

In-depth assessment of exhaustive extraction protocols for pesticide-polluted soil meta-metabolomics by LC-HRMS-based untargeted metabolic profiling

Hikmat Ghosson^{1,2,*}, Yannick Brunato^{1,2}, Delphine Raviglione^{1,2}, 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



Réseau Francophone de Métabolomique et Fluxomique

1ères Journées Scientifiques Numériques

25-26 Juin 2020

En Ligne via gotomeeting.com

<https://global.gotomeeting.com/join/318877725>

Context

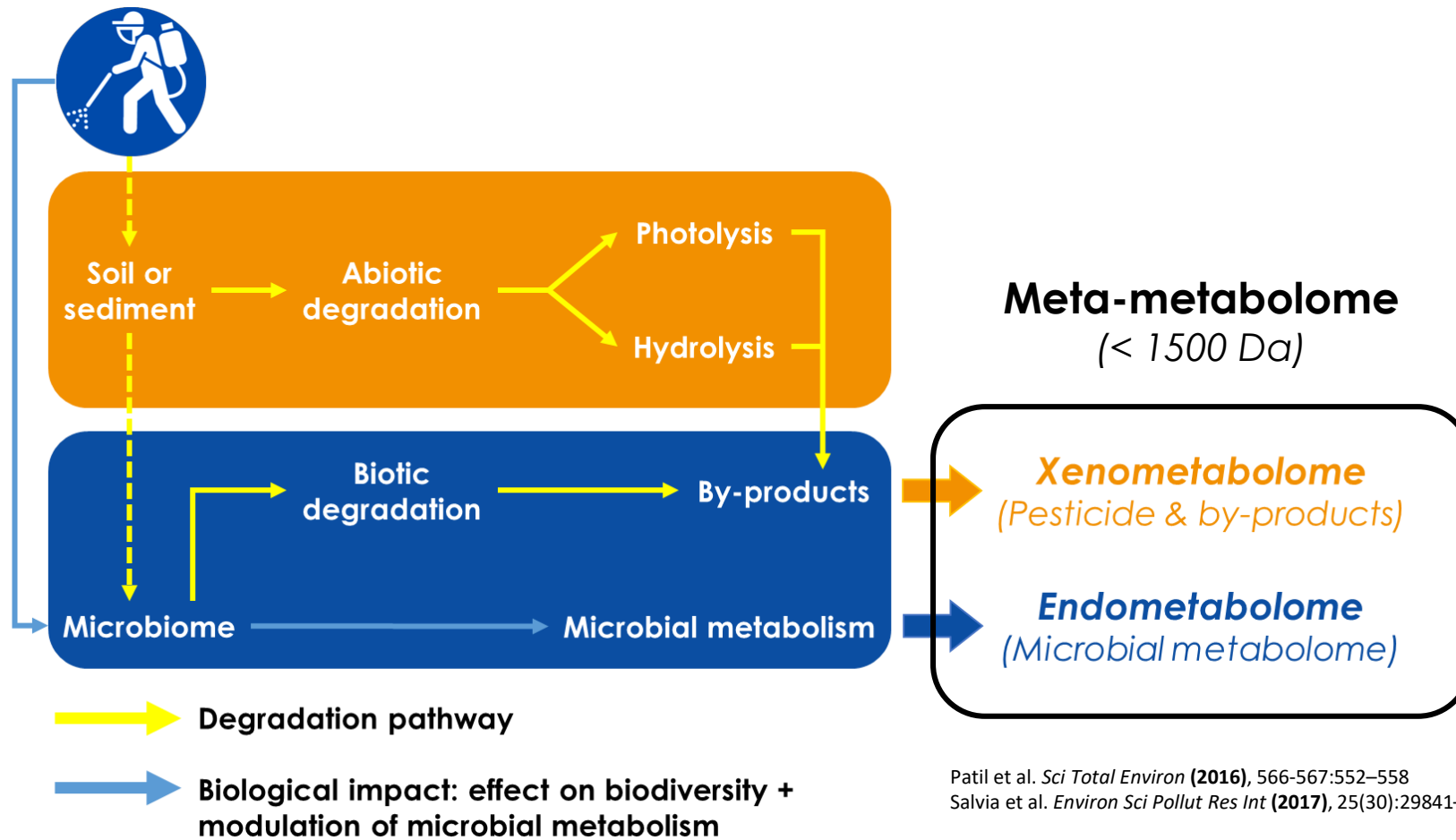
- **Biopesticides (*Biocontrol Agents*) are emerging in order to replace chemical pesticides**
- Usually, Biocontrol Agents have **complex Chemical/Biochemical nature**
- **Classical protocols are non-adaptable with this complexity**
 - ✓ Development of new approaches is needed
 - ✓ Assessing the environmental **fate** and **impact** is mandatory regarding the emerging ecological problems



Project co-financed by the European Regional Development Fund (ERDF)

Context

Environmental Metabolic Footprinting (EMF)



Patil et al. *Sci Total Environ* (2016), 566-567:552–558
Salvia et al. *Environ Sci Pollut Res Int* (2017), 25(30):29841–29847

Objectives

Meta-metabolome extraction

Towards maximizing the meta-metabolic information:

- ✓ Extracting **higher number** of both **xeno** and **endometabolites**
- ✓ Covering a **wide range of polarity** → various families of metabolites
- ✓ Reasonable **balance** between **wide polarity coverage** & **extraction yield**

Aims:

- ✓ Enhancing the detection of **pesticide** components and their TPs (fate)
- ✓ Increasing the **probability** to detect environmental **biomarkers** (Impact)

Development of extraction protocols

- ▶ 2 different types of soil are studied:
 - ✓ Perpignan (Pyrénées-Orientales)
 - ✓ Torreilles (Pyrénées-Orientales)
 - ✓ The aim is to compare two different soils with different physical-chemical properties
- ▶ 3 modalities of soil herbicide treatments with 5 replicates for each:
 - ✓ Untreated Control soil
 - ✓ Soil treated with Tartan Super 360™ / Formulated Glyphosate (*Gly*)
 - ✓ Soil treated with Beloukha® / Formulated Nonanoic acid (*Bel*)
 - ✓ To compare two different soil treatments with different natures & polarities



Development of extraction protocols

- ▶ 5 different protocols with different solvents and reagents were applied:
 - ✓ **E1: Extraction based on acidified (HCl) Ethyl Acetate**
 - ✓ **E2: Extraction based on Methanol**
 - ✓ **E3: Extraction based on Methanol + 1% Formic Acid (FA) (v/v)**
 - ✓ **E4: Broadband extraction based on 4 miscible solvents**
 - ✓ **E5: Broadband extraction based on 4 miscible acidified solvents**



Romdhane et al. *Front Microbiol* (2016), 7:775
Anastasiades et al. *EURL-SRM* (2016), V9.1

Total number of samples: 150

Project co-financed by the European Regional Development Fund (ERDF)

LC-HRMS analyses (Modified C18 - ESI+/QToF)

Soil: Perpignan - Control

Soil: Torrelles - Control

Codes:

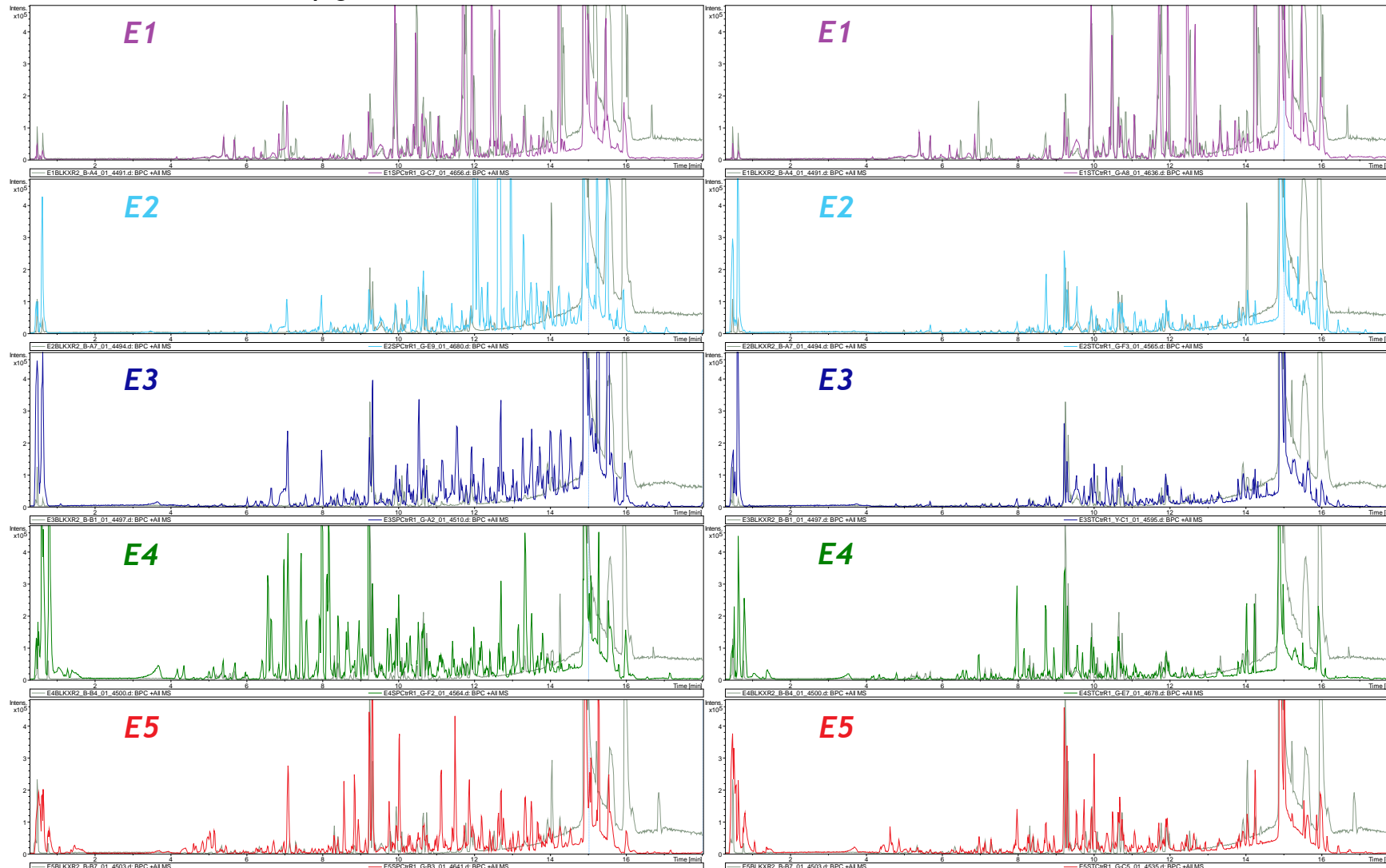
E1: EtOAc + HCl

E2: MeOH

E3: MeOH + FA

E4: BB-4 Solv.

E5: BB-4 Acid Solv.



BPCs: m/z 90-1000, Intensity fixed at 4.8E5
Grey background: Blank Extraction



Project co-financed by the European Regional Development Fund (ERDF)

LC-HRMS analyses (Modified C18 - ESI+/QToF)

Soil: Torreilles - Beloukha® (Bel)

Soil: Torreilles - Tartan Super 360™ (Gly)

Codes:

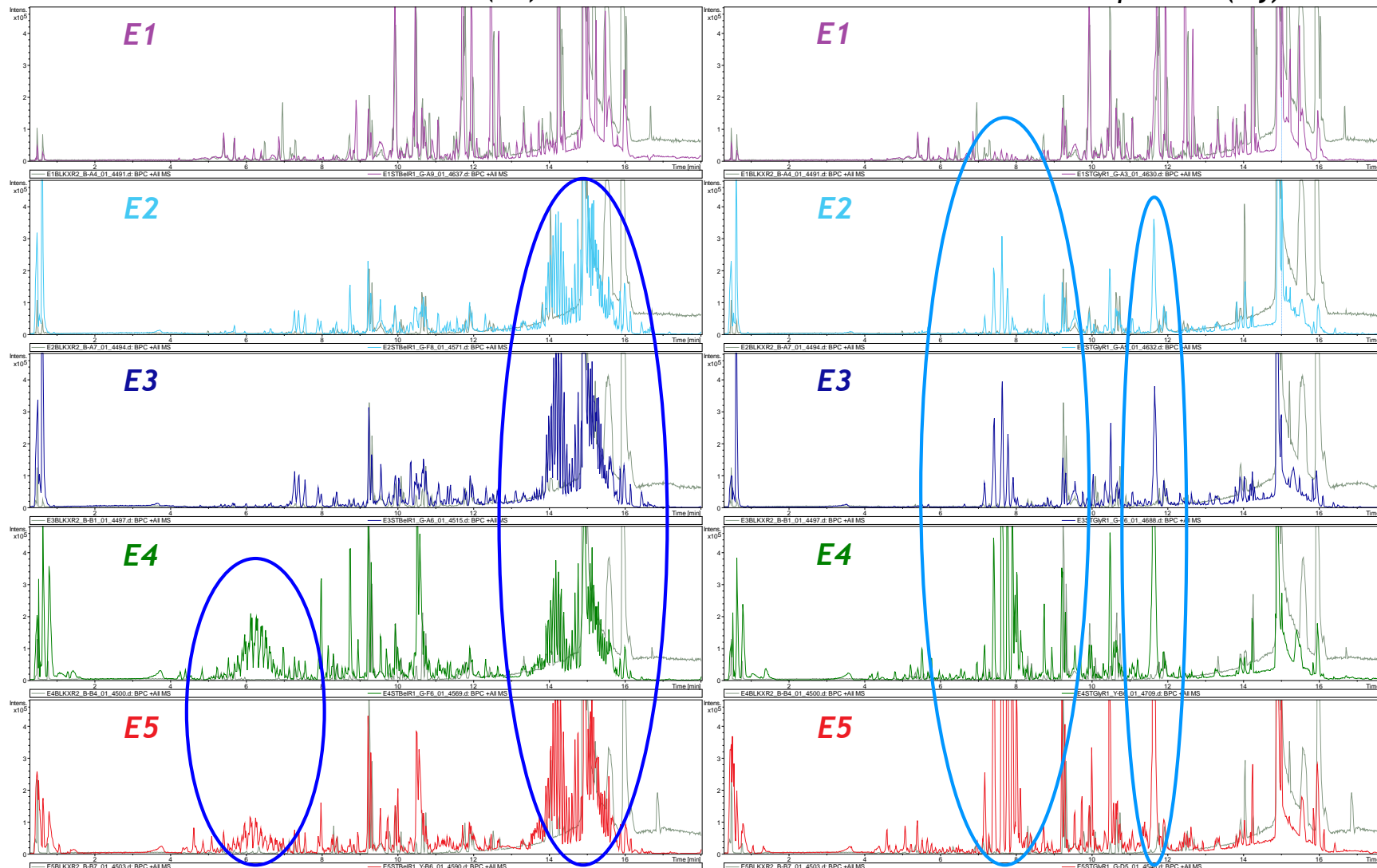
E1: EtOAc + HCl

E2: MeOH

E3: MeOH + FA

E4: BB-4 Solv.

E5: BB-4 Acid Solv.



BPCs: m/z 90-1000, Intensity fixed at 4.8E5
Grey background: Blank Extraction



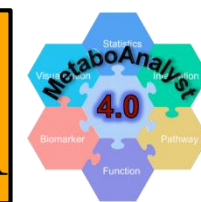
Problematic

How to define the optimal extraction?

► Criteria to judge the optimal extraction:

1. The ability to cover the **largest range of polarity** (qualitative)
2. The best **extraction yield** (quantitative)
3. The best **reproducibility**
4. The **performance in discrimination** between spiked and control groups (EMF objective)

*Untargeted metabolomics preprocessing & statistical tools:
To be exploited...*



Project co-financed by the European Regional Development Fund (ERDF)

Data Analyses

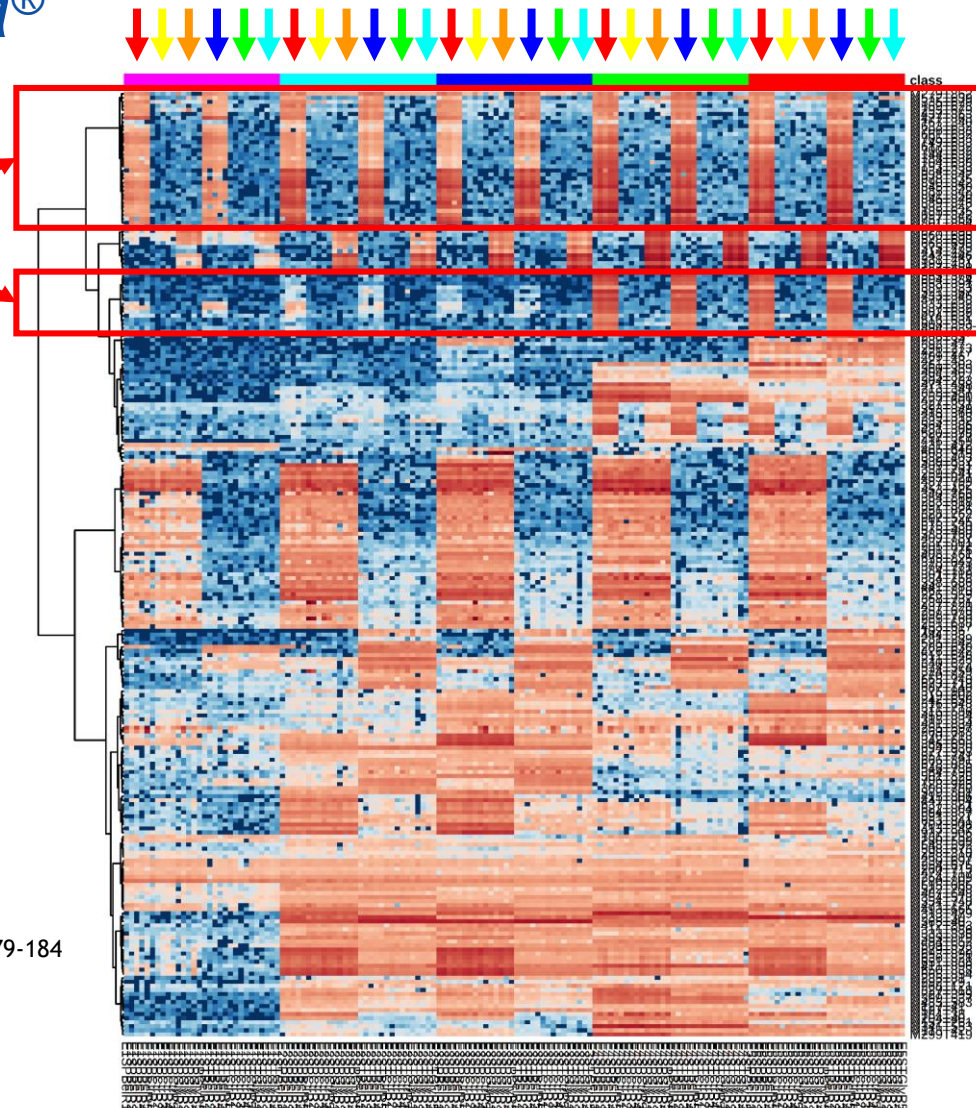
Heatmap - Beloukha®

Xenometabolome originating from Beloukha® herbicide

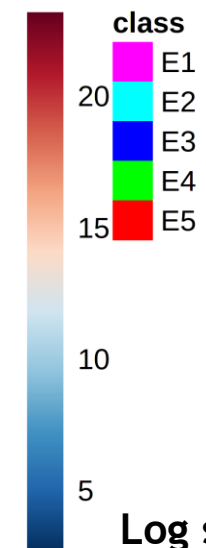
Condition indicators:

- ➔: Soil Perpignan - Bel
- ➔: Soil Perpignan - Ctr
- ➔: Soil Perpignan - Gly
- ➔: Soil Torreilles - Bel
- ➔: Soil Torreilles - Ctr
- ➔: Soil Torreilles - Gly

Wilkinson & Friendly. *Am Stat* (2009), 63(2):179-184



- ✓ Intensity increasing from E1 to E5
- ✓ Xenometabolites only detected in E4 and E5
- ✓ Highest intensities in E4



- E1: EtOAc + HCl*
- E2: MeOH*
- E3: MeOH + FA*
- E4: BB-4 Solv.*
- E5: BB-4 Acid Solv.*

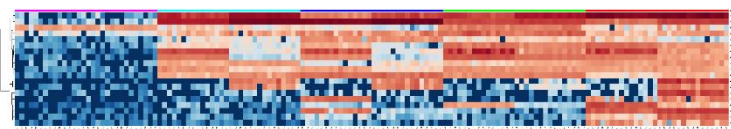
Data Analyses

Heatmap - Endometabolome

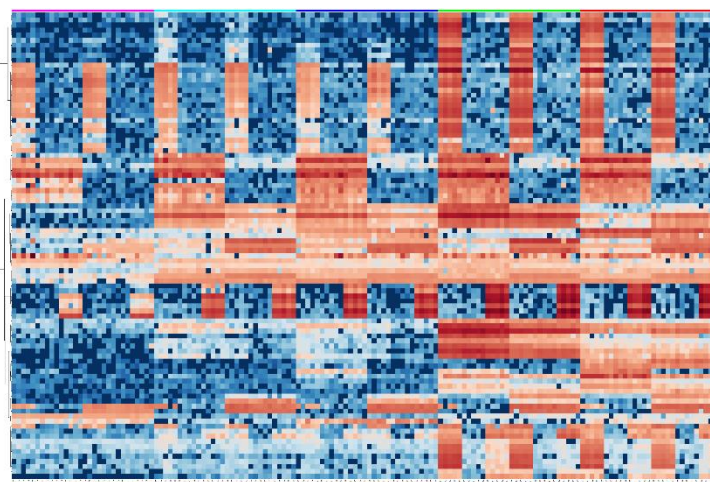
- Data matrix was split into 3 sub-data matrices according to the polarity scale (LogP3):

LogP3: -0.5
RT: 4.20 min
2'-Deoxyadenosine (MS/MS, Lev2)

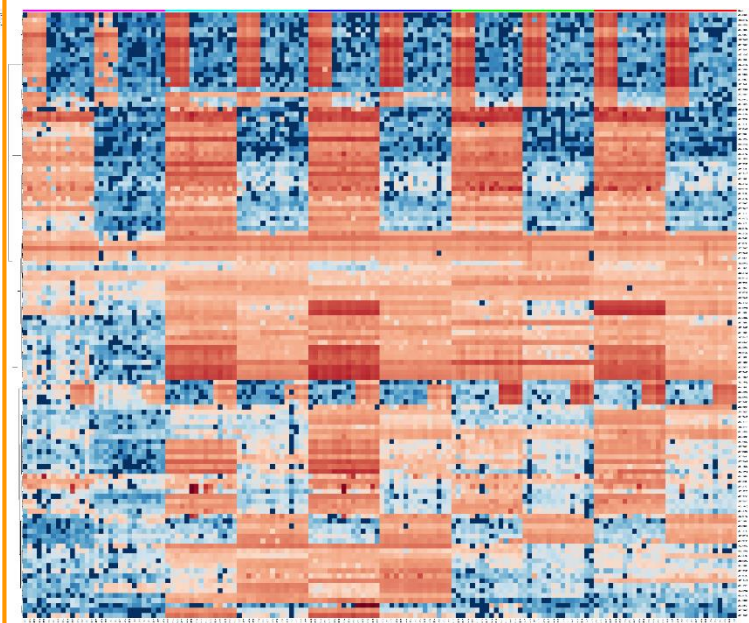
LogP3: 4.4
RT: 10.75 min
Diclofenac (Internal Standard)



Polar zone
19 metabolites



Semi-Polar zone
93 metabolites

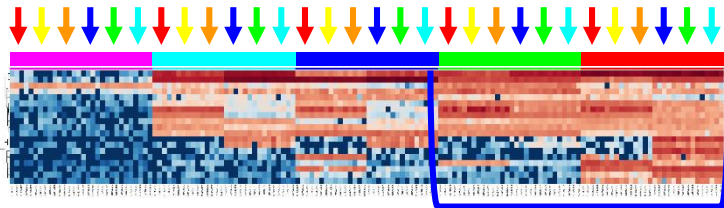


Non-Polar zone
122 metabolites

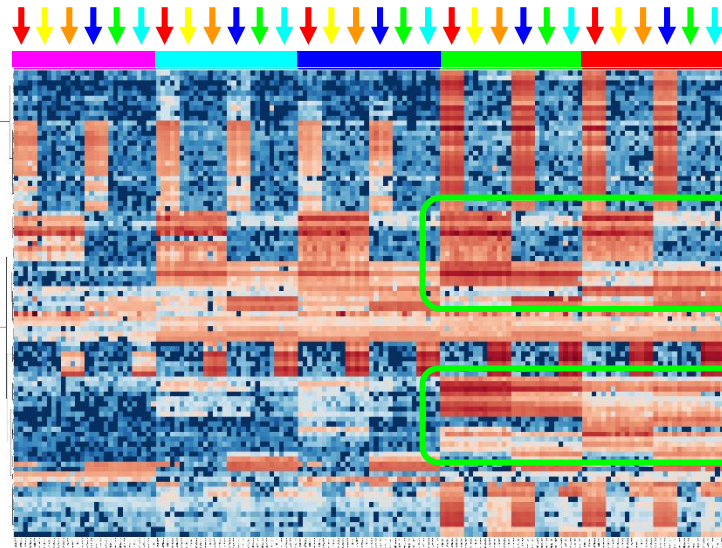
Project co-financed by the European Regional Development Fund (ERDF)

Data Analyses

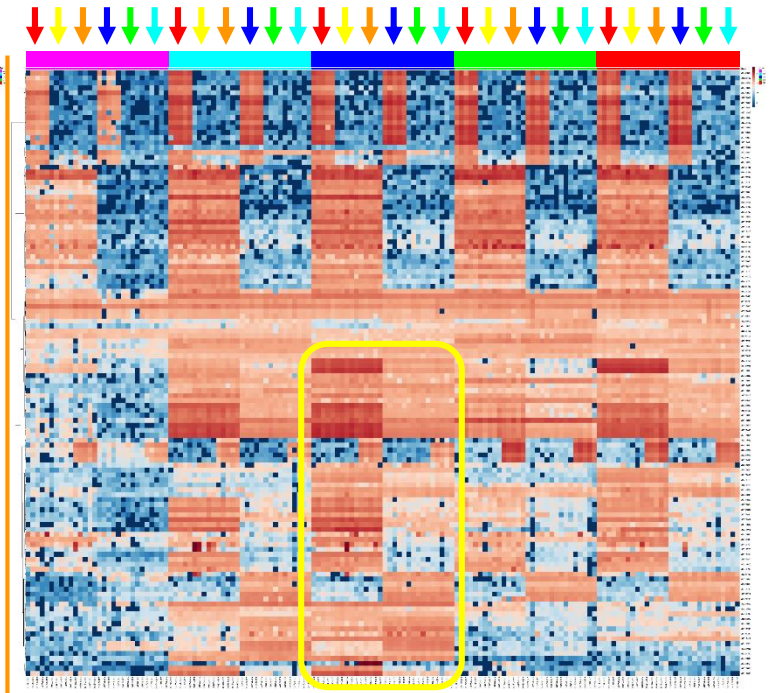
Heatmap - Endometabolome



- ✓ Highest number and intensities in E5, then in E4
- ✓ Only one metabolite is more intense in E3



- ✓ Highest number and intensities in E4, then in E5



- ✓ Highest number and intensities in E3

Condition indicators:

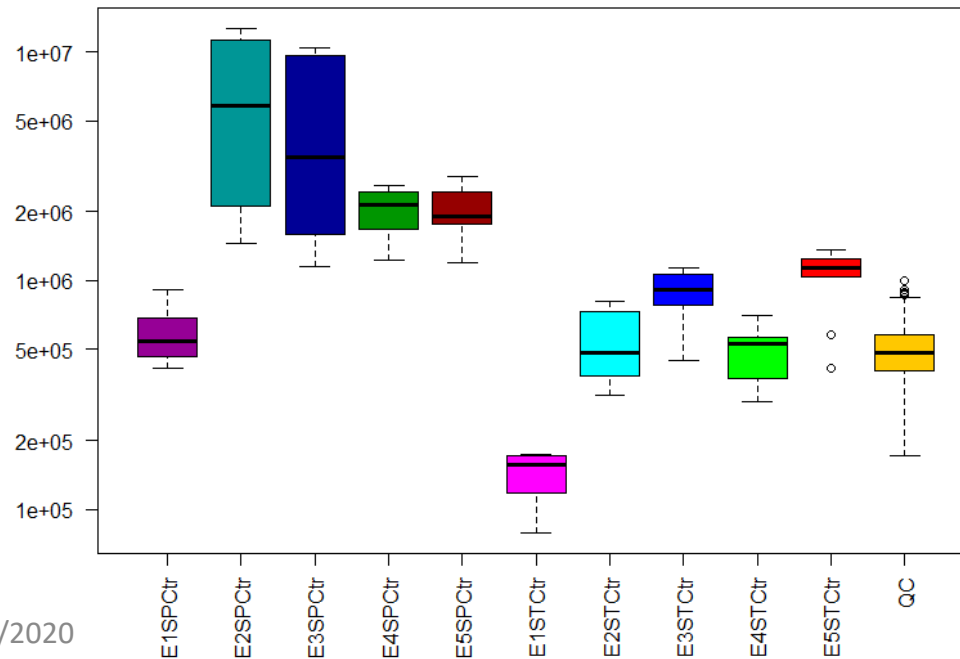
- ➔: Soil Perpignan - Bel
- ➔: Soil Perpignan - Ctr
- ➔: Soil Perpignan - Gly
- ➔: Soil Torreilles - Bel
- ➔: Soil Torreilles - Ctr
- ➔: Soil Torreilles - Gly
- : E1 - EtOAc + HCl
- : E2 - MeOH
- : E3 - MeOH + FA
- : E4 - BB-4 Solv.
- : E5 - BB-4 Acid Solv.

Data Analyses

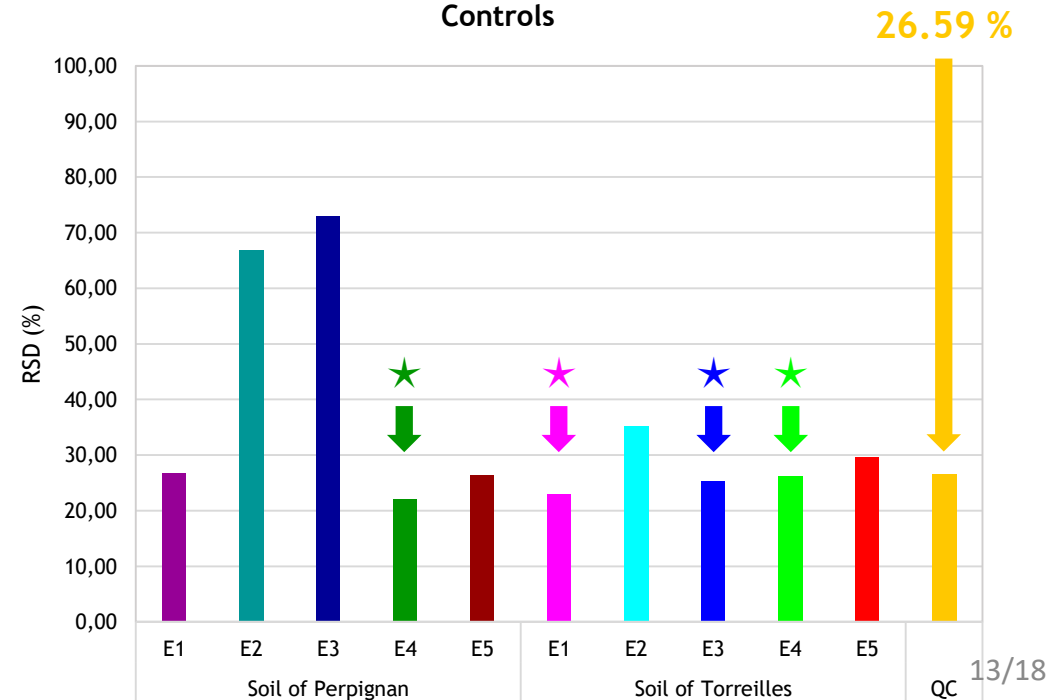
Euclidean Distances - Assessment of extractions reproducibility

- ▶ Euclidean Distances calculation aim to assess extraction's "inter-replicates" reproducibility
- ▶ For Controls: E4 showed the best score for the SP (Soil of Perpignan): 22.07 % of RSD
- ▶ For ST (Soil of Torrelles), best scores of E1, E3 and E4 were comparable: 23.03-26.29 % of RSD

Boxplots for Euclidean Distances Controls



RSD (%) of Euclidean Distances Controls

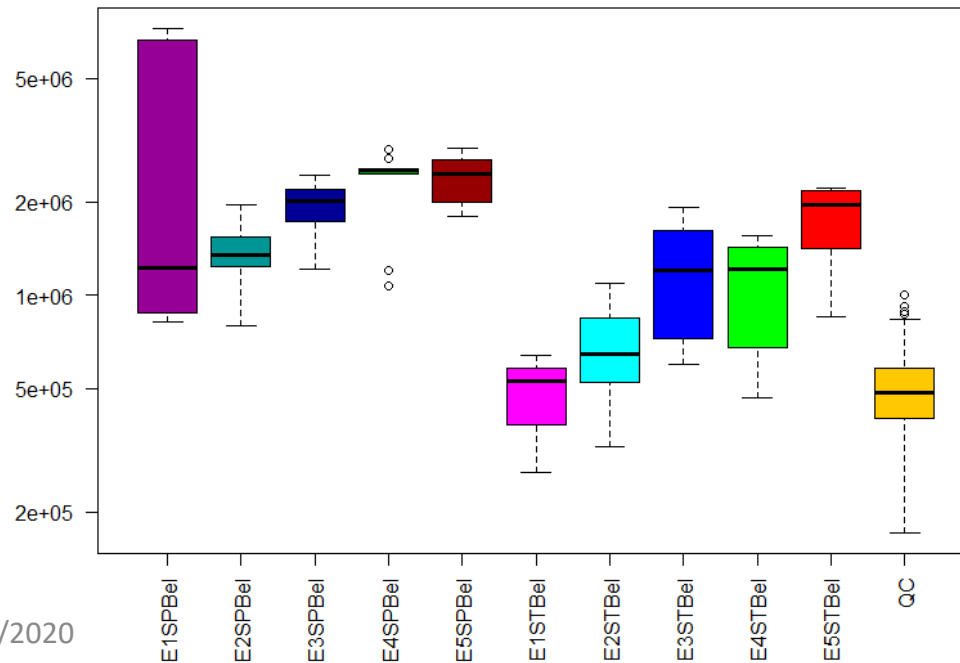


Data Analyses

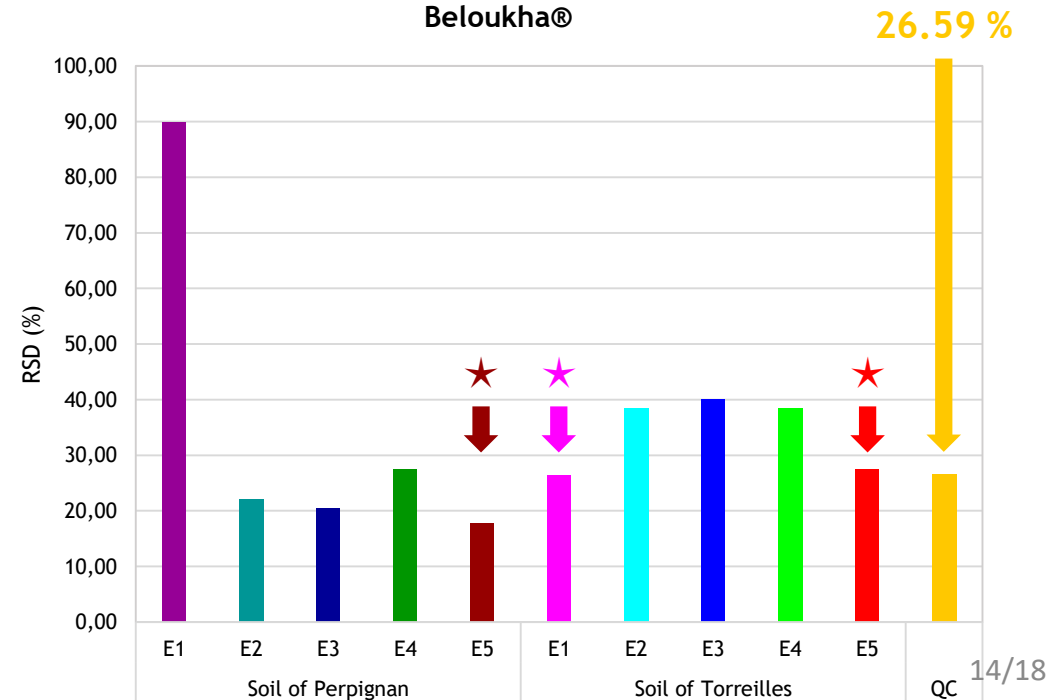
Euclidean Distances - Assessment of extractions reproducibility

- ▶ Euclidean Distances calculation aim to assess extraction's "inter-replicates" reproducibility
- ▶ For Beloukha®: E5 showed the best score for the SP: 17.66 % of RSD
- ▶ For ST, best scores of E1 and E5 were comparable 26.50-27.50 % of RSD

Boxplots for Euclidean Distances
Beloukha®



RSD (%) of Euclidean Distances
Beloukha®

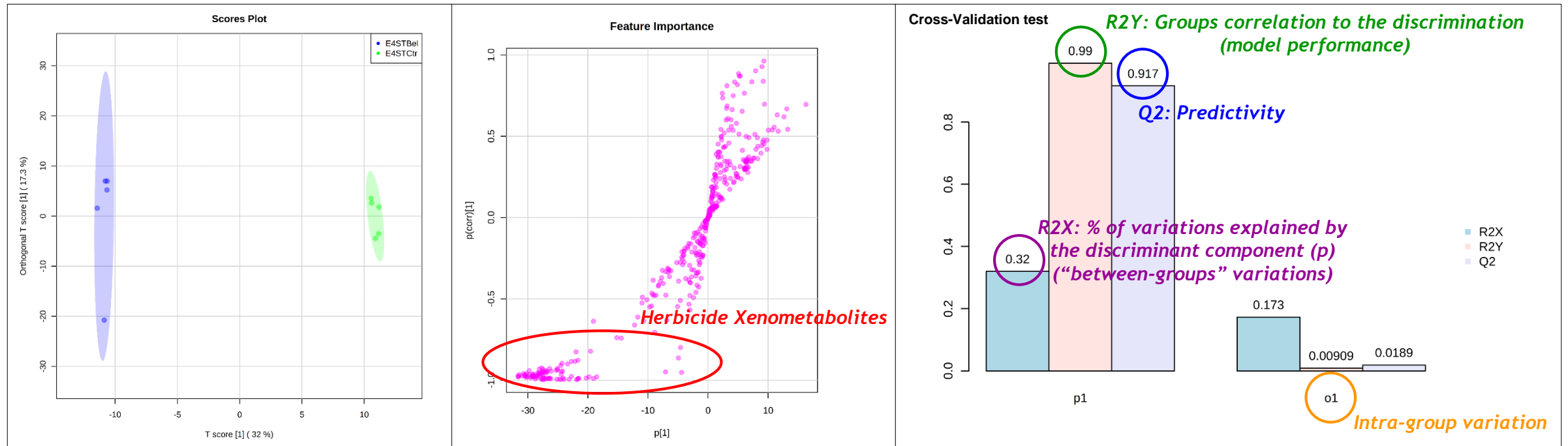


Data Analyses

OPLS-DA - Extractions discrimination power

- ▶ OPLS-DA is an **explicative, supervised** multivariate statistical analysis
- ▶ It can allow to assess the **ability** and the **predictivity** of an extraction to **discriminate** between a **control group** of soil samples and a **herbicide-spiked group** of soil samples

Trygg & Wold. *J Chemometrics* (2002), 16:119-128
Boccard & Rutledge. *Anal Chim Acta* (2013), 769:30-39



Data Analyses

OPLS-DA - Beloukha®

- For Beloukha®, E4 is the most discriminant and predictive in both soils (*according to R2X & Q2*)
- E4 is also the most discriminant and predictive for Tartan Super 360™ in both soils (*data not shown*)

Beloukha®	Soil of Perpignan						Soil of Torreilles					
	OPLS-DA (p1)			OPLS-DA (o1)			OPLS-DA (p1)			OPLS-DA (o1)		
	R2X	R2Y	Q2	R2X	R2Y	Q2	R2X	R2Y	Q2	R2X	R2Y	Q2
E1	22.30	99.20	78.80	10.80	00.70	04.37	20.30	99.00	73.10	10.70	01.02	02.77
E2	29.90	99.40	89.30	12.80	00.57	02.03	29.30	98.80	87.40	10.40	01.13	01.81
E3	26.40	98.50	82.40	14.30	01.40	03.20	25.60	97.50	81.70	13.00	02.43	02.79
E4	36.20	99.70	94.20	12.00	00.30	01.06	32.00	99.00	91.70	17.30	00.91	01.89
E5	34.10	98.50	90.30	11.10	01.43	01.26	31.20	99.70	91.10	11.90	00.25	01.99

Conclusions

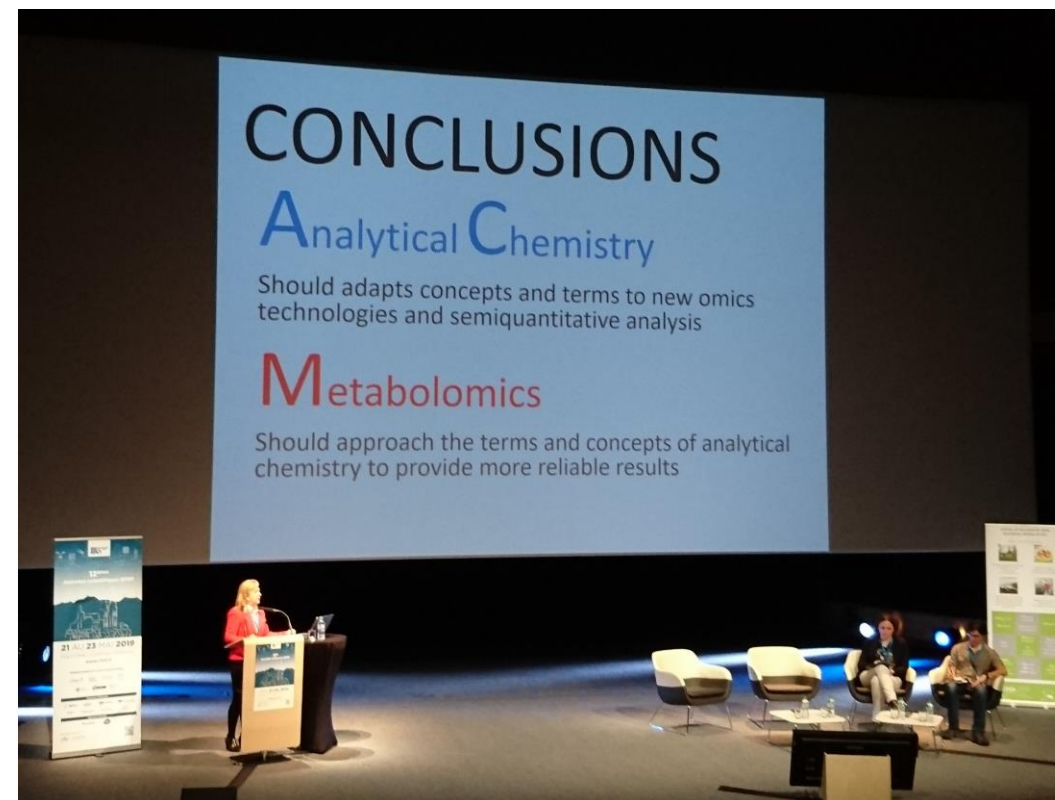
- “Optimum”: compromise between **analytical chemistry basics** and the **objectives of the study**
- Computational tools can be used to assess this “optimum”:
 - ✓ **Heatmap** → **exhaustive qualitative and quantitative** assessment of methods
 - ✓ **Euclidean Distances** → **assess methods’ reproducibility**
 - ✓ **OPLS-DA** → **methods’ ability to respond to the environmental objective:**
the discrimination between two conditions
- ★ **Broadband extractions based on miscible solvents are potentially promising for a highly-exhaustive Environmental Metabolic Footprinting approach**

Ghosson et al. *In-prep.*

Conclusions

Not only Analytical Chemistry is in favor of Metabolomics, Metabolomics and its tools can also be in favor of Analytical Chemistry that, in turn, is dedicated to respond for Metabolomics needs.

Analytical Chemistry & Metabolomics are thus in a “dialectic” relation.



Prof. Coral Barbas' Plenary Session (P1) at the 12JSRFMF May 21, 2019, Clermont-Ferrand, France

Acknowledgments

CRIOBE, Perpignan

Pr. Cédric Bertrand

Dr. Marie-Virginie Salvia, H.D.R.

Mélina Ramos, Ph.D. Std.

Yannick Brunato, M.Sc. Std.

Delphine Raviglione, M.Sc.

Jennifer Sola, M.Sc.

Marie-Louise Mascunano, Admin.

CRMPO, Rennes

Dr. Nicolas Le Yondre

Philippe Jehan, Engr.

CEFREM, Perpignan

Dr. Thierry Courp

Dr. Bruno Charrière

LABERCA, Nantes

Dr. Yann Guitton

Ecologie Microbienne, Lyon

Dr. Anne-Emmanuelle Hay, H.D.R.

BAE-LBBM, Perpignan

Pr. Thierry Noguier

#BBBS

Thank you!

Interreg
POCTEFA
PALVIP



UNIÓN EUROPEA
UNION EUROPÉENNE



EUROPEAN UNION
European Regional Development Fund



Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE



Ecole Doctorale UPVD
UNIVERSITÉ PUY DU FOU



Biodiversité & Biotechnologies Marines



RESEAU FRANCOPHONE DE METABOLOMIQUE ET FLUXOMIQUE