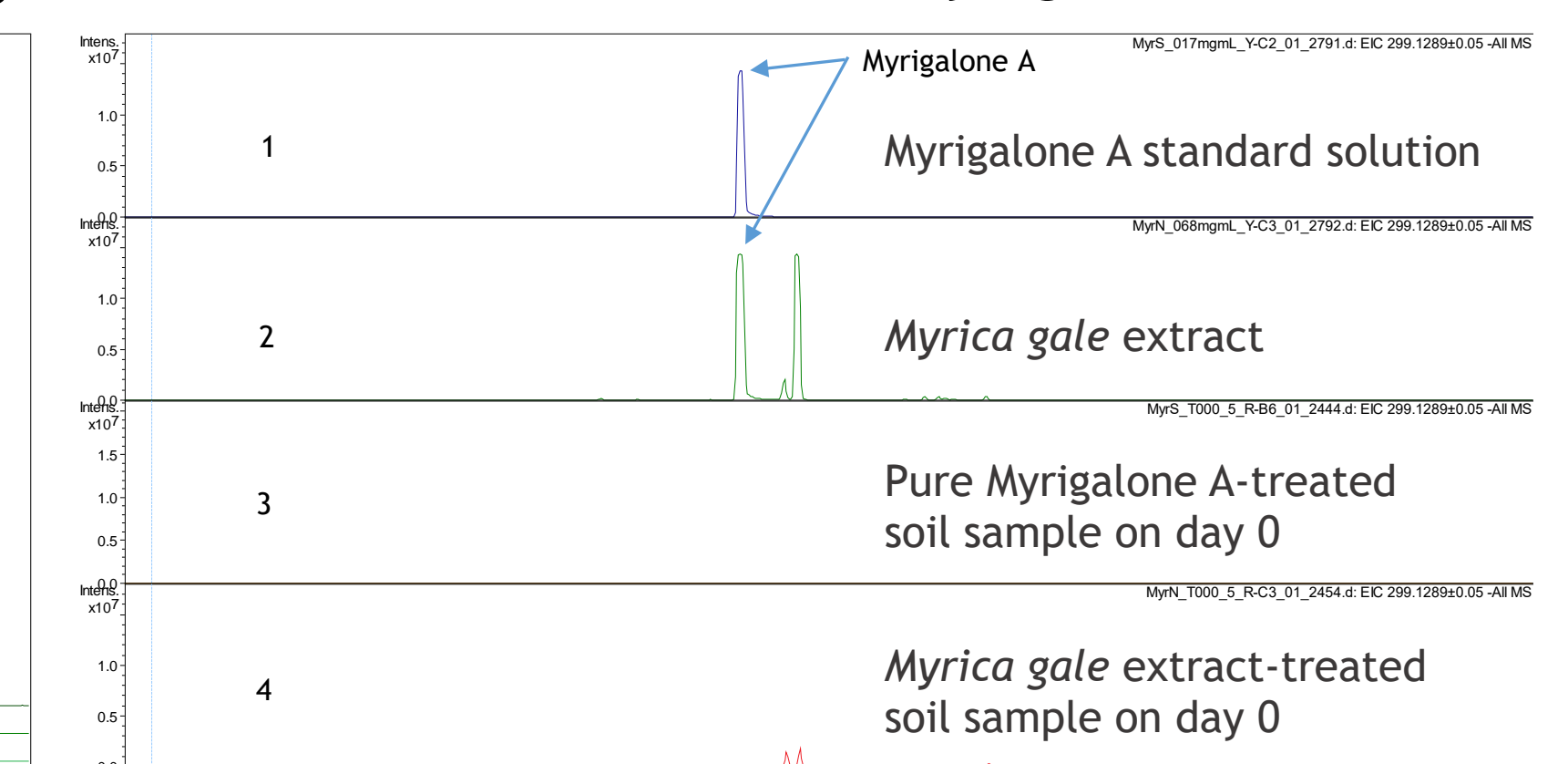
Experimental m/z	Potential ion formula	Theoretical m/z	Error (ppm)	Putative metabolite ^{[3],*}
299.1301	[C ₁₈ H ₁₉ O ₄] ⁻	299.1289	4.01	Myrigalone E/B/P/D
297.1142	[C ₁₈ H ₁₇ O ₄] ⁻	297.1132	3.37	n.i.
285.1146	[C ₁₇ H ₁₇ O ₄] ⁻	285.1132	4.91	Demethoxymatteucinol
283.0989	[C ₁₇ H ₁₅ O ₄] ⁻	283.0976	4.59	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

➤ Targeted kinetics monitoring:



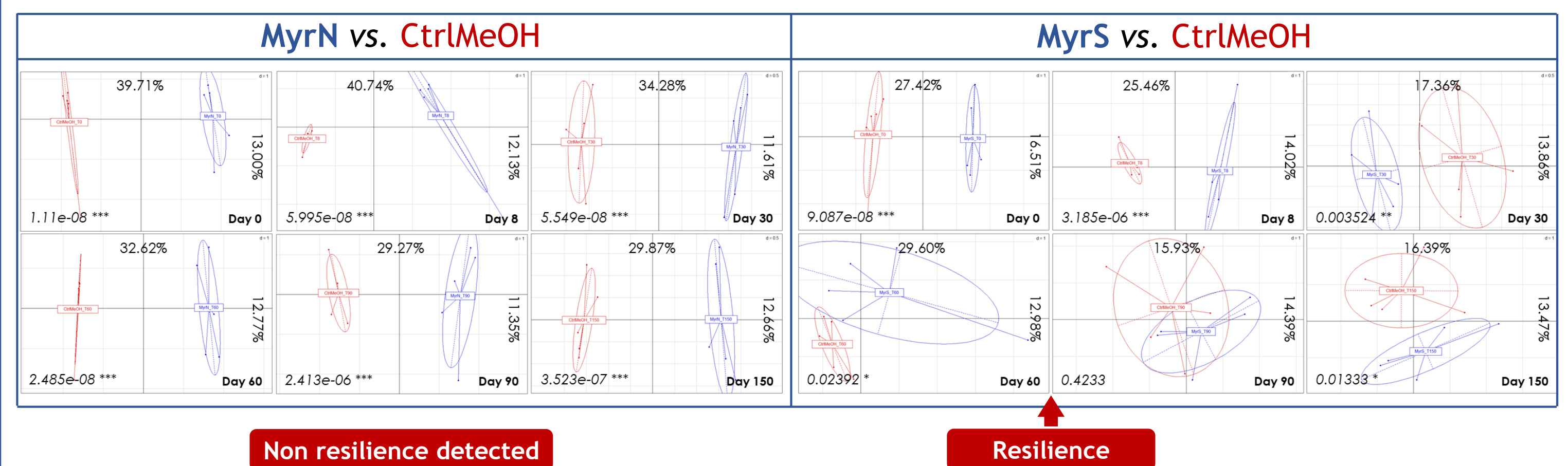
➤ Non-extractible Myrigalone A:



Comparison of m/z 299.1289 EIC between standard solution, *Myrica gale* extract and different treated soil extracted samples on day 0

4.2. Untargeted statistical analysis

- 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

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Acknowledgments

