Workshop:
How to commercialise research results
in the Western Balkans?

Development of a standard at the International Standard
Organization in the frame of a JERP

Fabrice Martin-Laurent; INRA
National Institute for Agricultural Research (France)
Development and evaluation of innovative tools to estimate the ecotoxicological impact of low dose-pesticide application in agriculture on soil functional microbial biodiversity (SEE-ERA-PLUS 216)

Ecofun-Microbiodiv http://www4.inra.fr/ecofun_microbiodiv
Introduction to SEE-ERA-PLUS-216

• Partners

F Martin-Laurent (FML), Coordinator, Dijon

S Djuric (SD), Faculty of Agriculture, Novi-Sad

I Petric (IP), Rudger Boskovic institute, Zagreb

Pr E Kandeler, Uni Hohenheim, Hohenheim

D Karpouzas, Uni of Thessaly, Thessaly
Introduction to SEE-ERA-PLUS-216

• **Context:**

Soil microbiota: a unique reservoir of biodiversity

The soil: - reservoir of microbial diversity
- natural patrimony

1 g of soil

1 billion of bacteria
1 million of species
1.500 t/ha

Microbiota = component of soil quality

Quality of the environment

1 million of fungi
10,000 to 100,000 species
3.500 t/ha
Introduction to SEE-ERA-PLUS-216

**Context:**

- Soil protection strategy
  - Good agricultural practices
  - Regulation on chemical and pesticides

**Soil protection**

- Pesticide waste management
- Agricultural practices
- Pesticides applications, fertilizer and organic amendments de boues in agro-systems.

**Indirect:**
- Environmental threat:
  - Modification of soil living biodiversity.

**Direct:** (on soil ecosystem functions)
- Modifications of the filtering and biotransformation abilities of soil.

- Soil contamination with organic (pesticides,...) and inorganic (heavy metals,...) pollutants

**Soil rehabilitation**

- Restriction of soil usage
- Bioremediation program

Towards the EU directive for Soil Protection

Novi-Sad, May 22-24, 2013
Introduction to **SEE-ERA-PLUS-216**

**Context:**

<table>
<thead>
<tr>
<th>Service*</th>
<th>Functions</th>
<th>Value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climate regulation</td>
<td>C &amp; N cycles</td>
<td>684</td>
</tr>
<tr>
<td>Nutrient cycles</td>
<td>Cycle nutriments</td>
<td>17,075</td>
</tr>
<tr>
<td>Waste treatment</td>
<td>Purifying</td>
<td>2,277</td>
</tr>
<tr>
<td>Run-off control</td>
<td>Soil preservation</td>
<td>576</td>
</tr>
<tr>
<td>Soil formation</td>
<td>Pedogenese</td>
<td>53</td>
</tr>
</tbody>
</table>

**Sub-Total** 20,665

17 known services **Total** 32,268

Defines and gives economic value to ecosystemic services including soil ecosystemic services depending on microbial communities (Costanza et al. 1997, Nature, vol 387).

---

[Table and text related to ecosystem services and their economic value.]

---

**MILLENNIUM ECOSYSTEM ASSESSMENT**

---

**INRA**

WBC-INCO.net Workshop  Novi-Sad, May 22-24, 2013
Introduction to SEE-ERA-PLUS-216

- Pesticide registration *a priori* estimation of the impact


Estimation of the impact of pesticides on soil microbiota:

- **Modified Sturm test**: estimation of pesticide biodegradability and of pesticide on the biodegradation of a reference substrate (sodium acetate or benzoate) [OECD 301B]

- **Carbon mineralization** [OECD 217]

- **Nitrogen mineralization** [OECD 216]

☞ Global tests which may not fully revealed the impact of pesticides on functional soil microbial communities and consequences on soil ecosystem services.
Introduction to **SEE-ERA-PLUS-216**

- *a posteriori* estimation of the impact of pesticides

European Union (EU) **water framework** (Directive 2000/60/EC) successively revised (2455/2001/EC, 2008/32/EC, 2008/105/EC et 2009/31/EC) fixed water potability for human consummation at -0.1 µg.l$^{-1}$ for a single compound, -0.5 µg.l$^{-1}$ for a mixture of compounds.

European Union (EU) has been preparing the **soil protection framework** but this project remains blocked and as a consequence - no obligations have to be followed to preserve soil quality for a sustainable agriculture.
Introduction to **SEE-ERA-PLUS-216**

- **Aim:** Estimation of the impact of ‘sulfonylureas’ (low dose herbicides) on soil ecosystemic services

![Diagram showing ecosystemic services]

- **Density**
- **Activity**
- **Diversity**
- **Soil microbiota functioning**
- **Pesticide application**
- **Impact**

**Soil ecosystemic services**
Work plan of **SEE-ERA-PLUS-216**

Development and evaluation of innovative tools to estimate the ecotoxicological impact of low dose pesticide application in agriculture on soil functional microbial biodiversity.

- **WP1 Management, coordination and process review**
- **WP2 Dissemination - Stakeholder Oriented and Policy Implications**
- **WP3 Set up of field and greenhouse experimentations**
- **WP4 Agronomical monitoring**
- **WP5 Pesticide monitoring**
- **WP 6 Microbial monitoring:**
  - 6.1 microbial biodiversity
  - 6.2 functional biodiversity
  - 6.3 acetohydroxyacid synthase
  - 6.4 adaptation to sulfonyle urea biodegradation
**Estimation of the impact of low dose herbicides on soil ecosystemic services**

WP2 Dissemination - Stakeholder Oriented and Policy Implications

**International Standard Organization** (ISO) and national standardization bodies (DIN for Germany and AFNOR for France) [21 countries: Austria, Belgium, Czech Republic, Denmark, Egypt, Finland, France, Germany, Italy, Japan, Republic of Korea, Mongolia, Netherlands, Norway, Poland, Portugal, Russian Federation, Sweden, Turkey, Ukraine, United Kingdom]

1-Overview of existing standards and evaluation of their pertinence for estimating pesticide impact on soil microbial communities,

2- Propose new methods for standardization.

**European Food Safety Agency** (EFSA)

- Pesticides Unit and the Panel on Plant Protection Products and their Residues (PPR): EFSA’s task is to carry out the risk assessment for pesticides and to provide the European Commission with scientific support in decision making processes

- Presentation of the results of Ecofun Microbiodiv
Estimation of the impact of low dose herbicides on soil ecosystemic services

WP 6 Microbial monitoring:

1- Test the pertinence of existing standards for estimating pesticide impact on soil microbial communities,

2- Develop, test and propose new methods for estimating pesticide impact of soil microbial communities for standardization.
WP 6 Microbial monitoring: 1- Test the pertinence of ISO standards

Over the 13 existing standards 7 were used in the Ecofun Microbiodiv to test their suitability to estimate the impact of low-dose herbicides onto the functional diversity of soil microflora.

Pr Ellen Kandeler, Uni Hohenheim

C Hénault and C Mougin, INRA

F Martin-Laurent, INRA
WP 6 Microbial monitoring: Development and test of innovative methods

Methodology

Soil samples → DNA extraction ISO 11063 → Real time-PCR

16S and 18S rRNA (11 bacterial groups)

Functional groups (endomycorrhiza, AHAS and amoA)

Amplification 16S–23S ISR/18S-5S ISR AHAS

A-RISA Fingerprint → RFLP analysis → PCA

Density analysis NWIP ISO 17601

Structure analysis
Steps for standardizing a new method within ISO Framework

A six steps process including two key steps (go/no go):

- Acceptance of the new work item
- International interaboratory test

MINIREVIEW

Standardisation of methods in soil microbiology: progress and challenges
Laurent Philippot¹, Karl Ritz², Pascal Pandard³, Sara Hallin⁴ & Fabrice Martin-Laurent⁴,⁵

Fig. 1. Flow chart summarising the different steps for standardising a new test method within the ISO framework.
Chronology of the emergence of the ISO 11063

2004 Presentation of soil DNA extraction method to the AFNOR
2005 Presentation of the project to the ISO (London)
2006 Preparation of the working draft
2007 Discussion about the Working draft (Deft and Berlin)

2011 Unanimous approval of the standard
2012 Publication of the ISO standard and translation to French (AFNOR) and German (DIN). CEN standard
WP2 Dissemination - Stakeholder Oriented and Policy Implications

☑ Presentation to AFNOR project of standard ‘Estimation of abundance of selected microbial gene sequences by quantitative realtime PCR from DNA directly extracted from soil’ (Paris, France, March 2011)

☑ Presentation to ISO TC190/SC4/WG4 project of standard (Adelaide, Australia, September 2011): **NWI ISO 17601**


Resolution Berlin-1

F Martin-Laurent is kindly asked for producing the revised draft of NWI 17601 at the beginning of July 2012.

Resolution Berlin-2

A ring test on qPCR will be organised. Partners of the EU-Projects ECOFINDERS, TRAINBIODIVERS and ECOFUN-MICROBIODIV will be invited to join the ring test.

☑ Presentation of revised draft of NWI 17601 and opening of the call for international reing-test at annual meeting of ISO TC190/SC4/WG4 (Helsinki, Finland, 16-17 March 2012)
Emergence of NWI 17601 within the framework **SEE-ERA-PLUS-216**

**WP2 Dissemination - Stakeholder Oriented and Policy Implications**

- Resolution of the annual meeting of Helsinki (September 2012)
  
  **RESOLUTION N 4/2012 (Helsinki)**
  **ISO 17601 – Soil quality – Estimation of abundance of selected microbial gene sequences by quantitative realtime PCR from DNA directly extracted from soil**

  ISO/TC 190/SC 4 “Soil quality – Biological methods” asks Ms Najoi El-AZHARI and Mr Fabrice MARTIN-LAURENT to revise the document in the light of discussion at the meeting by the end of October 2012. This Draft will be submitted to the Secretariat of SC 4 for submission to CD voting (and be the beginning of half of 2013).

  _The resolution is adopted unanimously_

- Revision was submitted to ISO in 2012

- Result of the vote 04-10-2013

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>abstain</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

**Answers to Q.1: “Do you agree to the circulation of the draft as a DIS?”**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14 x</td>
<td>Yes</td>
<td>Egypt (EOS) France (AFNOR) Germany (DIN) Italy (UNI) Japan (JIS) Korea, Republic of (KATS) Mongolia (MASM) Norway (SN) Poland (PKN) Russian Federation (GOST R) Sweden (SIS) Turkey (TSE) Ukraine (DSSU) United Kingdom (BSI)</td>
</tr>
<tr>
<td>1 x</td>
<td>Yes with comments</td>
<td>Czech Republic (UNMZ)</td>
</tr>
<tr>
<td>0 x</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6 x</td>
<td>We abstain</td>
<td>Austria (ASI) Belgium (NBN) Denmark (DS) Finland (SPS) Netherlands (NEN) Portugal (IPQ)</td>
</tr>
</tbody>
</table>
Organization of the international ring test within the framework **SEE-ERA-PLUS-216**

- First call for international ring test aiming at evaluating a method to estimate the abundance of selected microbial gene sequences by quantitative realtime PCR from DNA directly extracted from soil published by ISO the 11 december 2012.

<table>
<thead>
<tr>
<th>Partners</th>
<th>Responsible</th>
<th>EU program</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-INRA Dijon, France</td>
<td>F Martin-Laurent</td>
<td>Ecofun</td>
</tr>
<tr>
<td>B-Uni of Thessaly, Greece</td>
<td>D Karpouzas</td>
<td>Ecofun</td>
</tr>
<tr>
<td>C-ENOVEO, Lyon, France</td>
<td>C Malandain</td>
<td>Love-to-Hate</td>
</tr>
<tr>
<td>D-Uni de Santiago, Portugal</td>
<td>C Marques</td>
<td></td>
</tr>
<tr>
<td>E- Helmotz Zentrum, Munchen, Germany</td>
<td>M Scholter</td>
<td>Trainbiodivers</td>
</tr>
<tr>
<td>F-Uni of Florence, Italy</td>
<td>P Nanipieri</td>
<td>Trainbiodivers</td>
</tr>
<tr>
<td>G-Uni of Hohenheim, Germany</td>
<td>S Marhan</td>
<td>Ecofun</td>
</tr>
<tr>
<td>H-Institut Rudjer Boskovic, Croatia</td>
<td>I Petric</td>
<td>Ecofun</td>
</tr>
<tr>
<td>I-Uni of Uppsala, Sweden</td>
<td>S Hailin</td>
<td>Ecofinders</td>
</tr>
</tbody>
</table>

9 participants, 7 countries

- Kick off meeting of international ring test scheduled in June 2013.

- Revision of DIS draft to be submitted in July 2013

- Annual ISO meeting in Japan in September 2013
Communication of SEE-ERA-PLUS 216 consortium with EFSA

ECOFUN-MICROBIODIV: an FP7 European project for developing and evaluating innovative tools for assessing the impact of pesticides on soil functional microbial diversity towards new pesticide registration regulation?

- First contact established with EFSA (Theo Brock, 11/30/2012)

- Recently EFSA offered the coordinator of Ecofun-Microbiodiv the possibility to act as an external expert for the Working Group for the in soil Risk assessment of the Panel on Plant Protection Products and their Residues (PPR).

- Oral presentation of Ecofun-Microbiodiv results to Pesticide congress organized in York, UK, September 2013 (organized with the support of EFSA)

- Presentation of Ecofun-Microbiodiv results to PPR to be scheduled in the second half of year 2013.
What’s next step?

- Transfer of research results to develop standards to monitor the impact of pollutants on soil microbial communities diversity and activity

- This activity offers new opportunities for changing the regulation to preserve soil diversity and functioning
  - by improving pesticide registration (a priori evaluation)
  - by implementing soil monitoring (a posteriori evaluation)

- Implementation of new strategies for developing sustainable pesticides

- *in fine*, this may lead to the development of a business activity for assessing the quality of soil microbial community
  - Development of soil DNA extraction kit (ISO proof)
  - Development of qPCR kit for quantifying the abundance of microbial groups
  - ....
Many thanks to SEE-ERA-PLUS-216 partners

• Partners

S Djuric, Faculty of Agriculture, Novi-Sad

I Petric, Rudger Boskovic institute, Zagreb

Pr E Kandeler, Uni Hohenheim, Hohenheim

D Karpouzas, Uni of Thessaly, Thessaly
• Contact information

• url: http://www4.inra.fr/ecofun_microbiodiv
• MARTIN-LAURENT F 33 3 8069 3406
• fabrice.martin@dijon.inra.fr