



# Botanicals for bioherbicide development and metabolomics tools for E-fate evaluation of complex mixtures

Pr. Cédric BERTRAND

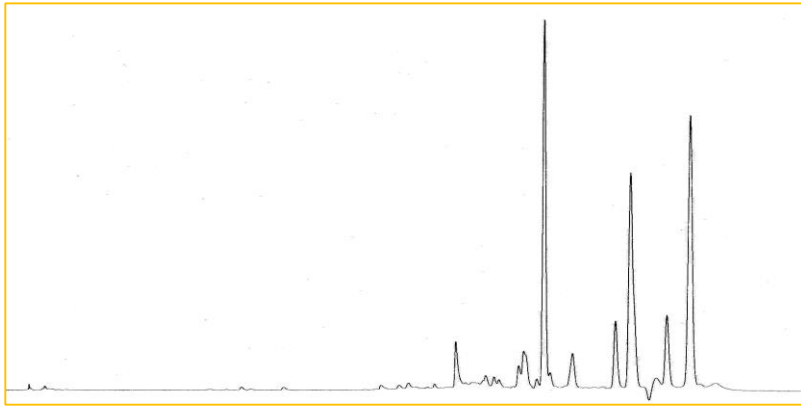
CRIOBE USR 3278 - Université de Perpignan  
Akinao – Scientific Director

Annual Biocontrol Industry Meeting - ABIM 2019,  
22 October 2019, Congress Center Basel, Switzerland.





# AkiNaO & CRIOBE - UPVD



- Identification of lead components
- Development of analytical methods for :
  1. Biomass quality assessment
  2. Accelerated degradation study
  3. Five batch analysis
  4. Toxins & metabolites quantification
- Analytical methods validation



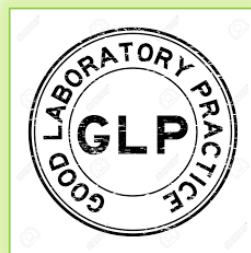
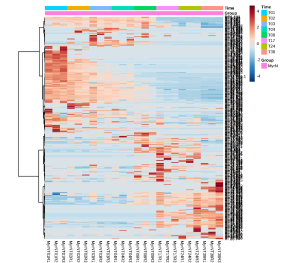
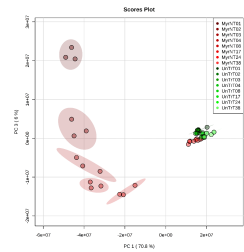
## Environmental chemistry

- Development of analytical system for e-fate study of conventional pesticide
- Development meta-metabolomics approach on soil and plant matrix (leaves, shoot, roots...)



## Metabolomic facilities

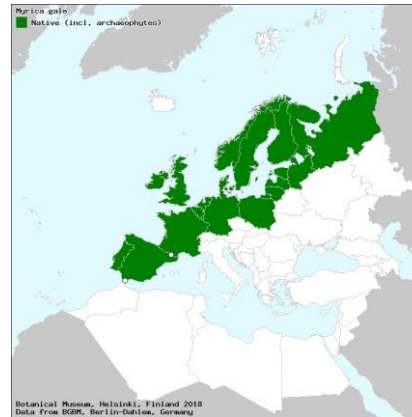
- UHPLC-HRMS Quadrupole-Orbitrap (Q-Exactive Plus)
- UHPLC-HRMS Quadrupole-temps de vol (Q-ToF)
- NMR 500 Hz (Jeol)
- .....





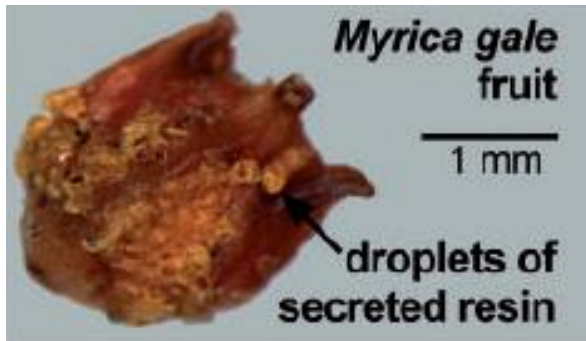
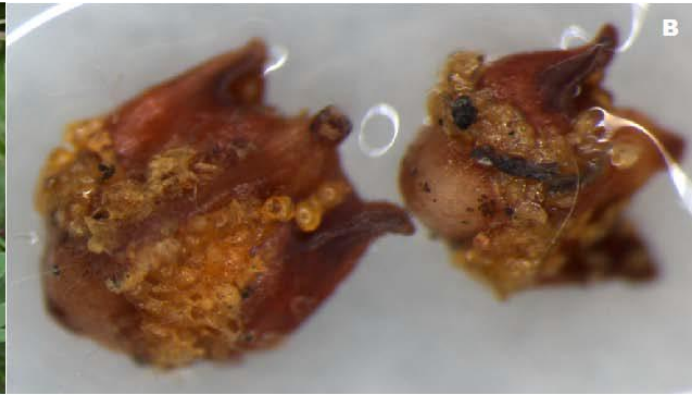
# *Myrica gale*

- *Myrica gale* L. (Myricaceae), commonly known as sweet gale and bog myrtle, is used as herbal tea...
- Widely distributed at high latitudes in the Northern hemisphere

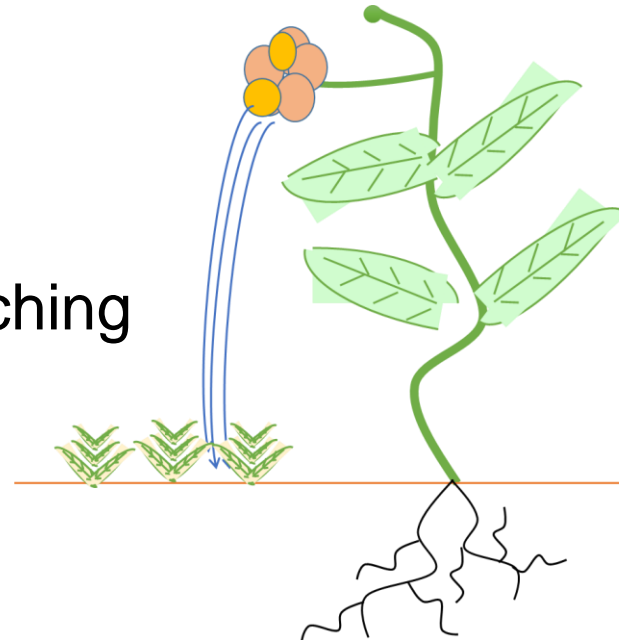




# “Allelochemical Hypothesis”



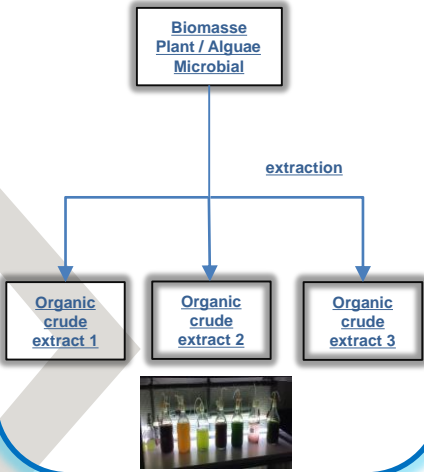
leaching



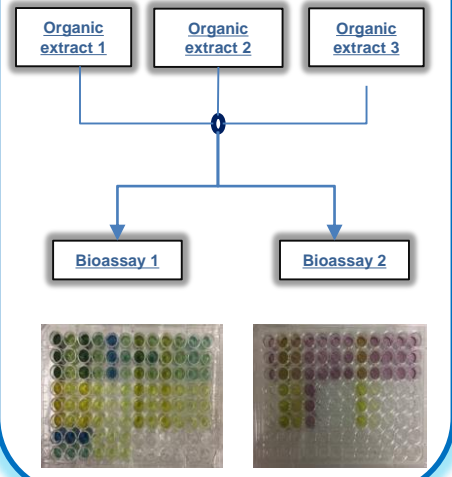


# Metabolomic workflow for Identification of active natural products

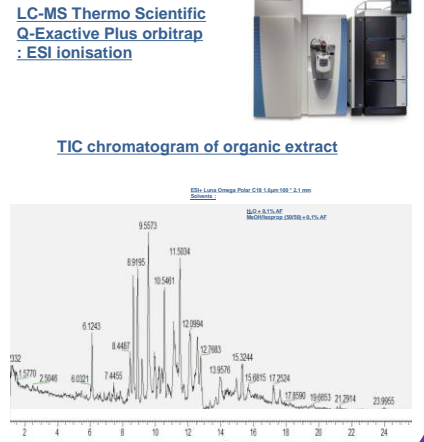
## Sample preparation



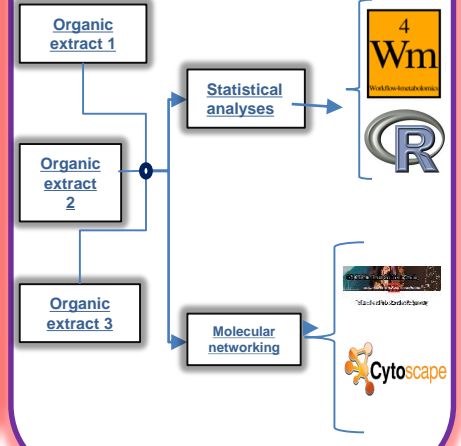
## Bioassays



## Metabolic fingerprints



## Data processings



## Mass spectra similarity networking and activity

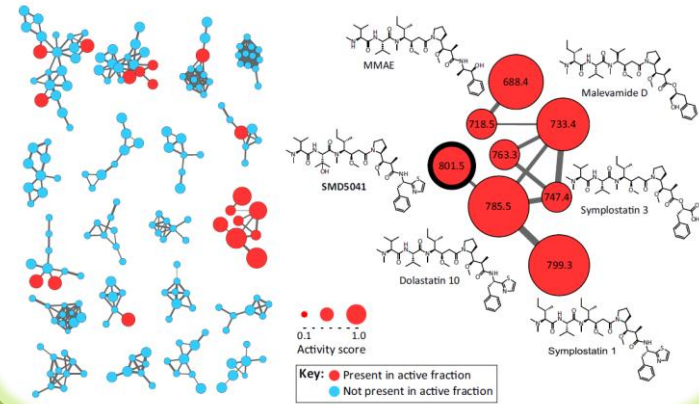


Figure 2. Molecular Networking in Natural Product Drug Discovery: Extracts of marine organisms are analyzed by liquid chromatography–tandem mass

# Myrica gale crude extract analysis

Extraction fruits leaching (MeOH/sonication)

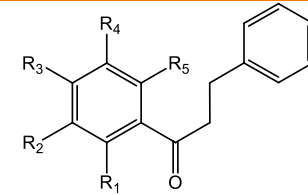


Chromatographic Fractionation



Structural Analysis of compounds from  
active fractions

(RMN <sup>1</sup>H et <sup>13</sup>C, HSQC, HMBC, LCMS, UV  
Mass spectra similarity networking)

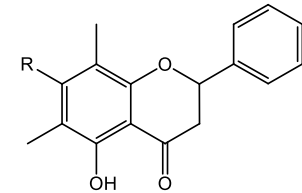


**Myrigalone E (MyE):** R<sub>1</sub> = OH, R<sub>2</sub> = CH<sub>3</sub>, R<sub>3</sub> = OCH<sub>3</sub>, R<sub>4</sub> = H, R<sub>5</sub> = OCH<sub>3</sub>

**Myrigalone B (MyB):** R<sub>1</sub> = R<sub>5</sub> = OH, R<sub>2</sub> = R<sub>4</sub> = CH<sub>3</sub>, R<sub>3</sub> = OCH<sub>3</sub>

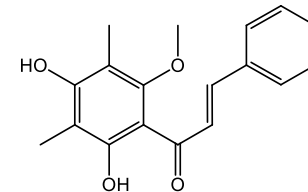
**Uvangoletin :** R<sub>1</sub> = R<sub>3</sub> = OH, R<sub>2</sub> = R<sub>4</sub> = H, R<sub>5</sub> = OCH<sub>3</sub>

**2',4',6'-trihydroxy-3',5'-diméthylidihydrochalcone:** R<sub>1</sub> = R<sub>3</sub> = R<sub>5</sub> = OH, R<sub>2</sub> = R<sub>4</sub> = CH<sub>3</sub>

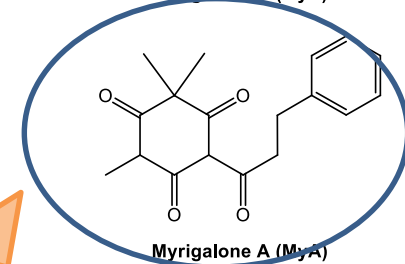


**Déméthoxymatteucinol:** R = OH

**Déméthoxymatteucinol-7-méthyl éther:** R = OCH<sub>3</sub>

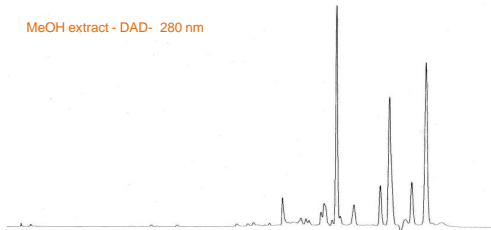


**Myrigalone D (MyD)**



**Myrigalone A (MyA)**

MeOH extract - DAD- 280 nm

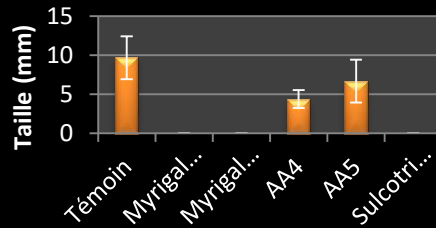


# Biological activity of crude extract

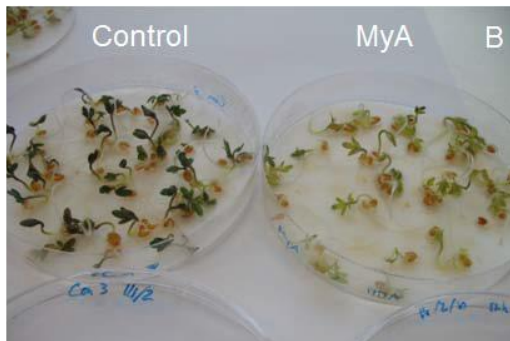
Dicotyledone road, garden



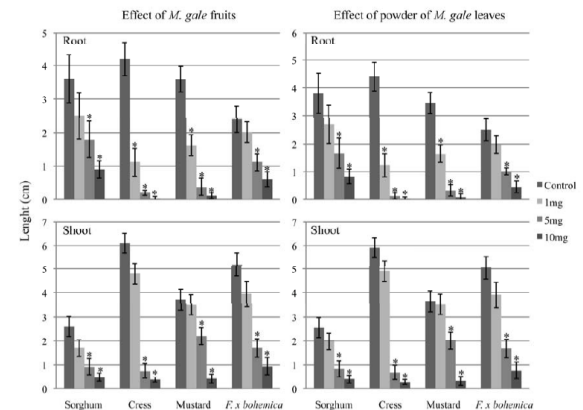
## Senecio vulgaris



18 hrs after treatment myrigalone treated seedlings of *L. sativum* were found to be slept on the soil surface and showed no signs of growth (and discoloration) while no effect was observed on solvent treated control greenhouse (7% DMSO).



Seedlings grown on Myrigalone A medium are bleached





# PATENDEE



(11) **EP 2 320 726 B1**

(12) **FASCICULE DE BREVET EUROPEEN**

(45) Date de publication et mention  
de la délivrance du brevet:  
**08.03.2017 Bulletin 2017/10**

(51) Int Cl.:  
**A01N 35/04** (2006.01) **A01N 35/06** (2006.01)  
**A01N 43/16** (2006.01) **A01N 65/00** (2009.01)  
**A01P 19/00** (2006.01) **A01P 13/00** (2006.01)

(21) Numéro de dépôt: **09740391.9**

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(87) Numéro de publication internationale:  
**WO 2010/012945 (04.02.2010 Gazette 2010/05)**

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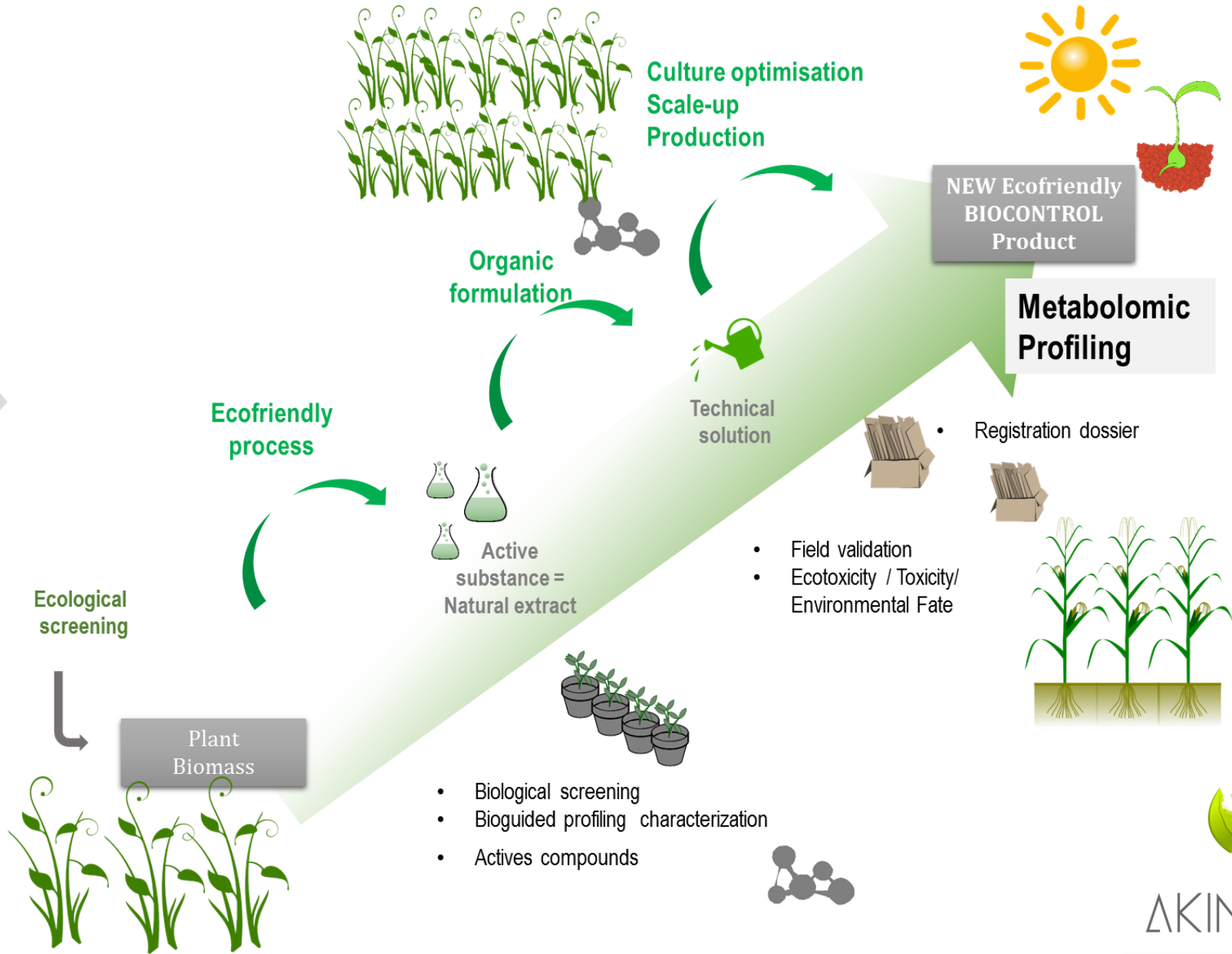
(54) **UTILISATION D'UNE PLANTE MYRICA GALE POUR LA PRODUCTION D'UN AGENT HERBICIDE**

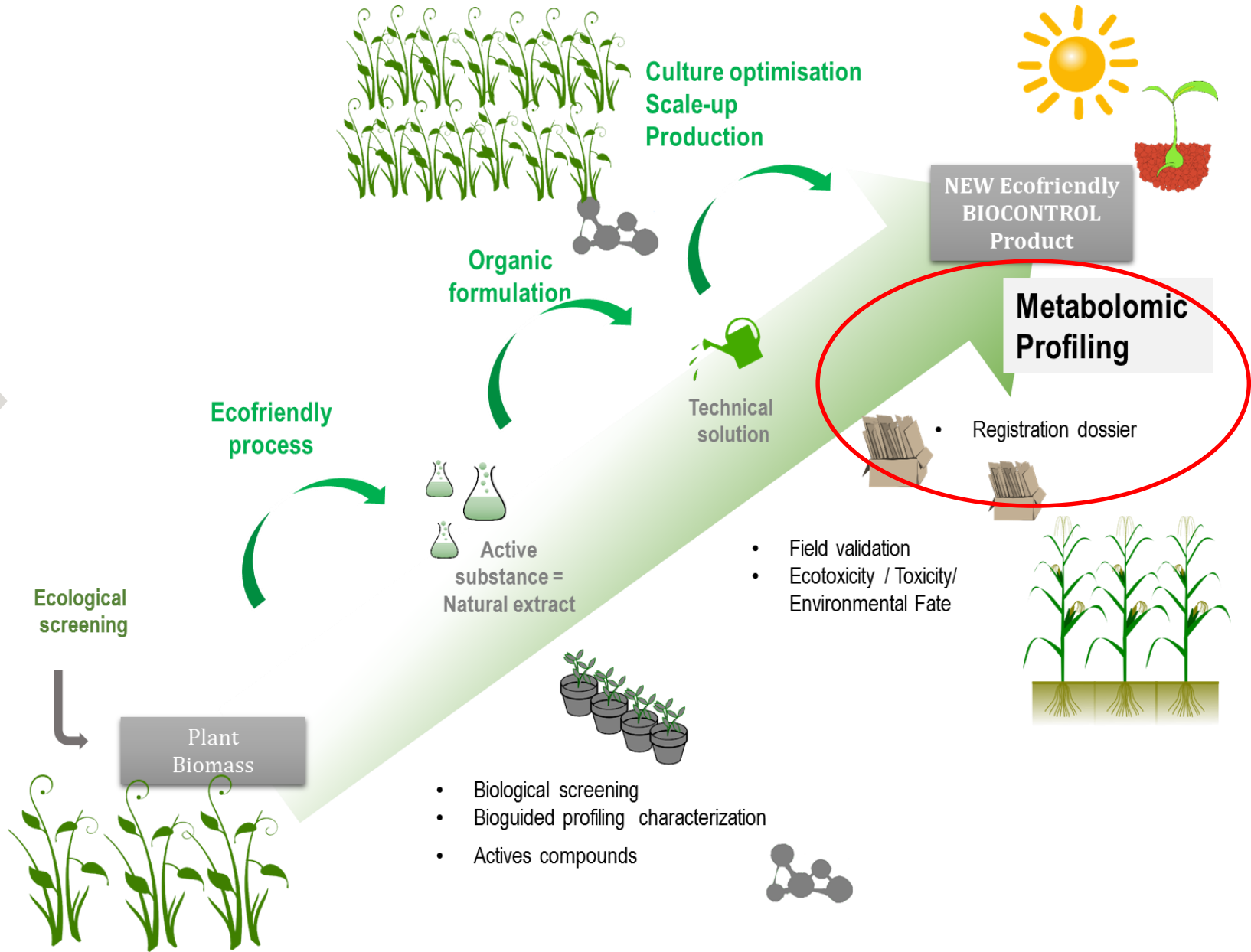
**VERWENDUNG EINER MYRICA GALE PFLANZE ZUR HERSTELLUNG EINES HERBIZIDEN  
MITTELS.**

**USE OF A MYRICA GALE PLANT FOR THE PRODUCTION OF AN HERBICIDAL AGENT.**











## Registration dossier

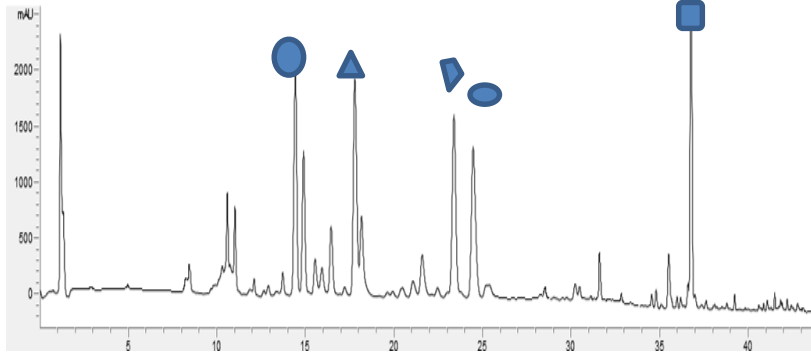
The European Food Safety Authority **requires**, within the framework of European legislation (SANCO/11470/2012– rev. 8), **the extended risk assessment and ecotoxicological monitoring of pure natural compounds as well as plant extracts.....**



→ **we need new analytical tools adapted to crude extract for environmental fate studies**



# How to manage environmental fate of crude extract?

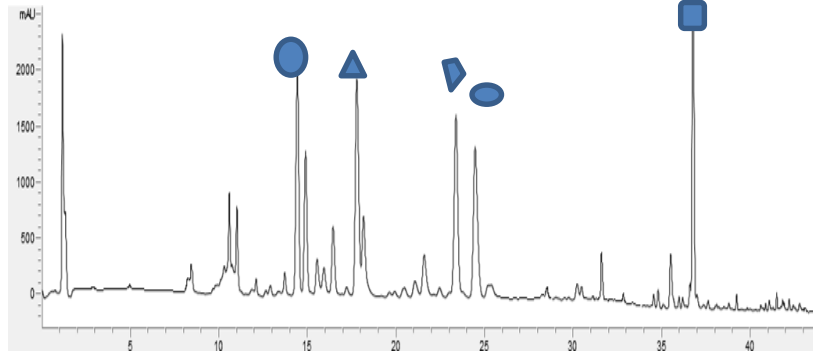


Crude extract  
Of *Myrica gale*





# How to manage environmental fate of crude extract?



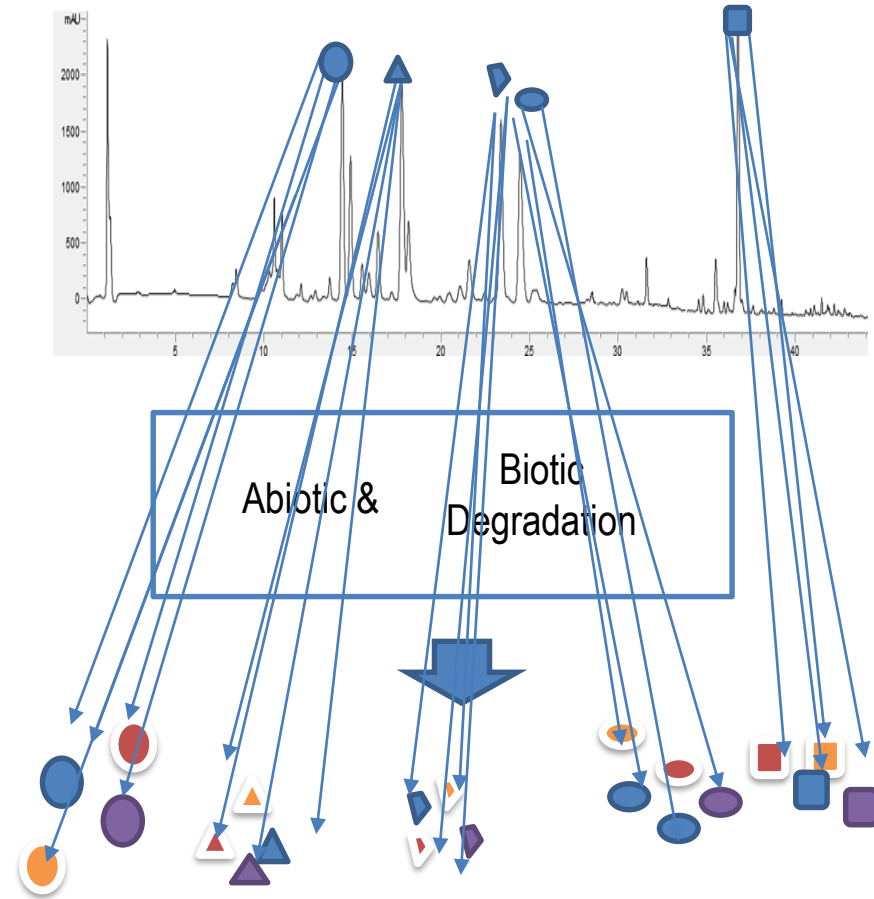
Crude extract  
Of *Myrica gale*

Abiotic & Biotic  
Degradation





# How to manage environmental fate of crude extract?



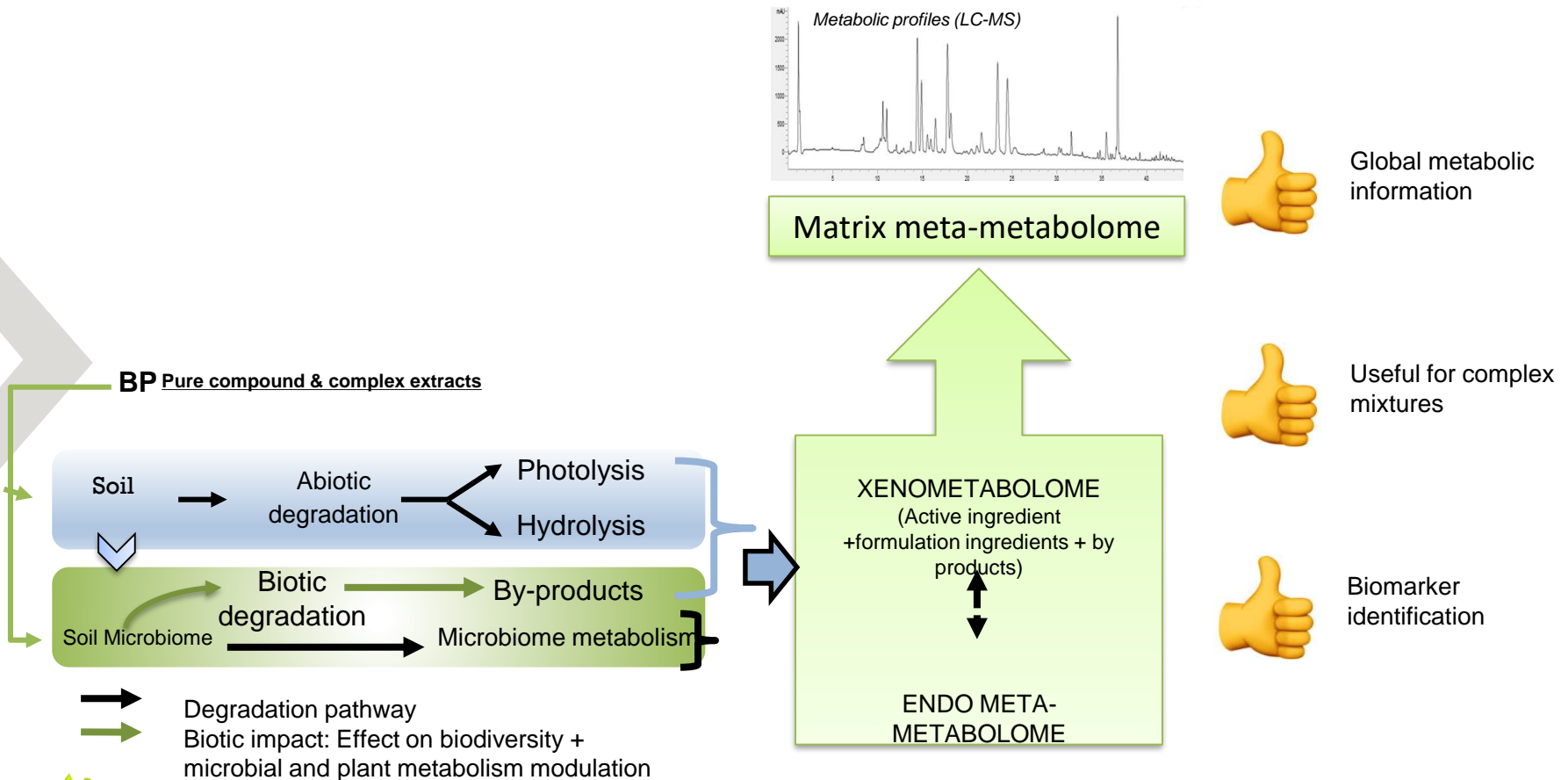
Crude extract  
Of *Myrica gale*

By-products of  
crude extract



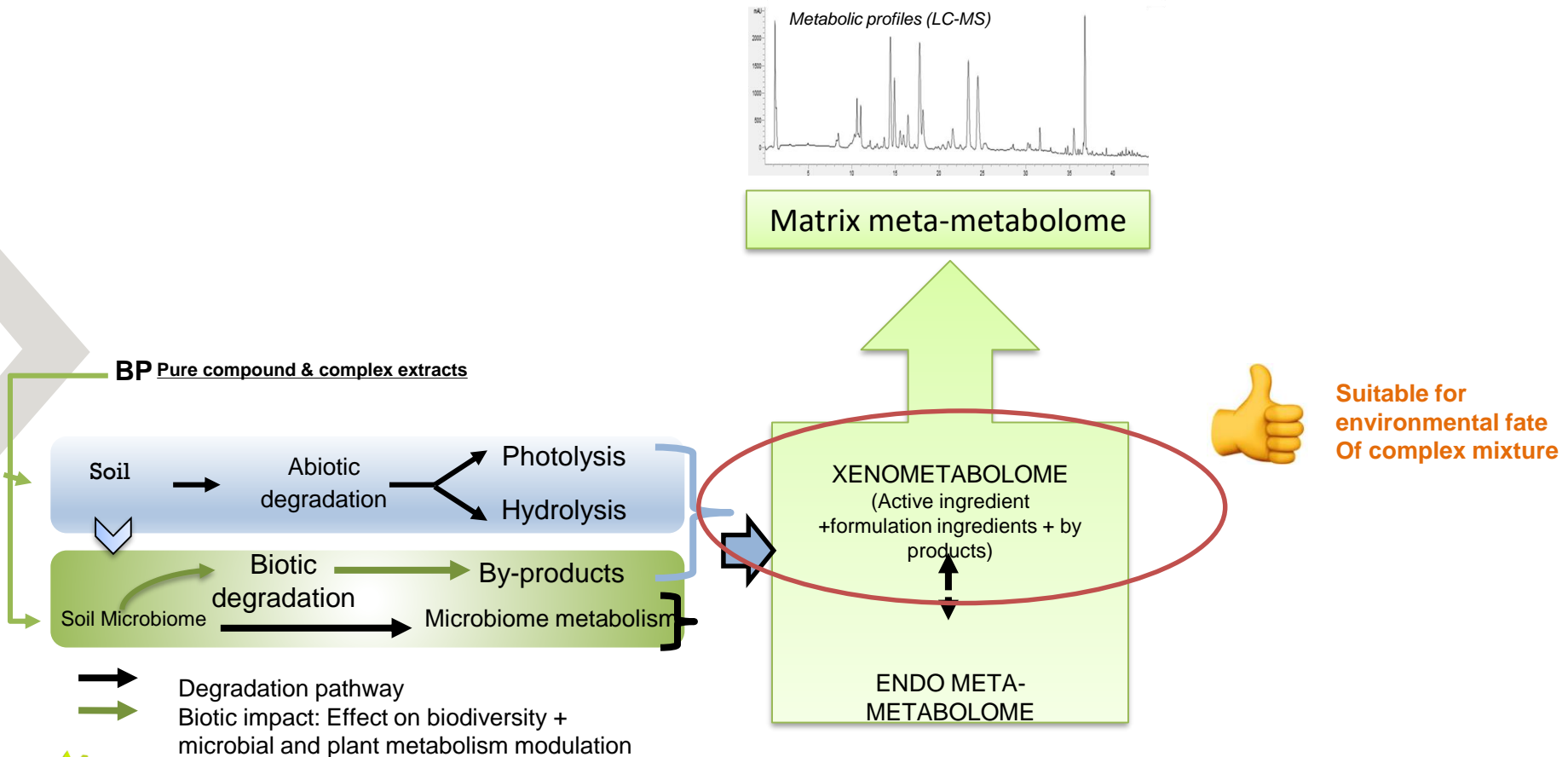


## Environmental Metabolic Footprinting (EMF) approach - Metabolomics





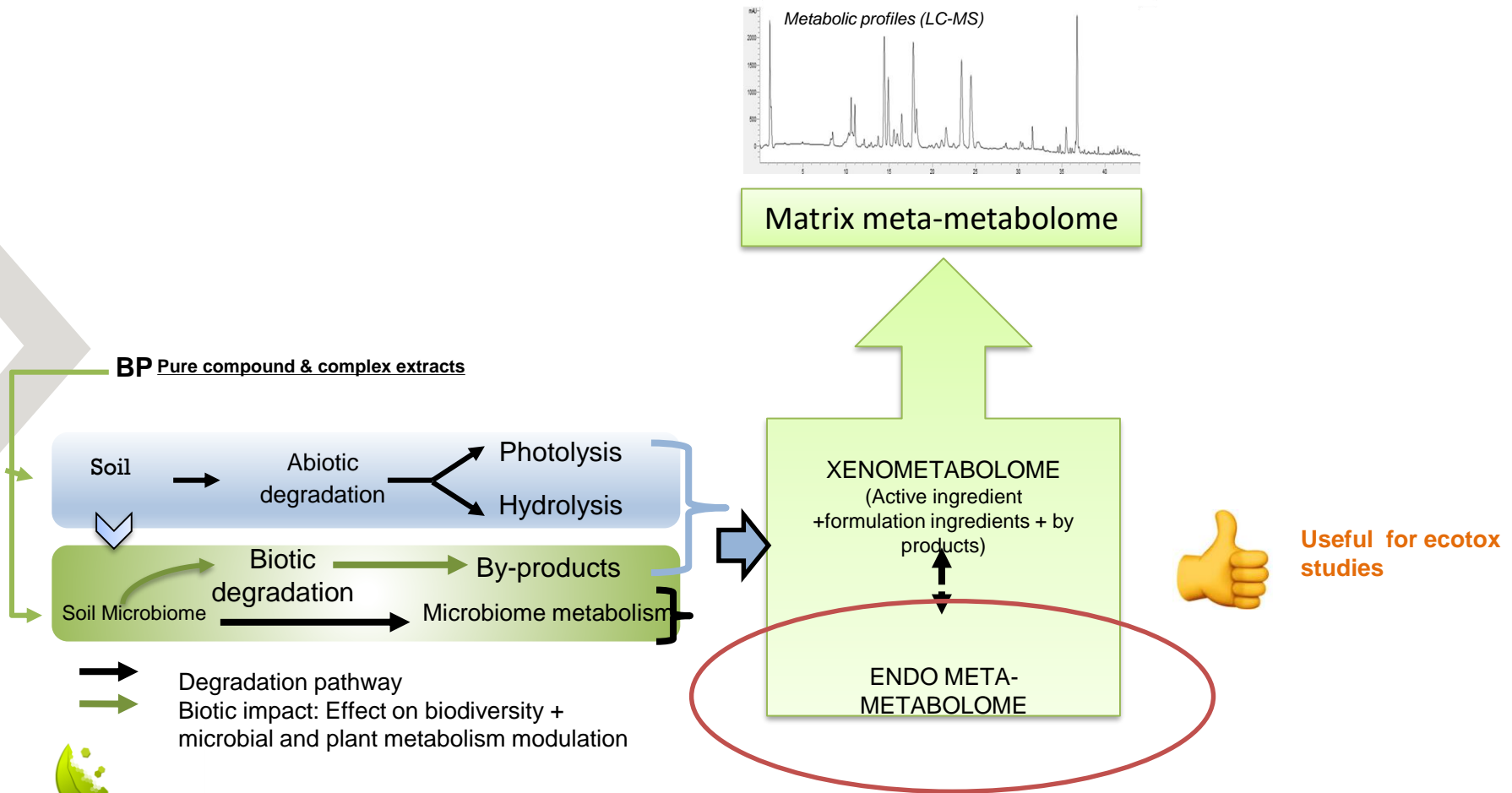
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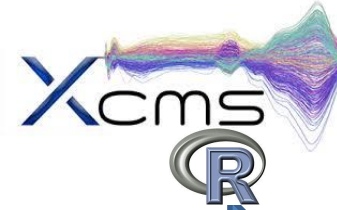
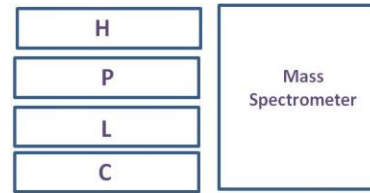
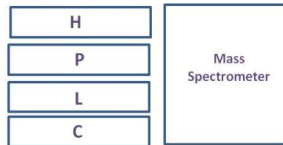


# Environmental Metabolic Footprinting approach (EMF) Work flow

- Soil (Control)
- Soil + crude extract  
(n = 5), Day : 0, 2, 4, 8, 15, 30, 45, 60, 90



Extraction



Ions matrix generation  
(800 variable)

name	class	treatment	time	M350725	M351726	M852T
BG_C_T0_1	C_T0	C	T00	96.021135602	24.529593331	111.49
BG_C_T0_2	C_T0	C	T00	136.72131347	50.38591657	73.899
BG_C_T0_3	C_T0	C	T00	635.7780000	172.1830610	55.224
BG_C_T0_4	C_T0	C	T00	333.99328695	51.60891304	154.82
BG_C_T0_5	C_T0	C	T00	109.37164306	119.39847231	122.71
BG_C_T2_1	C_T2	C	T02	17.65383184	90.47016834	190.86
BG_C_T2_2	C_T2	C	T02	752.21818181	279.20524995	189.90
BG_C_T2_3	C_T2	C	T02	210.42532344	165.18530271	140.50
BG_C_T2_4	C_T2	C	T02	139.2181008	133.74272805	129.32
BG_C_T2_5	C_T2	C	T02	100.27287074	44.90165405	115.22
BG_C_T4_1	C_T4	C	T04	218.22875976	76.65139493	36.693
BG_C_T4_2	C_T4	C	T04	376.47272727	94.76386951	47.381
BG_C_T4_3	C_T4	C	T04	62.79336983	89.15334647	62.737
BG_C_T4_4	C_T4	C	T04	237.96217564	58.07364908	106.09
BG_C_T4_5	C_T4	C	T04	16.29001964	31.47907326	68.411

Development of analytical methods suitable for analysis of active substances and potential by-products from soil Microcosms

Kinetics studies with LCMS profiling of soil extract / control and contaminated microcosms

Statistical analysis  
(PCA, PLS-DA, VIP...)

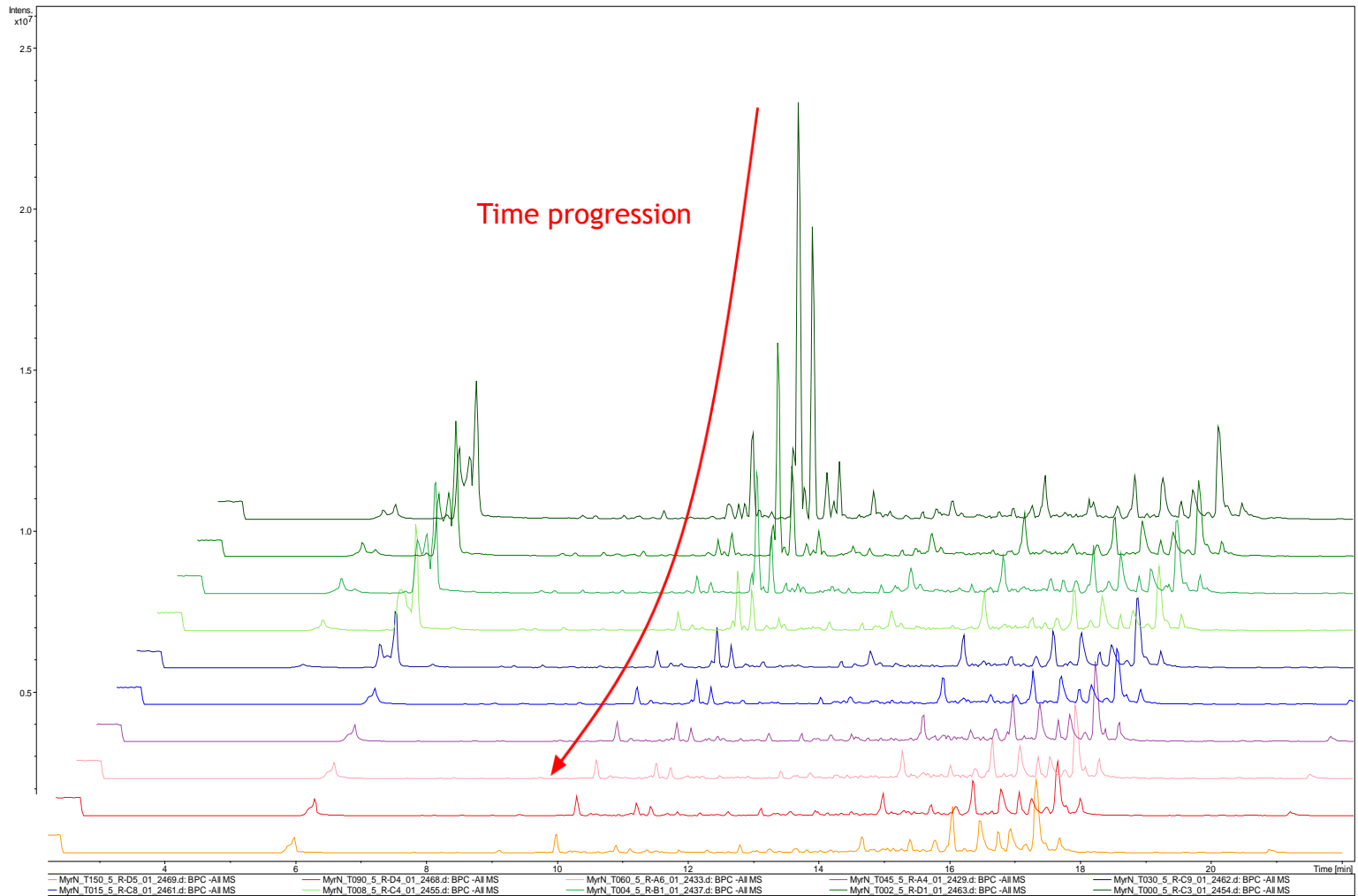
SOIL RESILIENCE ?  
PERSISTENT BY-PRODUCTS IDENTIFICATION

Selection of persistent compounds



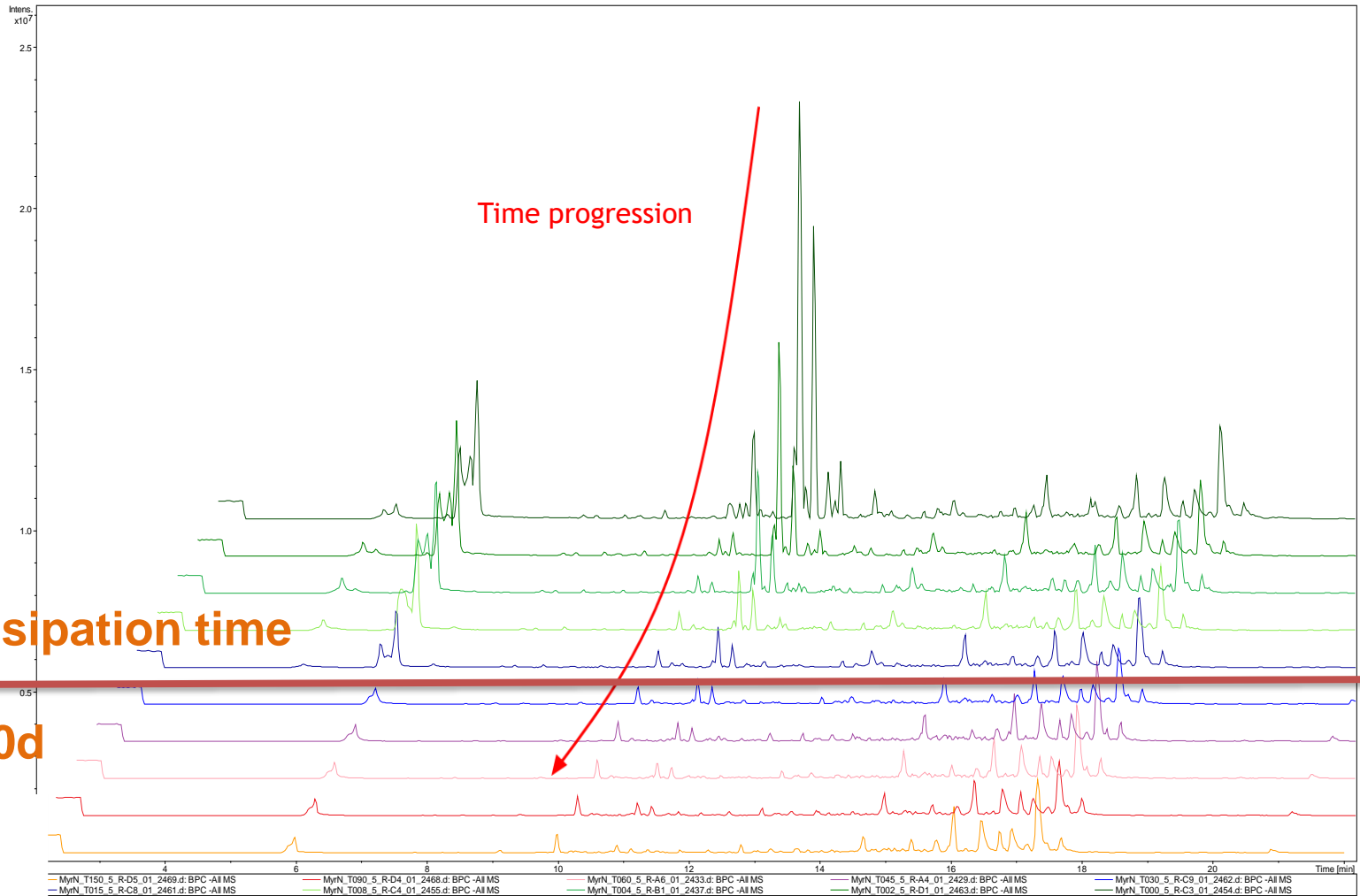


# Base Peak Chromatogram (BPC) of *Myrica gale* extract treated soil samples at different time points





# Base Peak Chromatogram (BPC) of *Myrica gale* extract treated soil samples at different time points



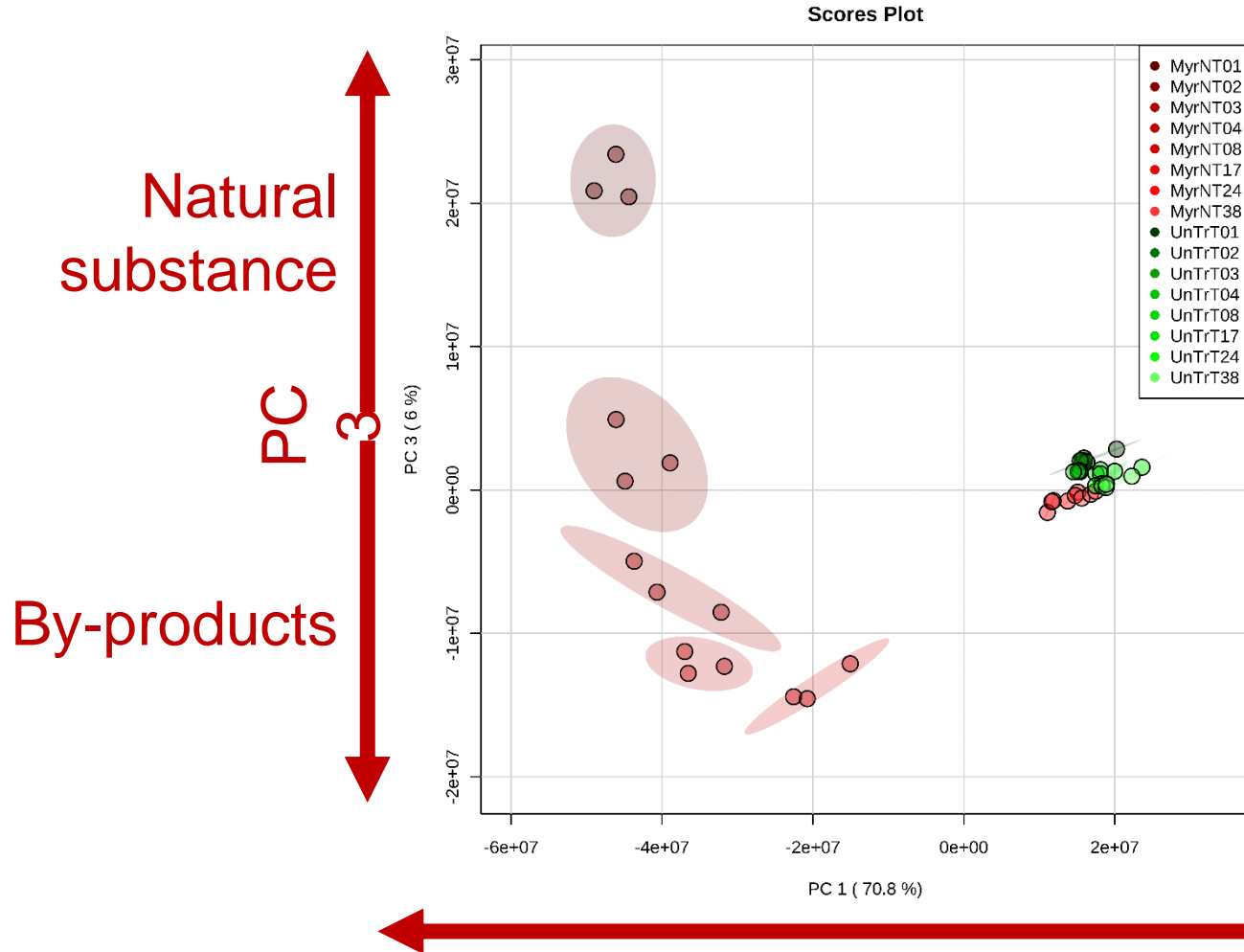
Total dissipation time

16d < t < 30d





# Xeno-metabolome evolution

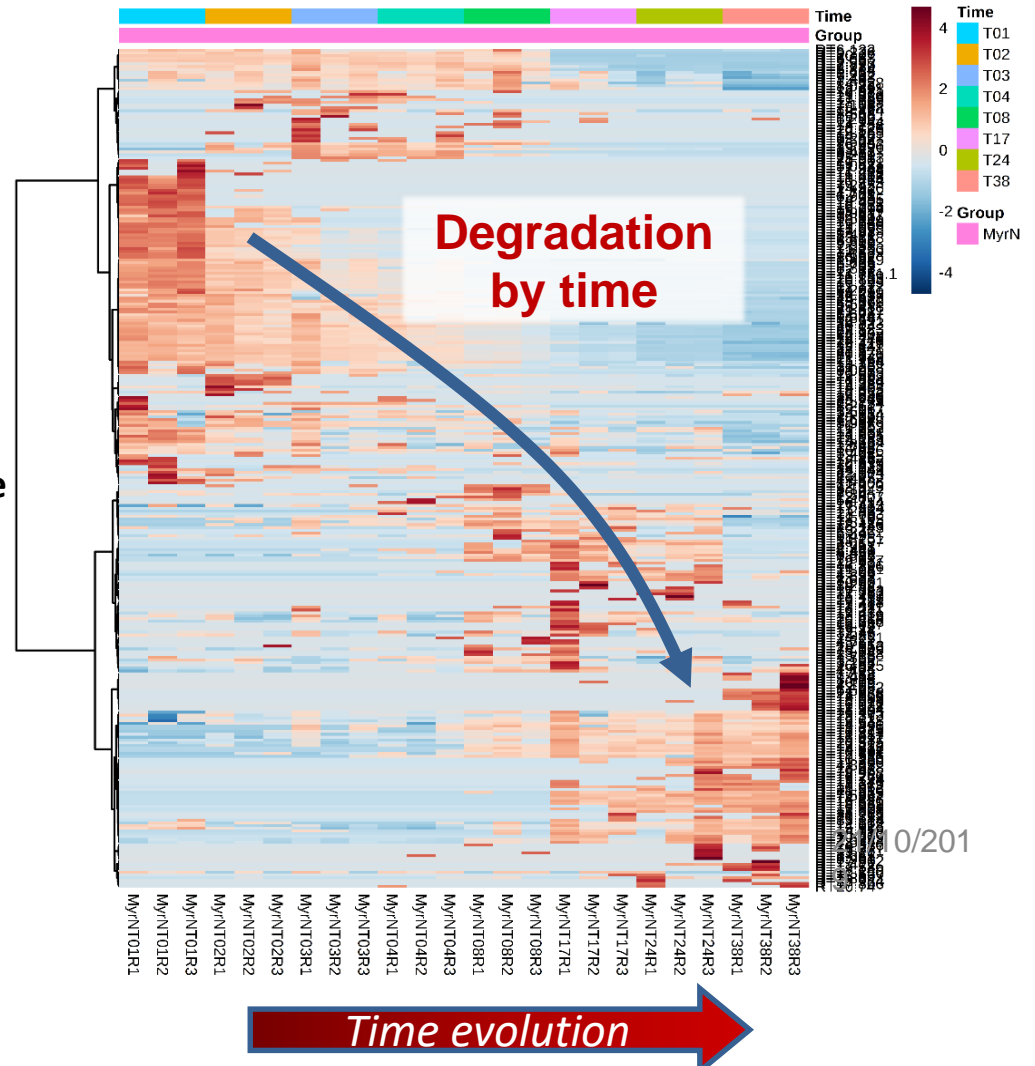




## HS-SPME-GC-MS Method

Results: "fishing" of degradation by-products

- Heatmap applied:
  - ✓ Distance Measure: **Pearson**
  - ✓ Clustering Algorithm: **Ward**
  - ✓ 1 factor time series: **MyrN**
- Plotting the **degradation by time evolution**

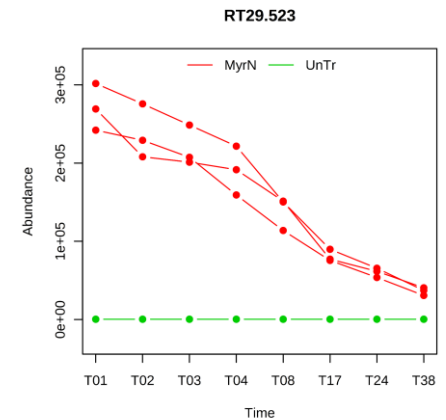
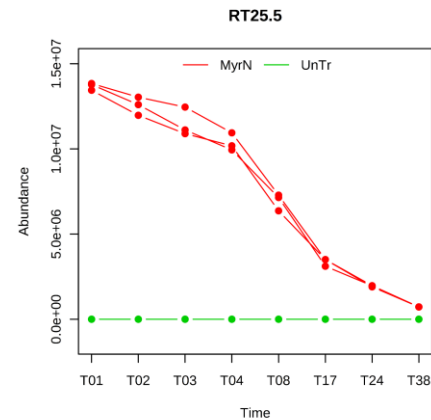
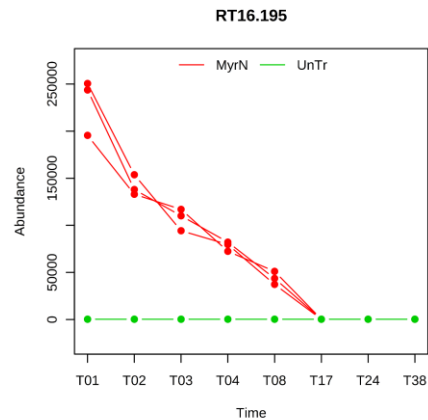
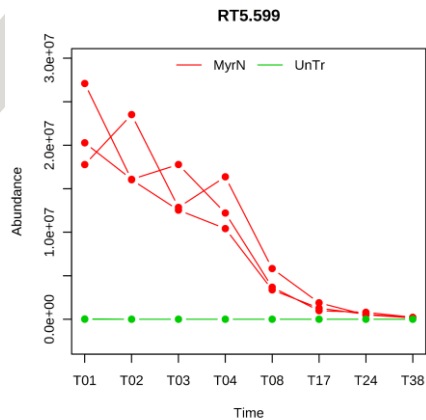


## HS-SPME-GC-MS Method

Results: "fishing" of degradation by-products

Kinetics tracking by time evolution:

- Natural substances : *Myrica gale* extract compounds





# CONCLUSION

- Identification of a new allelopathic compounds
- Mode of action identification
- Development of metabolomics tools for environmental fate evaluation of crude extract
- Environmental fate evaluation of crude extract
- Optimization of chemical synthesis of major active compounds (Myrigalone A)







# Acknowledgment



Hikmat Ghosson



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Sana Romdhane



Vanessa Andreu



Delphine Raviglione



Nicolas Inguibert



[www.biocontrol2020.com](http://www.biocontrol2020.com)



Natural products and Biocontrol 2020

Le colloque Natural products and Biocontrol aura lieu du 15 au 18 Septembre 2020 à Perpignan.

The conference Natural Products and Biocontrol will take place from 15 to 18 September 2020 in Perpignan.

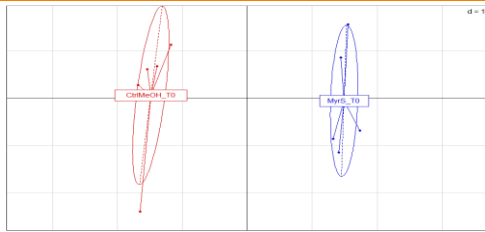
[www.biocontrol2020.com](http://www.biocontrol2020.com)





# Combined proxy : e-fate and ecotox

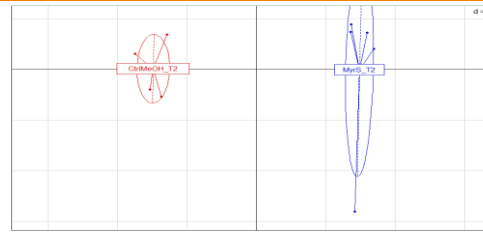
## Soil resilience after bioherbicide application vs Control



Day 0

MANOVA p-Value: 9.087e-08 \*\*\*

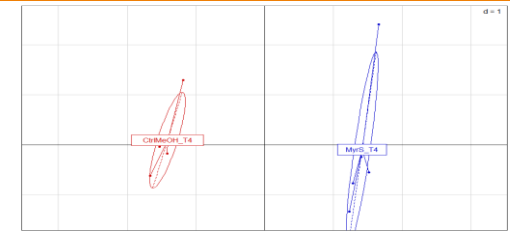
PC1: 27.42% - PC2: 16.51%



Day 2

MANOVA p-Value: 8.389e-08 \*\*\*

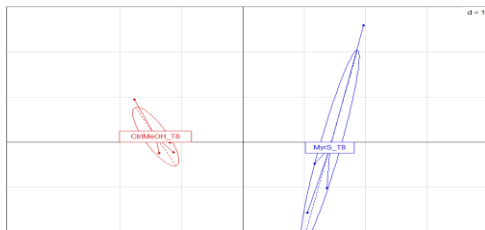
PC1: 27.12% - PC2: 13.30%



Day 4

MANOVA p-Value: 8.757e-09 \*\*\*

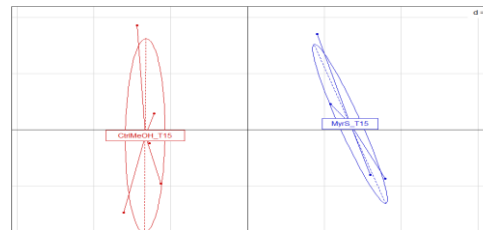
PC1: 24.75% - PC2: 12.85%



Day 8

MANOVA p-Value: 3.185e-06 \*\*\*

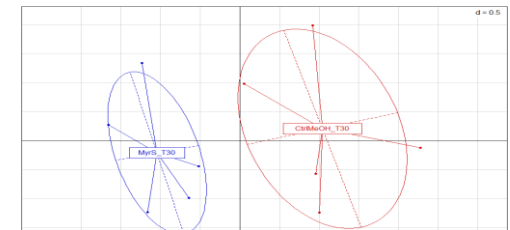
PC1: 25.46% - PC2: 14.02%



Day 15

MANOVA p-Value: 3.053e-06 \*\*\*

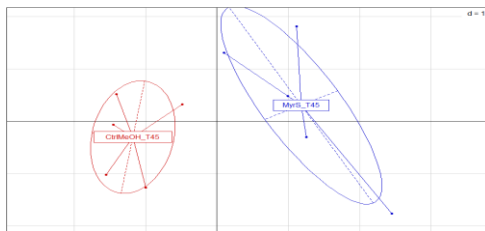
PC1: 22.54% - PC2: 13.73%



Day 30

MANOVA p-Value: 0.003524 \*\*

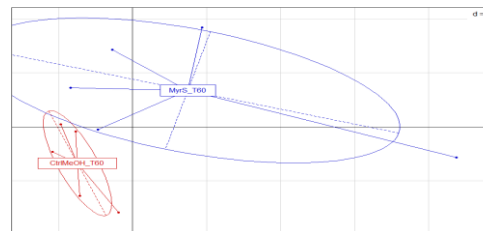
PC1: 17.36% - PC2: 13.86%



Day 45

MANOVA p-Value: 0.0006859 \*\*\*

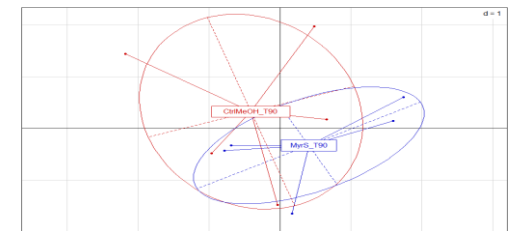
PC1: 21.38% - PC2: 14.03%



Day 60

MANOVA p-Value: 0.02392 \*

PC1: 29.60% - PC2: 12.98%



Day 90 (\*)

MANOVA p-Value: 0.4233

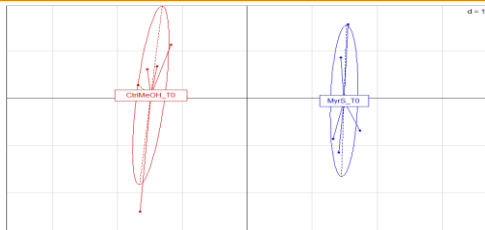
PC1: 15.93% - PC2: 14.39%





# Combined proxy : e-fate and ecotox

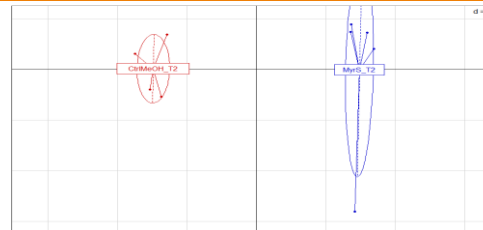
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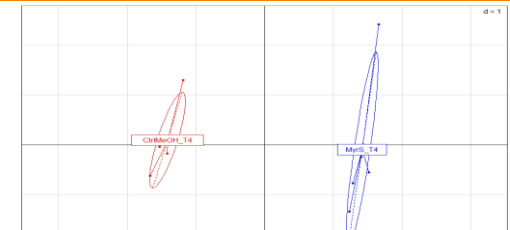
PC1: 27.42% - PC2: 16.51%



Day 2

MANOVA p-Value: 8.389e-08 \*\*\*

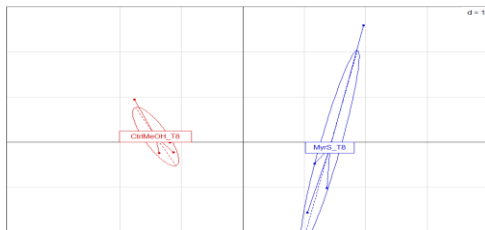
PC1: 27.12% - PC2: 13.30%



Day 4

MANOVA p-Value: 8.757e-09 \*\*\*

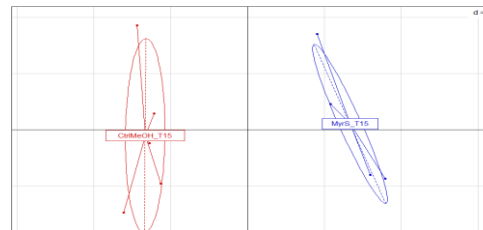
PC1: 24.75% - PC2: 12.85%



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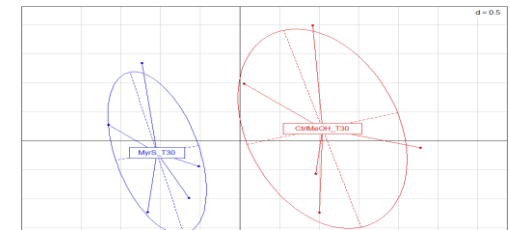
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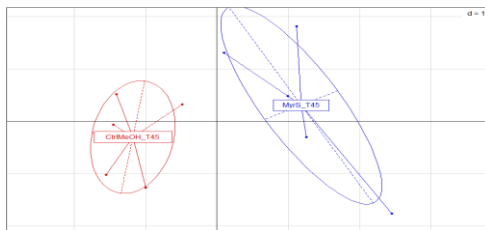
PC1: 22.54% - PC2: 13.73%



Day 30

MANOVA p-Value: 0.003524 \*\*

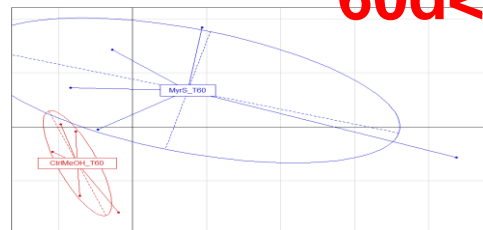
PC1: 17.36% - PC2: 13.86%



Day 45

MANOVA p-Value: 0.0006859 \*\*\*

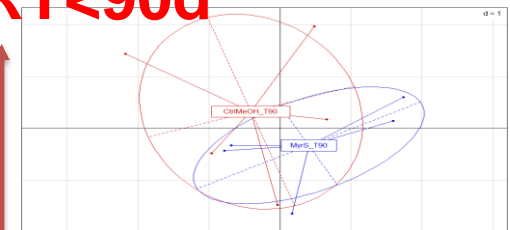
PC1: 21.38% - PC2: 14.03%



Day 60

MANOVA p-Value: 0.02392 \*

PC1: 29.60% - PC2: 12.98%



Day 90 (\*)

MANOVA p-Value: 0.4233

PC1: 15.93% - PC2: 14.39%

**60d < RT < 90d**

