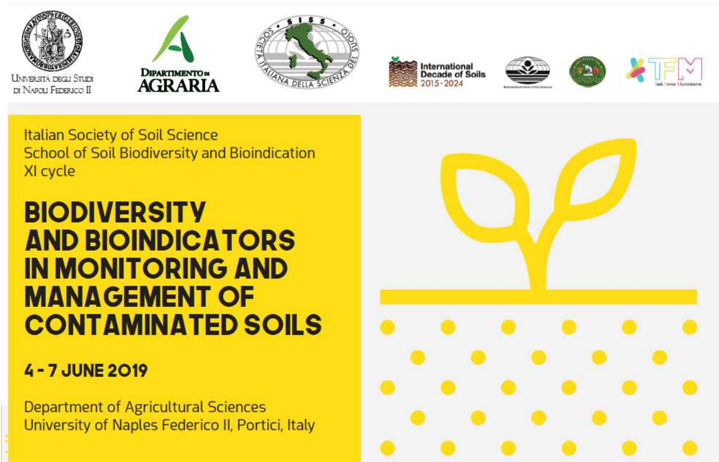
Bioindicators suitable for soil quality monitoring and risk assessment



Guénola Pérès
Agrocampus Ouest
UP Sciences du sol
UMR SAS









- **Introduction**
  - Definition of bioindicator/ biomarker
  - Why do we need bioindicators ?
  - Interest of soil organisms (weight, diversity, functions)
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  Diagnostic (Risk assessment / soil quality), management (monitoring)

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- Conclusion

Definitions

Bioindicateurs and biomarkers

➢ Bioindicateur

Organism (or part of an organism or community of organisms) which indicates by its presence or absence and its behaviour, the state and functionning of an ecosystem

Blandin (1986) ; van Gestel et van Brummelen (1996)

➢ Biomarker

Any biological response to an environmental chemical at the below individual level, measured inside an organism or in its products (urine, faeces, hairs, feathers, etc.).

Term restricted to biochemical, physiological, histological and morphological (including appearance, pigmentation, surface deformation, etc.) measurements of 'health' and exclude behavioural effects (van Gestel et van Brummelen, 1996)









Context

Why do we need bioindicators ?

❖ Soil = a non-renewable resource that must be protected

❖ Millenium Ecosystem Assessment (MEA, 2005) reinforced importance of soil for human well-being

➔ Soils are major component of ecosystems for delivering goods and ecosystem services (services of support, provision, regulation, cultural)

❖ Soil degradation = still accelerating des sols = en accélération

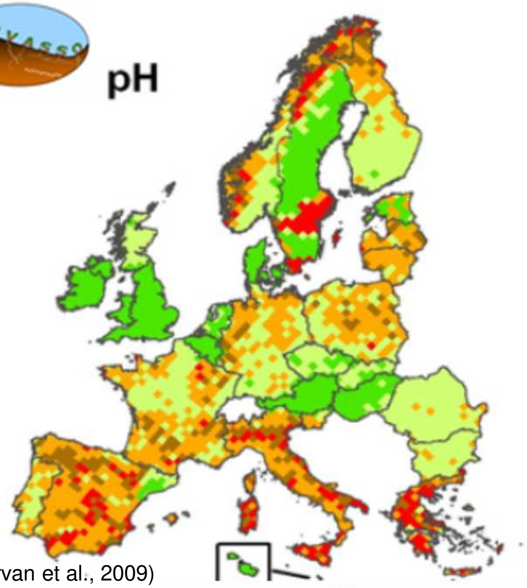
➔ Risk: erosion, contamination, compaction, loose of organic matter, loose of biodiversity.

↳ Need of indicators that reveal and evaluate soil quality degradation and/or soil restauration

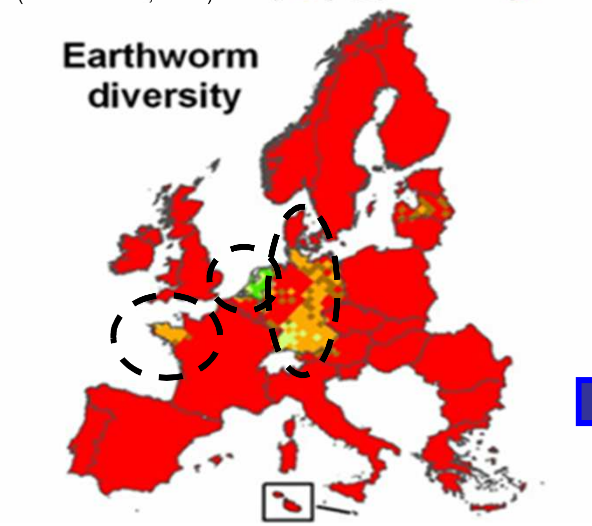
        

Which indicators are used for soil quality assessment?

At european scale



(Morvan et al., 2009)



European project ENVASSO (2007)

Objectives :

- *List the survey networks dealing on soil quality*
- *List the indicators which are used*

➔ **Main Results**

- Classical Indicators: physical and chemical, used all over Europe
- Lack of bioindicators to describe soil quality *(D, NL, F)* (Bispo et al., 2009)

Soil monitoring in Europe: A review of existing systems and requirements for harmonisation

X. Morvan[a,b,], N.P.A. Saby[a], D. Arrouays[a], C. Le Bas[a], R.J.A. Jones[c], F.G.A. Verheijen[c], P.H. Bellamy[c], M. Stephens[c], M.G. Kibblewhite[c]*

Real need to develop research programs dealing on soil biodiversity
= to complet chemical and physical indicators









The weight of soil organisms under your feet

Under soil pasture

260 millions of fauna/m²

150 g of invertebrates (earthworms)/m²

➔ 1.5 T per hectare

= the weight of 2 cows per hectare

300 g of microflora

➔ 3 T per hectare (4 cows)

➔ Huge biomass and abundance

Under forest soil
under a hiker foot

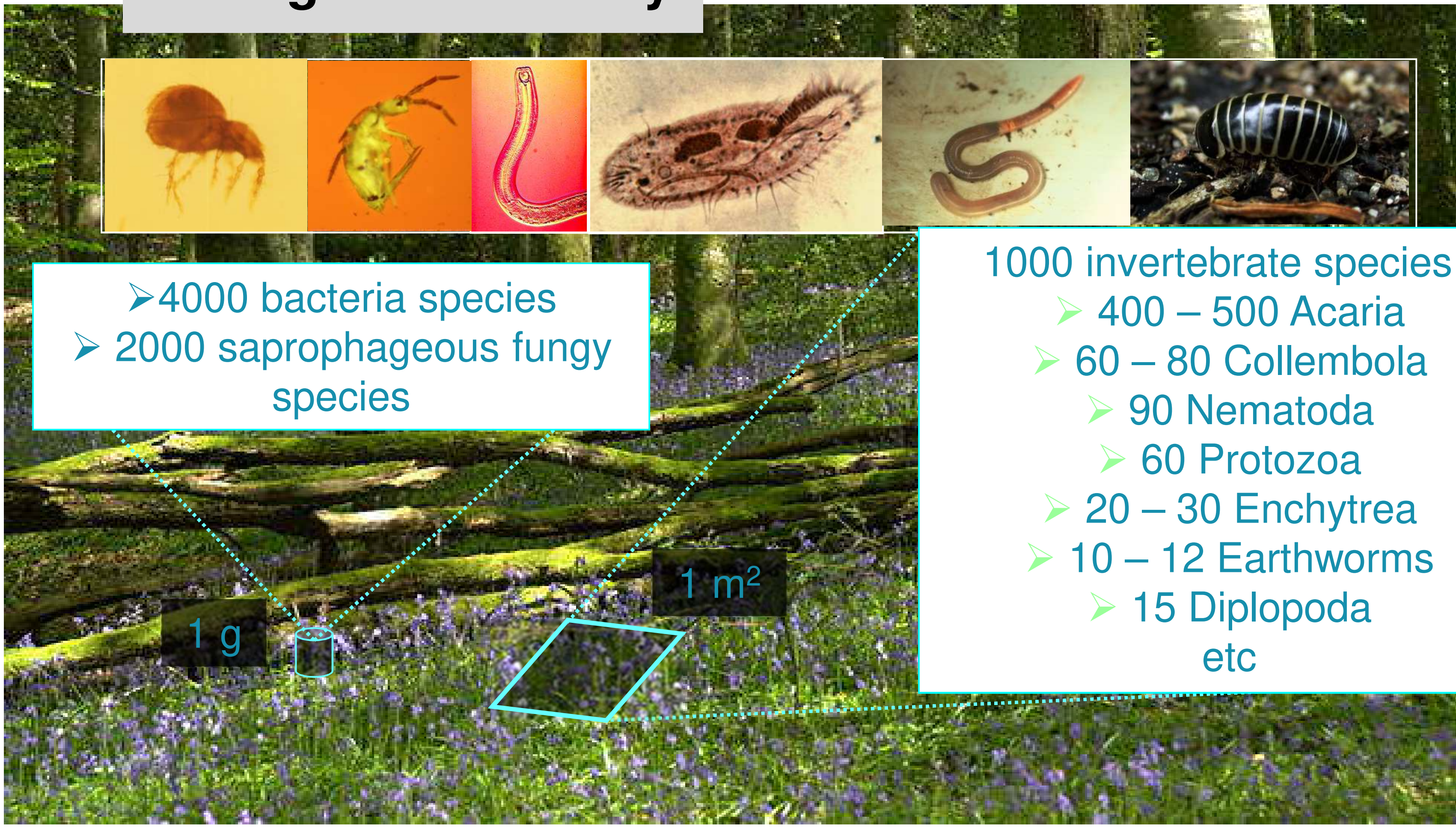
7 millions of animals

= Switzerland inhabitants

Soil organism diversity

- Soil organisms : 25 % of 1.5 millions of species described all over the world !!
- 90% of soil organisms are unknown

➔ Huge biodiversity



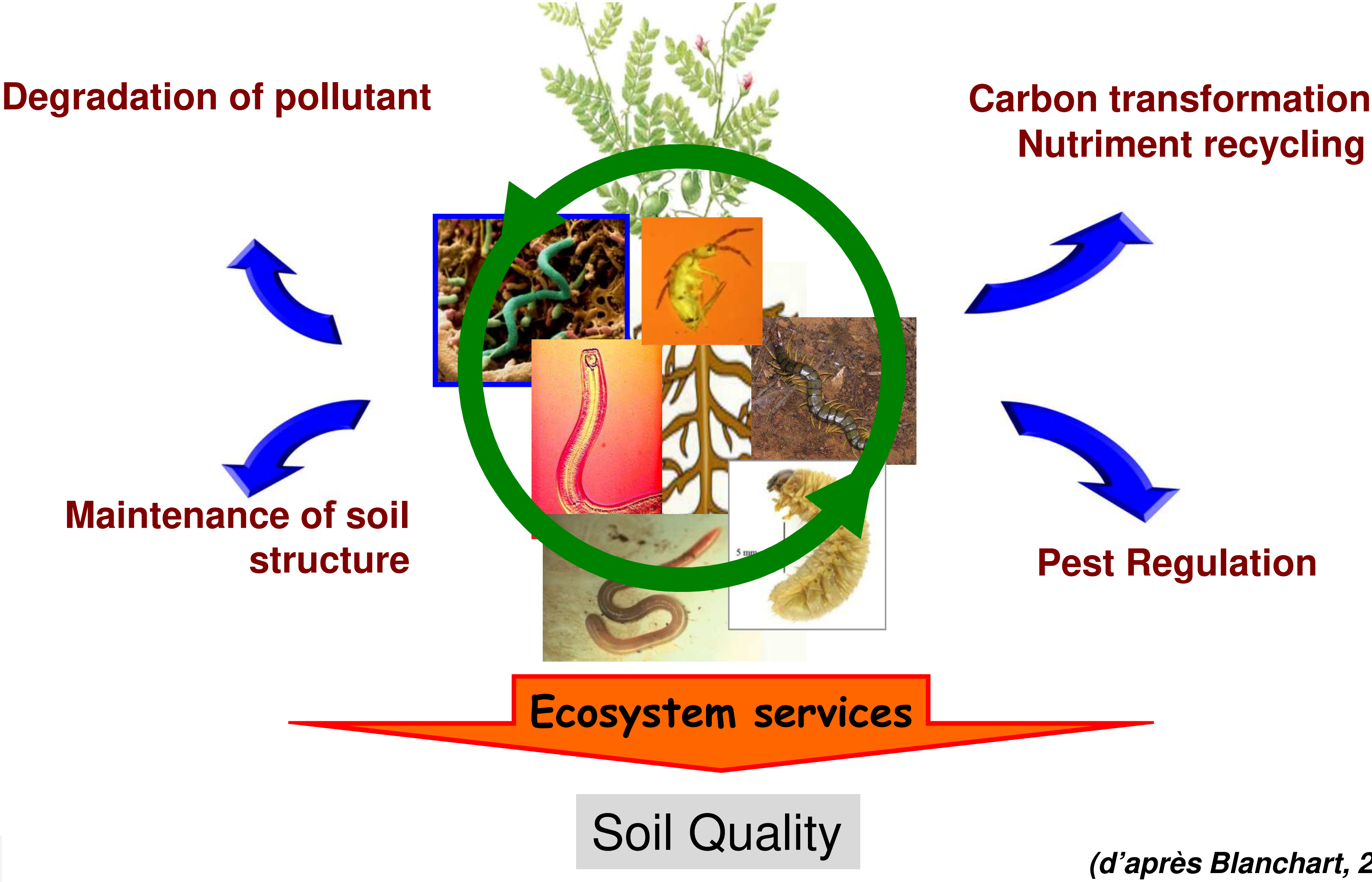
- 4000 bacteria species
- 2000 saprophageous fungy species

1000 invertebrate species :
- 400 – 500 Acaria
- 60 – 80 Collembola
- 90 Nematoda
- 60 Protozoa
- 20 – 30 Enchytrea
- 10 – 12 Earthworms
- 15 Diplopoda

etc

D'après Decaëns. Torsvick et al. (1994), Hawksworth (2001), Schaefer et Schauermann (1990)

4 functions provided by soil organisms



(d'après Blanchart, 2012,

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Microorganisms - Microflora

❑ **microbial indicators are based on** :

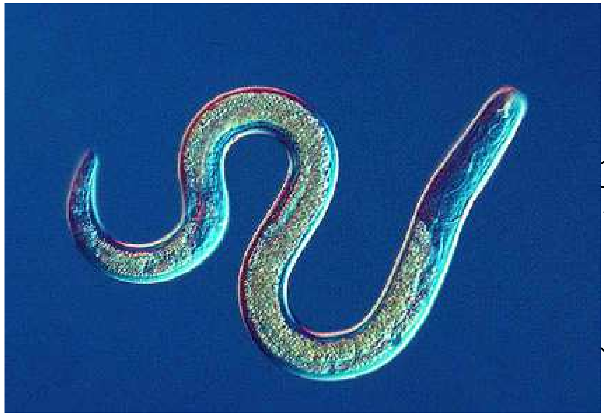
- density,
- biomass,
- diversity,
- activity (mineralisation, respiration, enzymatic, …)

Seen in detail on wednesday
(Edoardo Puglisi, Valeria Ventorino, Olimpia Pepe, Loredana Canfora)

Microfauna : nematodes

SoilBiodiversity - Microfauna (<0.2 mm)

Nematodes



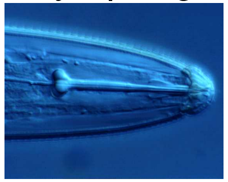
- Large group (+ 11 000 sp)
- Several millions / m²
- Untill 50 species / sample
- Have free life or parasit

Nematod indicators are based on:
- **Abundance**
- **Functionnal group**
  - ➔ **Biodiversity index**
  - ➔ **Food web index**

Prédateurs

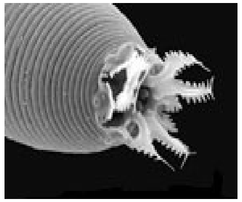
Positive effect on organic matter dynamic,
Soil fertlity, nutrient dynamic

Phytophages

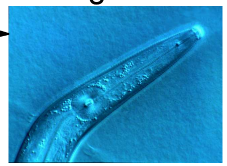


Bad effect on plant
Pests

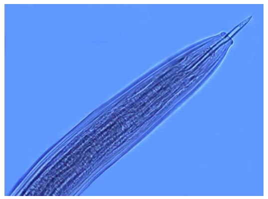
Bactérivores



Fongivores



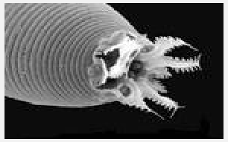
Omnivores



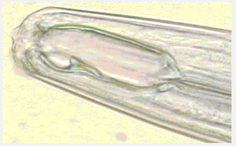
nematods index

1) nematod index based on BIODIVERSITY of Nematods

Bactérivores



Carnivores

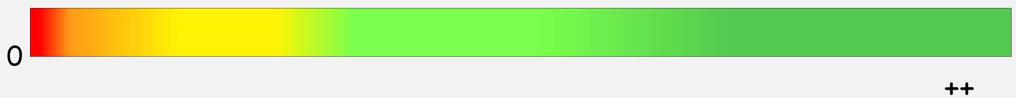


Fongivores

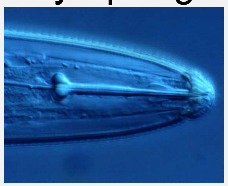
Omnivores

Number of free nematodes / 100g soil

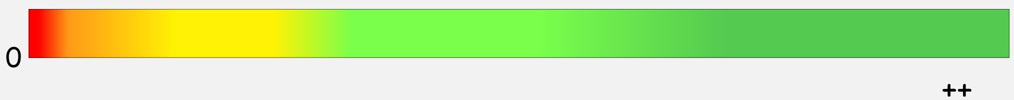
-> level of biological activity in the soil



Phytophages



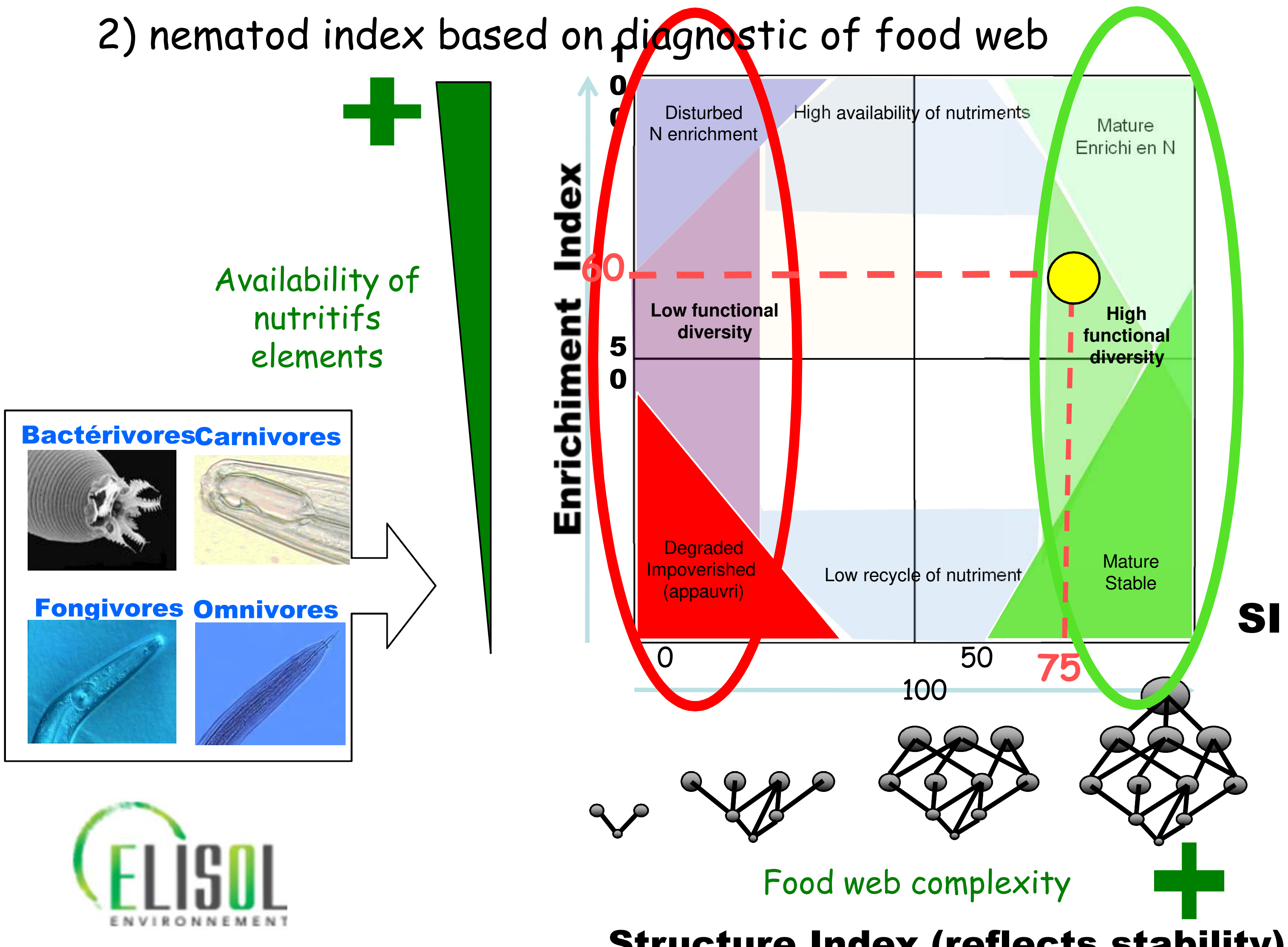
Number of phytophagous
Plant Parasitism Index
→ Level of risk for plant



SoilBiodiversity - Microfauna (<0.2 mm)

nematods index

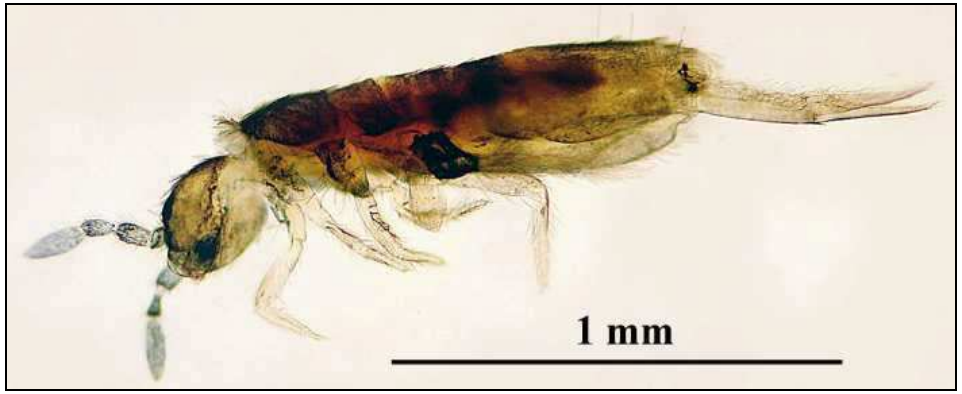
2) nematod index based on diagnostic of food web



SI=75
EI=60

Ferris et al., 2001

mesofauna : Microarthropds



Mite (acarina) indicators are based on :
- Density (nb/m²)
- Diversity

Collembola indicators are based on :
- Density (nb/m²)
- Diversity - Functional groups

Seen in detail on wednesday
(Christina Menta, Sara Remelli, Lucia Santorufo, Giulia Maisto)

Macrofauna : earthworms

SoilBiodiversity - Macrofauna (> 4 mm)

Earthworms

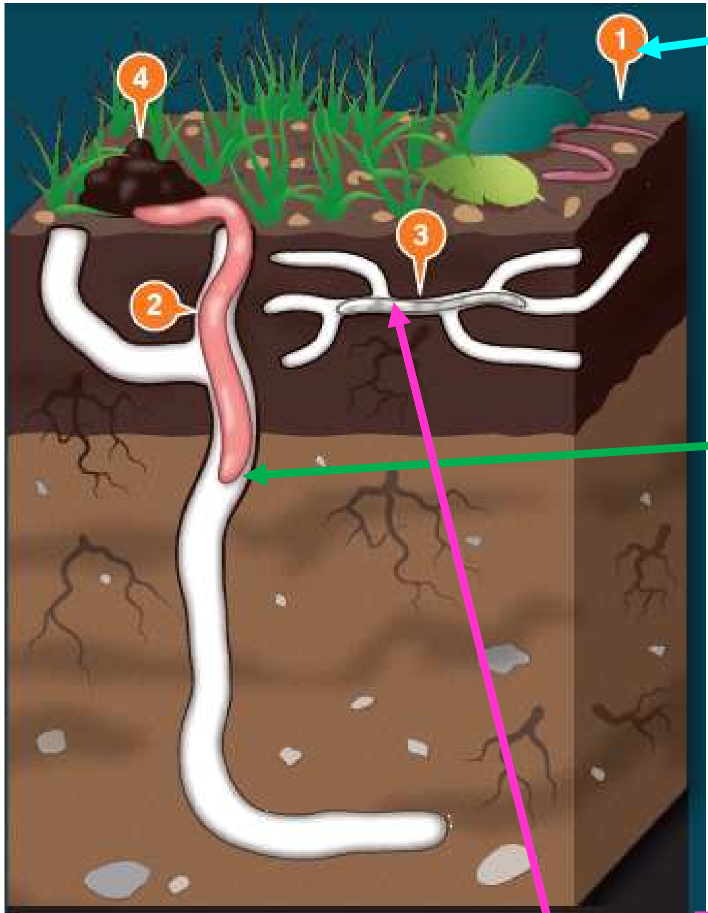
3000 species in the world
100 species in France

12-15 species in grassland
1-3 species in crop field

Earthworm indicators are based on :
- Density (nb/m²), Biomass (g/m²)
- Functional (ecological) groups
- Species structure (species richness, Diversity)

SoilBiodiversity - Macrofauna (> 4 mm)

Earthworms → 3 functional groups



EPIGEIC

1-3 cm (red)
Living at soil surface
Ingesting few mineral matter
No or few burrows

ANECIC

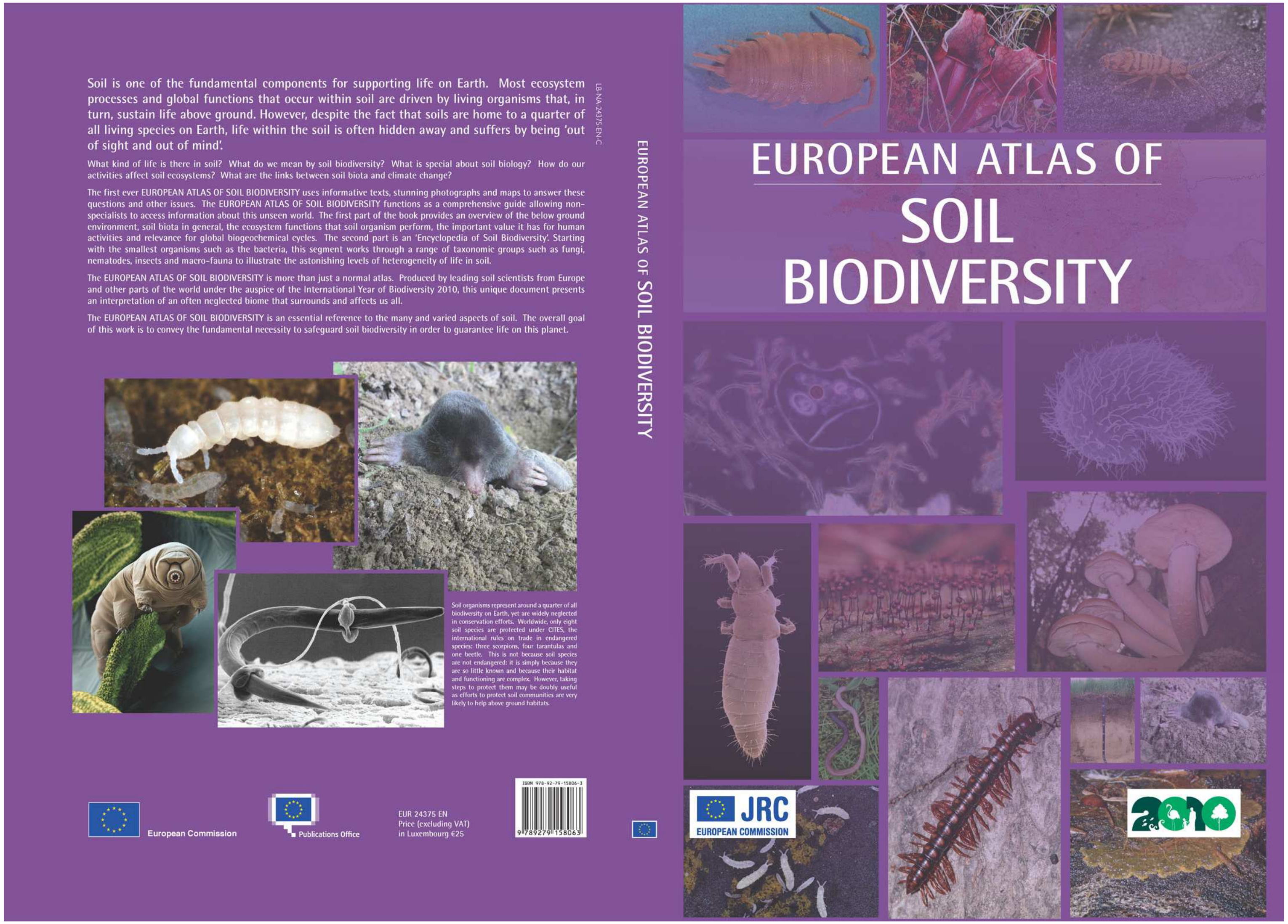
10-110 cm (red, grey)
Mixing in organic matter from surface with mineral soil
Sub-vertically permanent burrows
Casts on soil surface (middens)

ENDOGEIC

1-20 cm (white, green)
Living in the soil, Geophagous
Ephemeral burrow systems = Casts in soil

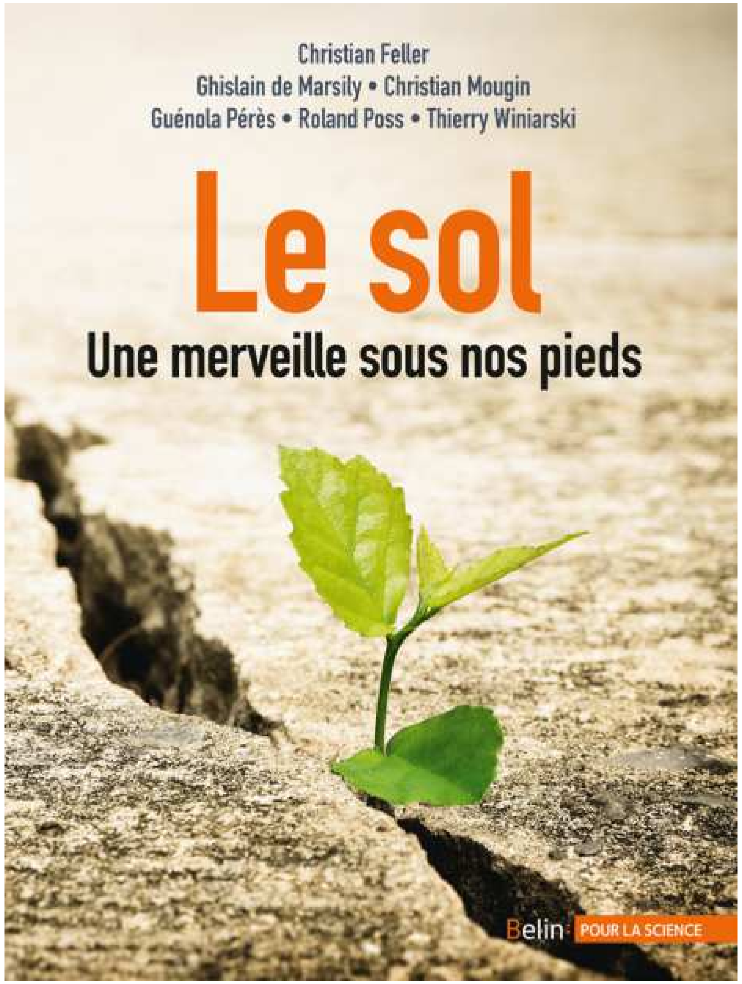
(Bouché, 1972, 1977 ; Lavelle, 1981)


Soil is one of the fundamental components for supporting life on Earth. Most ecosystem processes and global functions that occur within soil are driven by living organisms that, in turn, sustain life above ground. However, despite the fact that soils are home to a quarter of all living species on Earth, life within the soil is often hidden away and suffers by being 'out of sight and out of mind'.
What kind of life is there in soil? What do we mean by soil biodiversity? What is special about soil biology? How do our activities affect soil ecosystems? What are the links between soil biota and climate change?
The first ever EUROPEAN ATLAS OF SOIL BIODIVERSITY uses informative texts, stunning photographs and maps to answer these questions and other issues. The EUROPEAN ATLAS OF SOIL BIODIVERSITY functions as a comprehensive guide allowing non-specialists to access information about this unseen world. The first part of the book provides an overview of the below ground environment, soil biota in general, the ecosystem functions that soil organism perform, the important value it has for human activities and relevance for global biogeochemical cycles. The second part is an 'Encyclopedia of Soil Biodiversity'. Starting with the smallest organisms such as the bacteria, this segment works through a range of taxonomic groups such as fungi, nematodes, insects and macro-fauna to illustrate the astonishing levels of heterogeneity of life in soil.
The EUROPEAN ATLAS OF SOIL BIODIVERSITY is more than just a normal atlas. Produced by leading soil scientists from Europe and other parts of the world under the auspice of the International Year of Biodiversity 2010, this unique document presents an interpretation of an often neglected biome that surrounds and affects us all.
The EUROPEAN ATLAS OF SOIL BIODIVERSITY is an essential reference to the many and varied aspects of soil. The overall goal of this work is to convey the fundamental necessity to safeguard soil biodiversity in order to guarantee life on this planet.
LB-NA-24375-EN-C
Soil organisms represent around a quarter of all biodiversity on Earth, yet are widely neglected in conservation efforts. Worldwide, only eight soil species are protected under CITES, the international rules on trade in endangered species: three scorpions, four tarantulas and one beetle. This is not because soil species are not endangered: it is simply because they are so little known and because their habitat and functioning are complex. However, taking steps to protect them may be doubly useful as efforts to protect soil communities are very likely to help above ground habitats.
European Commission
Publications Office
EUR 24375 EN
Price (excluding VAT)
in Luxembourg €25
ISBN 978-92-79-15806-3
9 789279 158063
EUROPEAN ATLAS OF SOIL BIODIVERSITY
EUROPEAN ATLAS OF
SOIL
BIODIVERSITY
JRC
EUROPEAN COMMISSION
2010

Soil biodiversity and functions



Pérès et al., 2016









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  Risk assessment / soil quality monitoring

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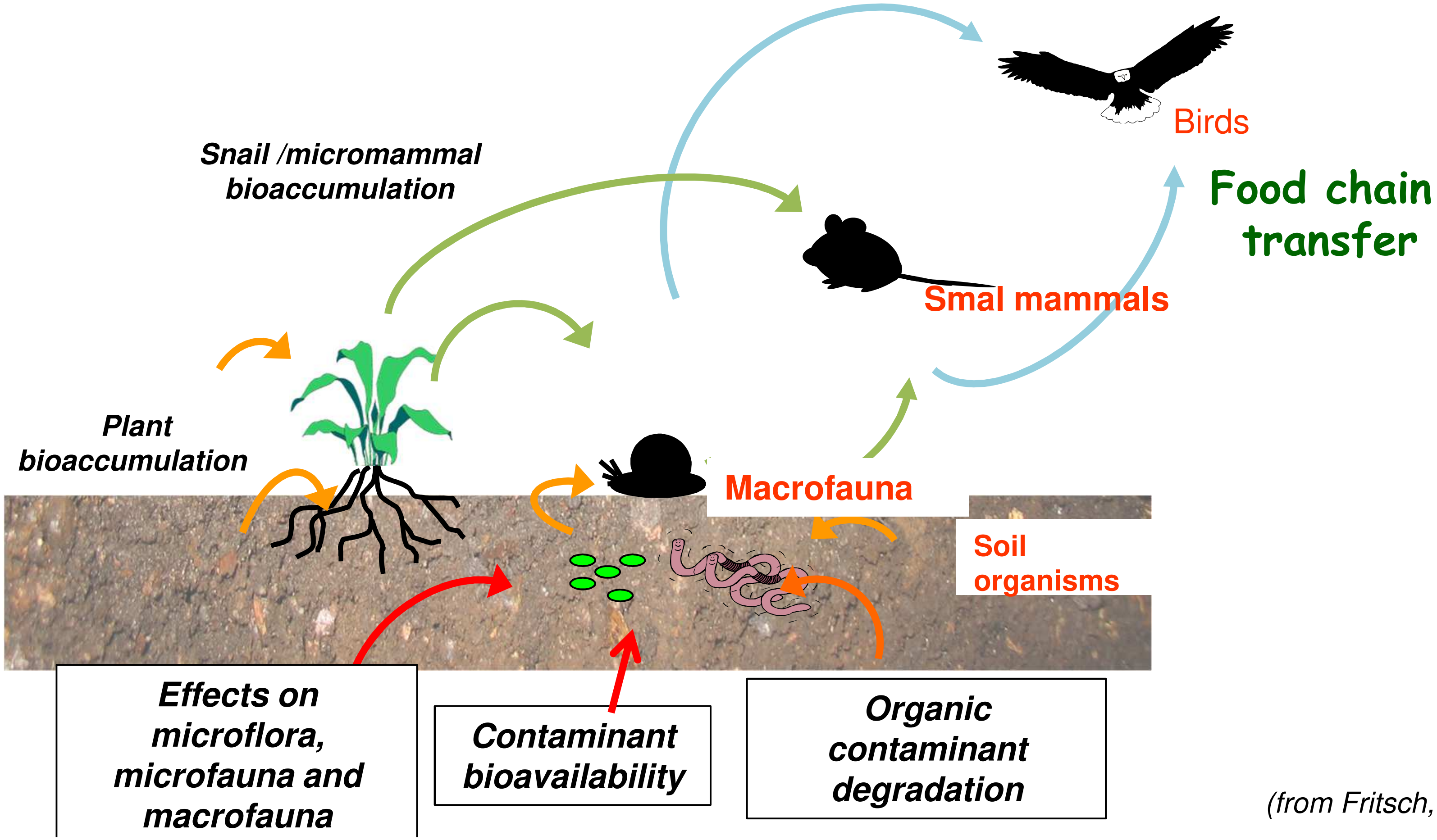
- Conclusion

What are the needs in contaminated soils ?

Need 1 – Diagnostic (risk assessment, bioavailability, transfer)

In order to improve soil diagnostic (risk assessment, bioavailability, transfer)

➔ Tools which can supplement physico-chemical analysis



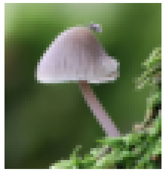
(from Fritsch, 2010)

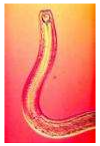
        

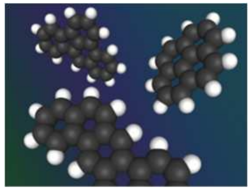
What are the needs in contaminated soils ?

Need 2 – Diagnostic (soil quality) and Soil management

Tools to measure biological state and **soil functions** and at least to evaluate soil **ecosystem services** before AND after soil remediation







Organic contaminant degradation

Organic matter degradation

Soil structuration

Ecosystem services evaluation

Natural attenuation

Nutrient cycling

Biomass production

Water regulation

(quality, quantity)

Providing Habitat

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ADEME (French Agency for Environment and Energy Management) initiated a national research programme to develop such indicators **:** **"Bioindicator Programme"** (2006-2012).

http://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur

BIOindicateurs

Objectives

- to **calibrate the bioindicators** by **providing a base of referential values and endpoints** in order to interprate the results

- to **provide relevant biological tools** (sensibility, accessibility, cost, ...) depending on the purpose :
  - soil quality monitoring
  - characterization
  - risk assessment (bioavailability, transfer)

- to **transfer** the tools to stakeholders (policy makers, farmers, technicians…)

47 parameter studied

1) Microflora

Parameters of quantity

- Global (Bm, DNA)
- No global (Fungial, Bacterial)

Parameters of activity (examples)

-12 Enzymatic Activities (N, C, P, S)
-Metabolical Functions (mineralisation, respiration ..)

Parameters of genetic structure

-PCR TTGE (Bacterial, Fungial)
-PCR Empreinte ARISA (Bacterial)
-Pyrosequencage

2) Flora

Parameters -> individual effects

- Lipid Biomarkers (Oméga 3)
-Metal Bioaccumulation

3) Fauna

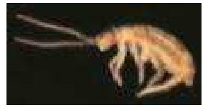
Global Parameter

- Total Macrofauna

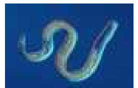
Parameters -> community level

- Earthworms,

- Collembola, mites



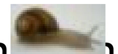
- Nematodes



Parameters -> Individual level

- Molecular earthworm biomarkers (metallothionein coding gene expression, Cd sensivity)

- Bioaccumulation (snail, micromammal)



Complementarity of indicators

- **different sizes (micro to macro)**
- **different levels (cellular, community)**
- **from state to activities, transfer indicator**

BIOindicateurs

ADEME

13 sites → 47 plots

ISO 23 611

→ 188 samples

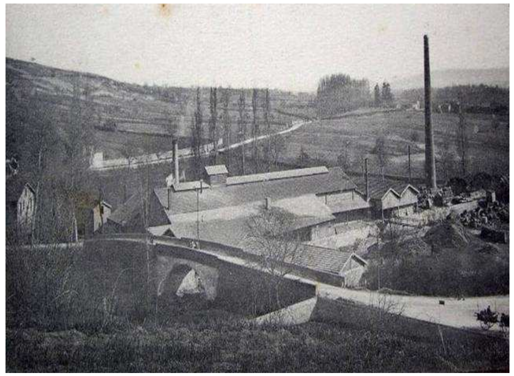
Forest sites

4 sites (4 plots)

Agricultural sites

5 sites (25 plots)

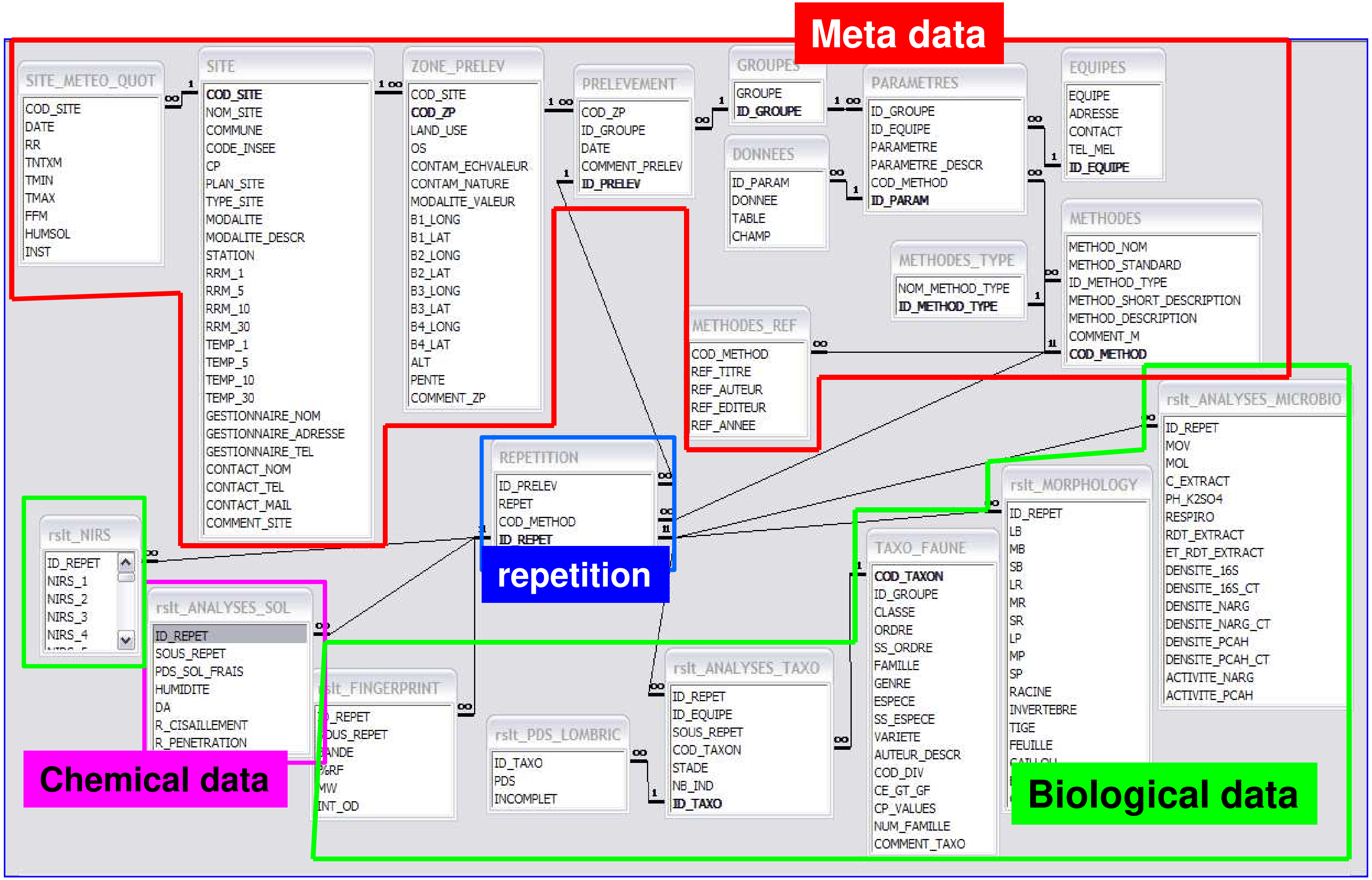
Contaminated sites



4 sites (19 plots)

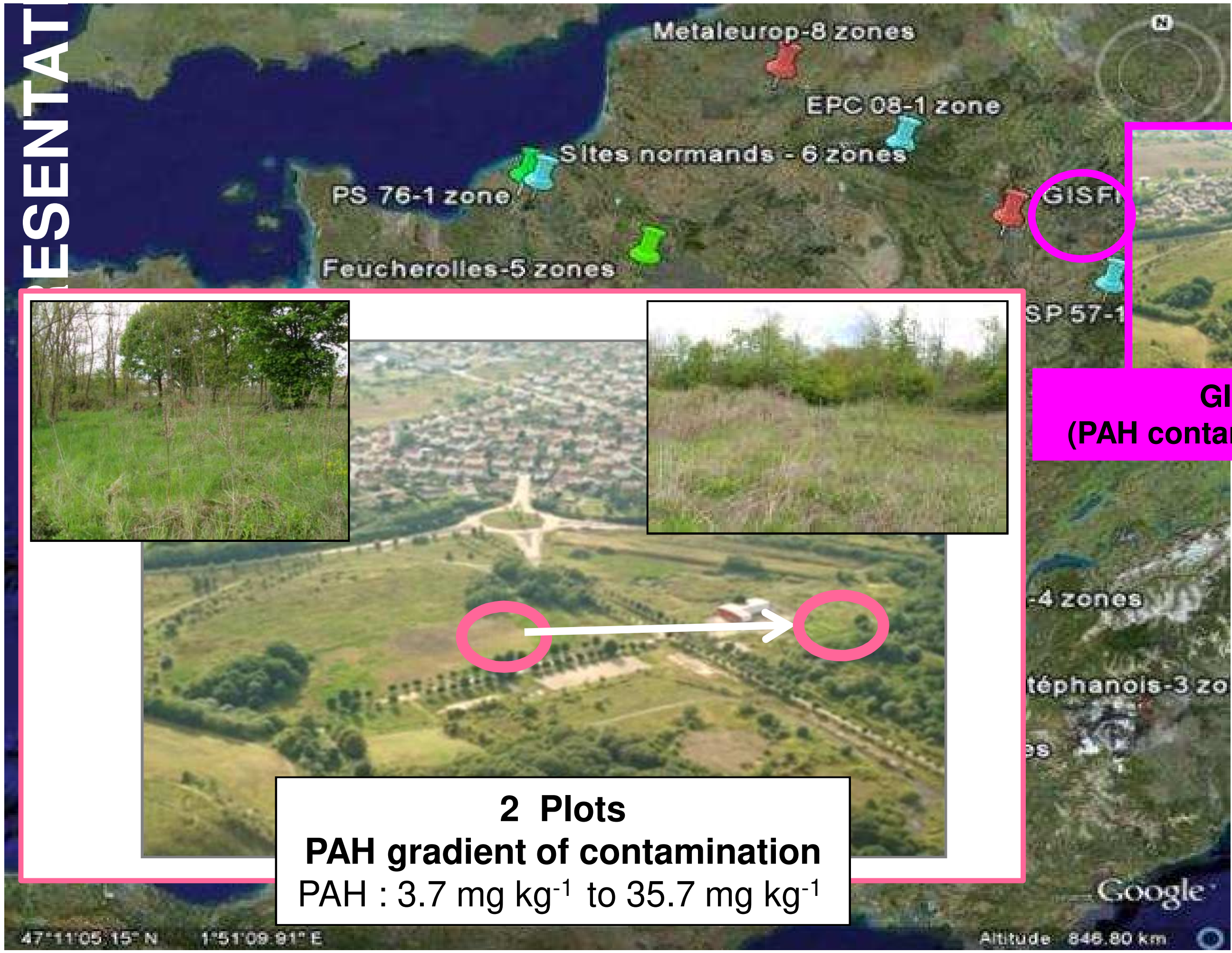
➔ More than 2000 data

Database with relational tables



BIOindicateurs ADEME

Site location (contaminated sites)



2 Plots
PAH gradient of contamination
PAH : 3.7 mg kg^{-1} to 35.7 mg kg^{-1}

GIS-FI Homécourt

GISFI

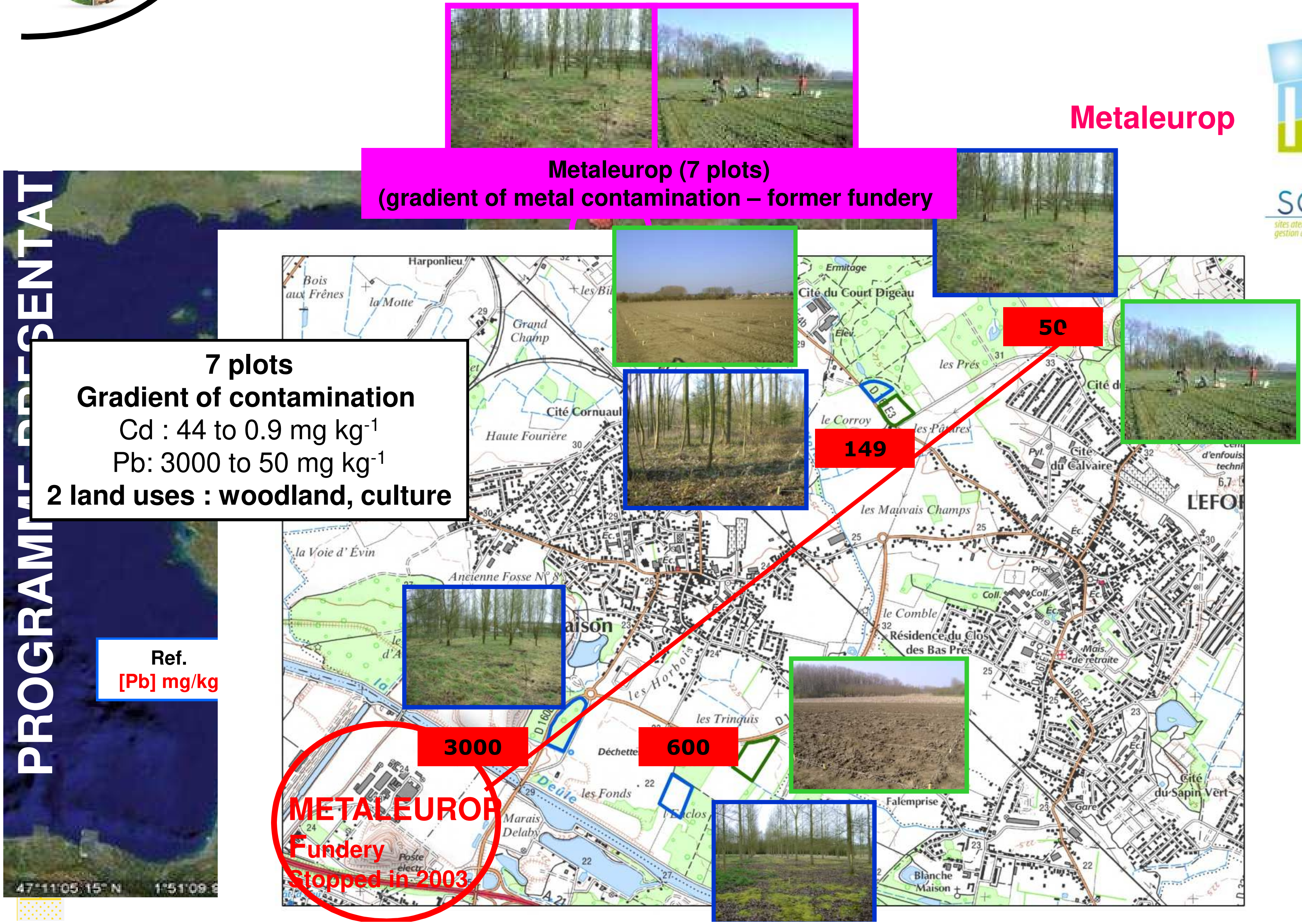
LIMOS

safir sites ateliers gestion des sols

GIS-FI Homécourt (2 plots)
(PAH contamination – industrial wastelands)

Site location (contaminated sites)

Metaleurop

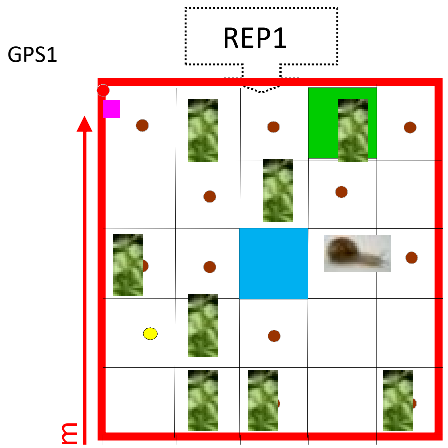


Sampling design

Based on - existing french regional sampling design (Cluzeau et al., 2009)

- recommandations from european programme ENVASSO (Bispo et al., 2007)

● GPS points



● Soil composit sample
(12 sampling points/replicate):
Microbiology
Nematofauna
Physico-chemical

Soil sample for Collembola, mites

Earthworms (community)

Earthworm (biomarker)

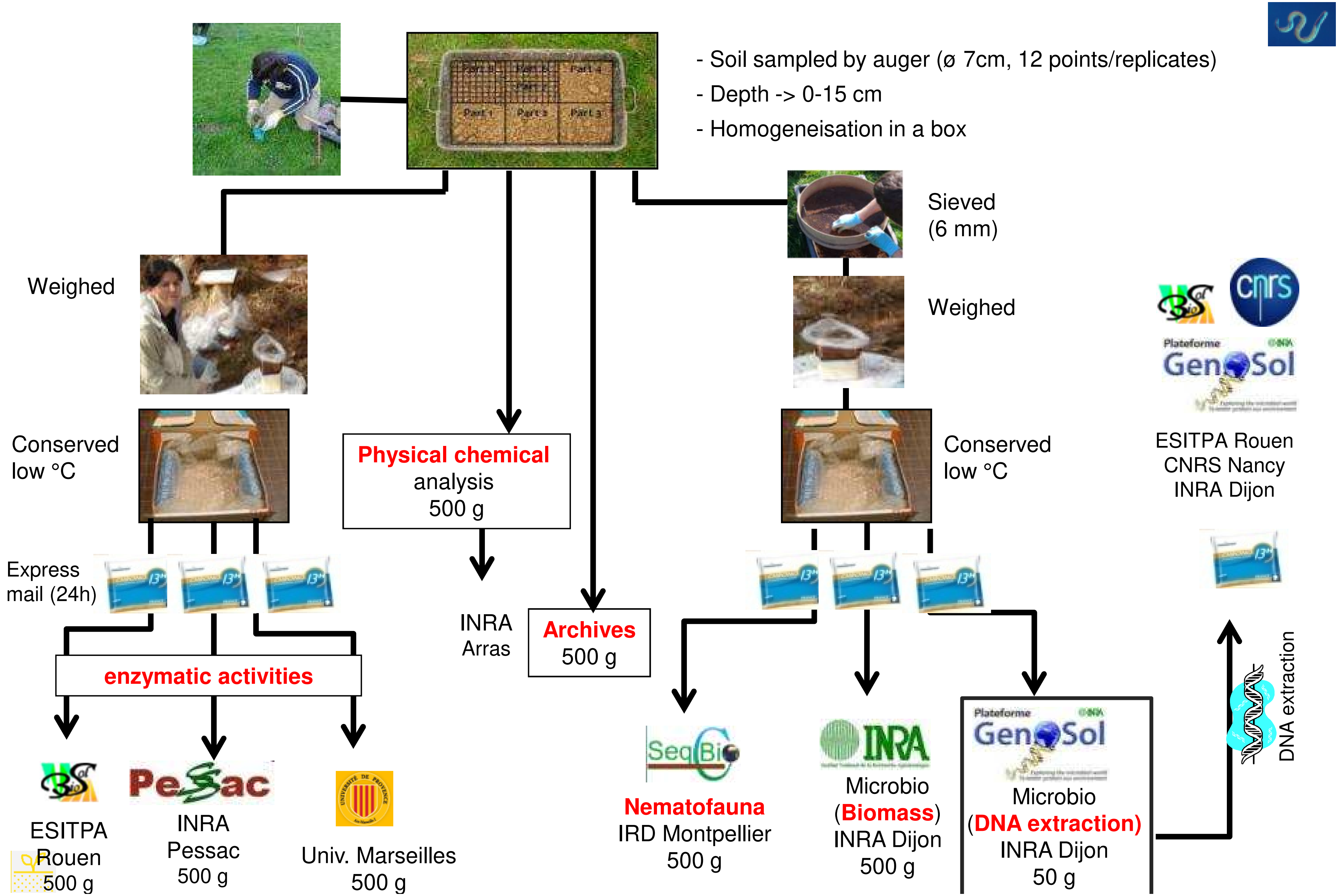
Total Macrofauna

Flora (8 individuals)

Snails *(Cantareus aspersus)* (microcosme)

Sampling protocole

Example → Soil sampling (microorganisms, nematofauna)



Results

1 – guide (referential) values

- 1.1. <u>for element transfer - bioaccumulation (snails, plant)</u>

- 1.2. for biological statement - biological quality (all organisms)

Bioindicators for element transfert - bioaccumulation

CHRONO ENVIRONNEMENT

Snail (*Cantareus aspersus)*
exposure 28 days

ICP MS

Quantity of Metal in snail
(C_{esc})

Ecole Nationale Supérieure des Mines SAINT-ETIENNE

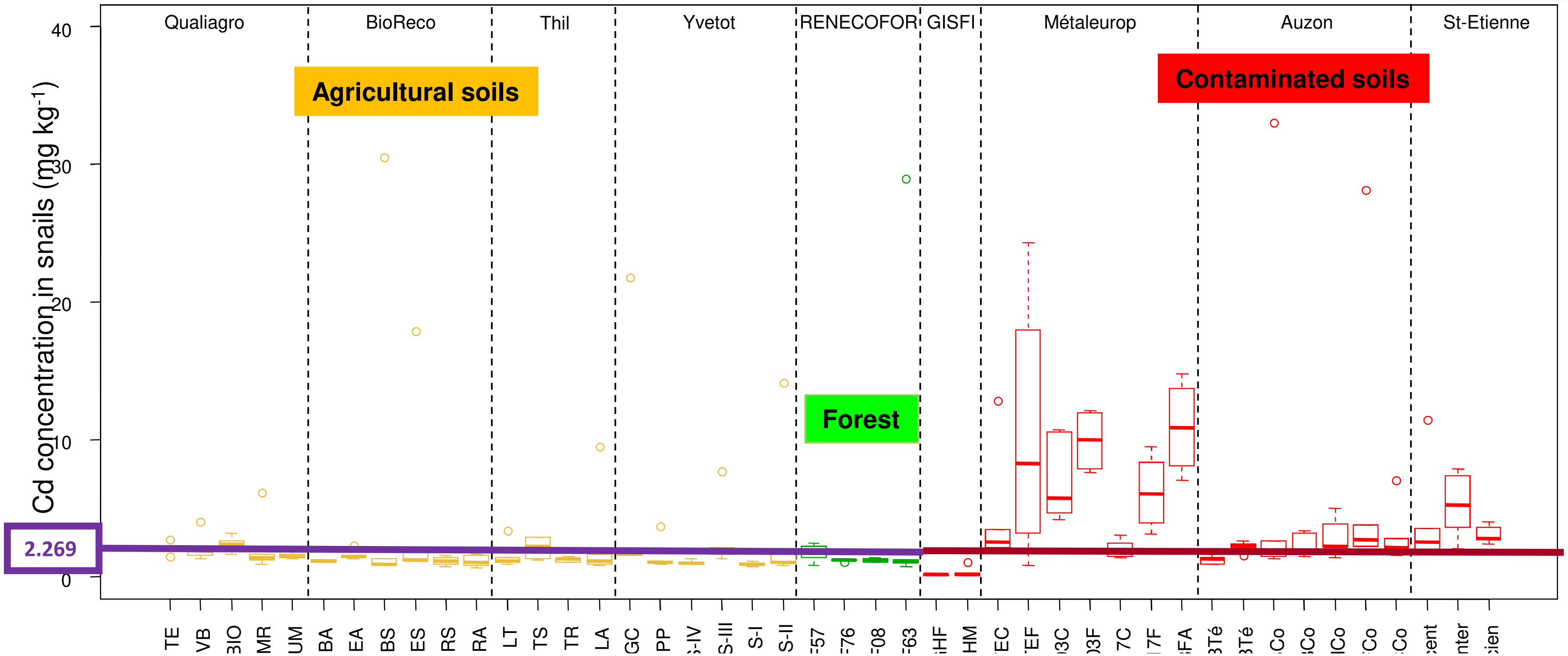
Sample of a pool of different plant species

ICP MS

Quantity of Metal in plant (C_{pl})

1.1 – Initiation of guide values for element (metal) transfer

Example: variation range of Cd concentration in snails after 28 days



Values of Cd concentration in snails at no contaminated soil

➔ gives a reference « referential value of intern concentration » CIRef for each metal

Pauget et al., 2013[a]. Ecological Indicator

Pauget et al., 2013[b] Ecological Indicator

1.1 – Initiation of guide values for element (metal) transfer

Metal	As	Cd	Co	Cr	Cu	Hg	Mo	Ni	Pb	Sb	Sn	Sr	Tl	Zn
CIRef ($mg.kg^{-1}$)	0.30	2.27	6.67	2.01	184.7	0.20	4.43	5.24	12.9	0.07	0.06	125.7	0.26	1490

(Grand et al., 2018)

Metal	As	Cd	Cr	Cu	Ni	Pb	Zn
MédT (mg.kg-1)	0.45	0.16	0.59	9.1	1.65	0.23	56.9

(Grand et al., 2018)

Results

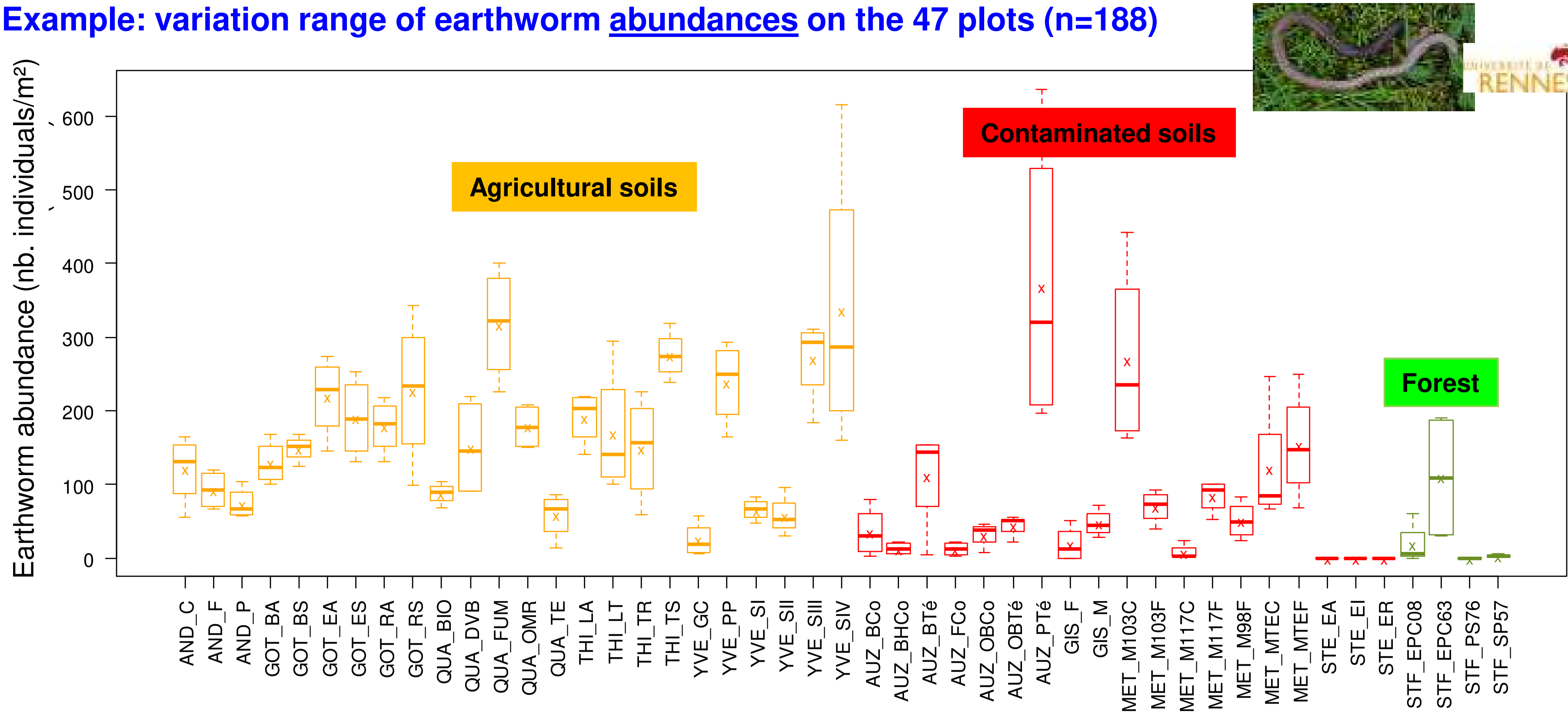
1 – guide (referential) values

- 1.1. for element transfer - bioaccumulation (snails, plant)

- 1.2. for biological statement - biological quality (all organisms)

1.2 – Initiation of guide values for soil biological statement

Example: variation range of earthworm abundances on the 47 plots (n=188)

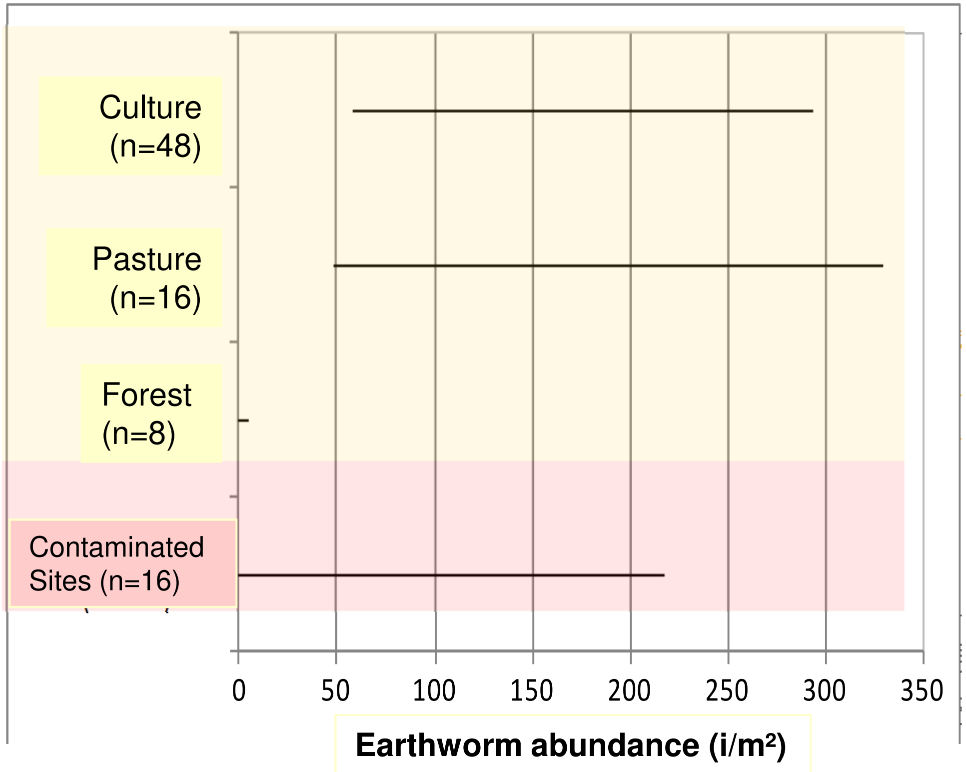


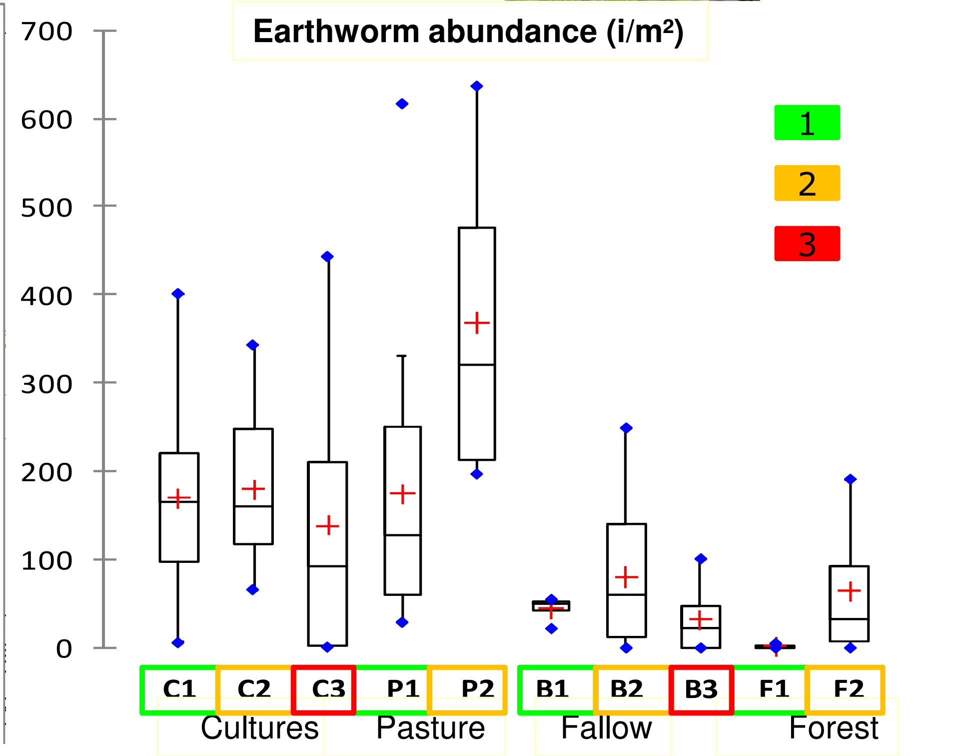
(Guernion et al., 2011)

1.2 – Initiation of guide values for soil biological statement

(Guernion et al., 2011)

Example: variation range of earthworm abundances on the 47 plots (n=188)





➔ calibration of the indicator (range of the biological responses depending on industrial, agronomic, pedological and climate contexts)

➔ guide values -> help in result interpretation

Results

1 – referential values

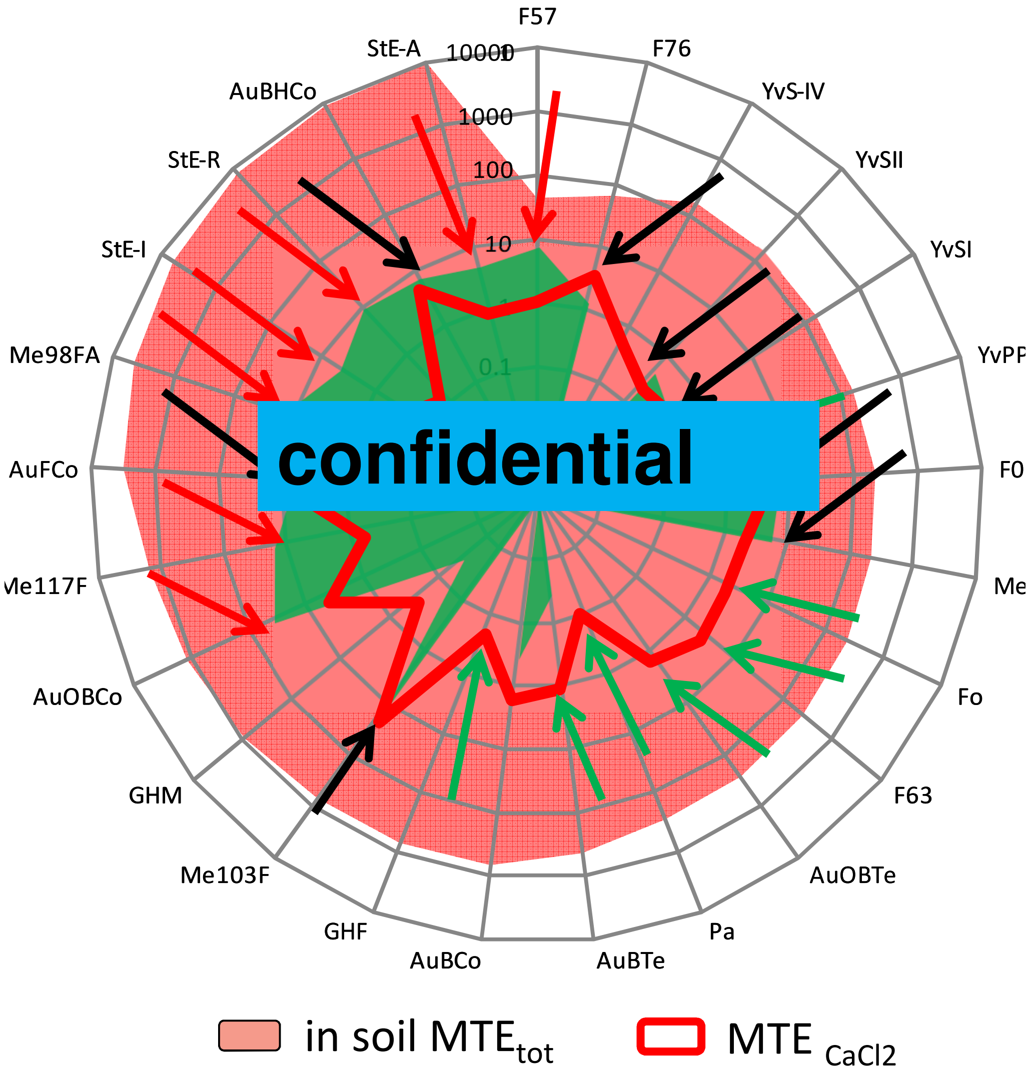
2 – Transfer and risk assessment tools

2.1 Transfer tool

2.2 Risk assessment tool

Metal Transfer in Plant (CMT)

2.1 – Tools for transfer assessment (Bioavailability)



MTE Total different MTE CaCl2

MTE CaCl2 : bioavailability ?

Metal Transfer in Plant (CMT)

➔ Consistence between CMT and MTE CaCl2

➔ Inconsistence between CMT and MTECaCl2 : transfer Soil-Plant

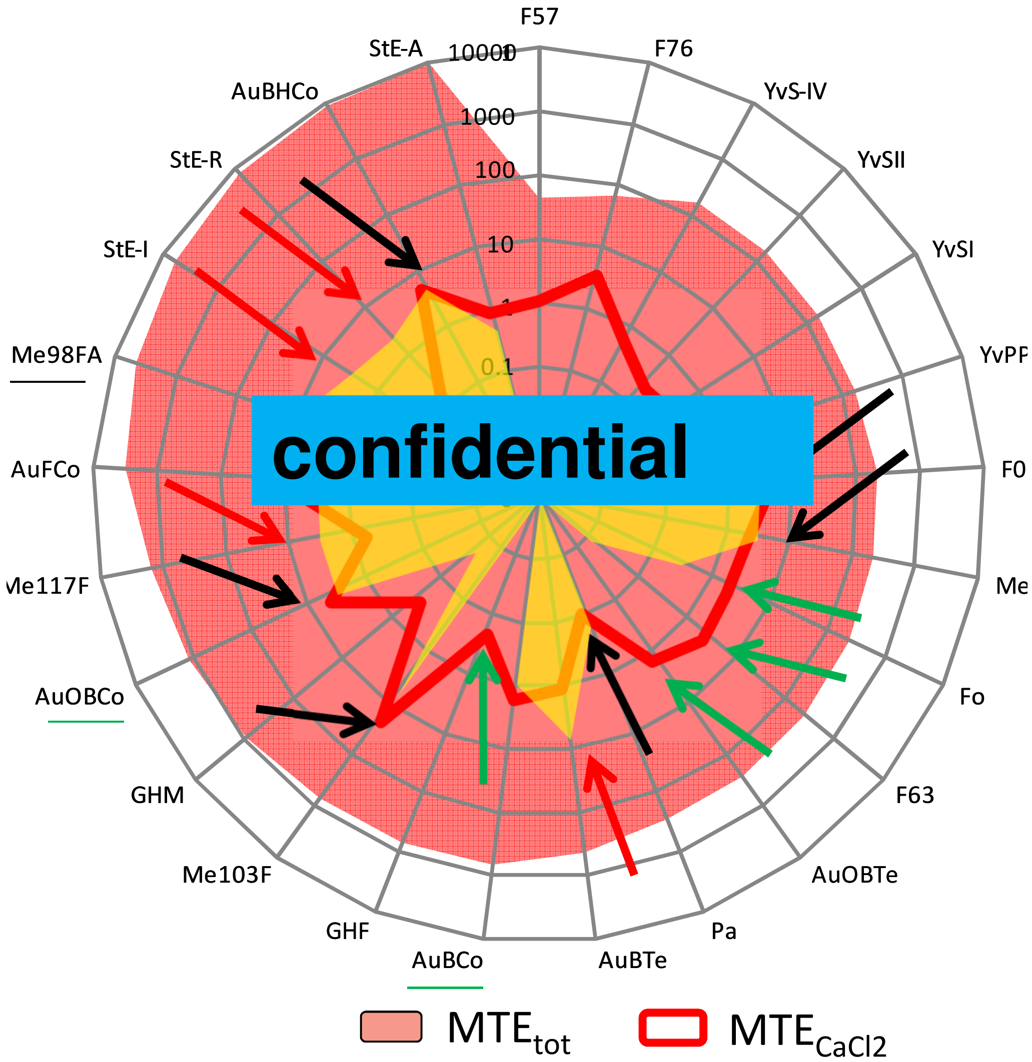
➔ Inconsistence between CMT and MTECaCl2 : NO transfer Soil-Plant

Transfer Soil-Plant different from MTE CaCl2 measures

(de Vaufleury et al., 2012)

2.1 – Tools for transfer assessment (Bioavailability)

Excess of Metal tranfer in Snail (SET_{mod})

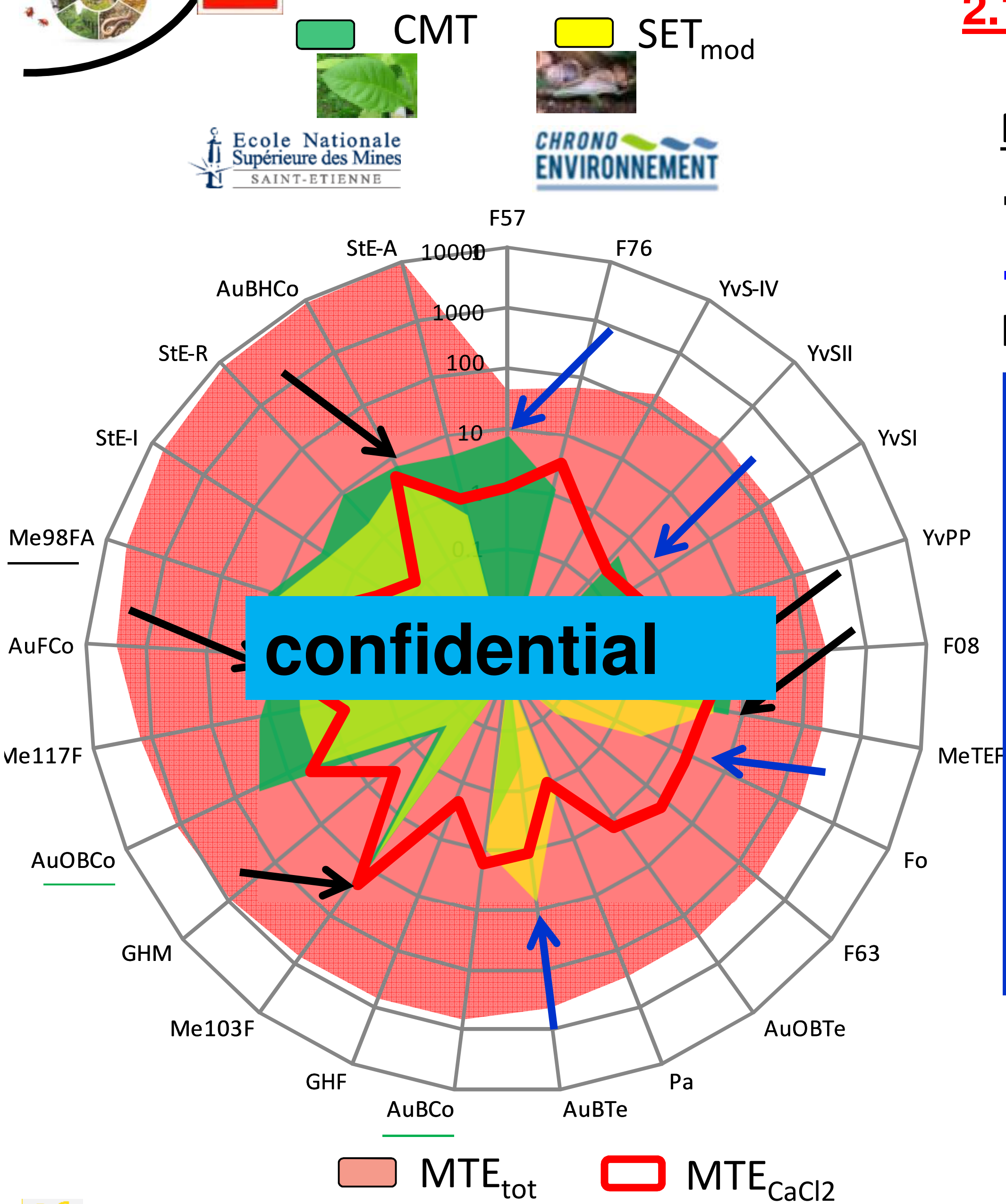


Snails (SET)

- ➔ Consistence between SET and MTECaCl2
- ➔ Inconsistence between SET and MTECaCl2 : transfer Soil-Snails
- ➔ Inconsistence between CMT and MTECaCl2 : NO transfer Soil-Snails

Transfer Soil-Snails different from MTECaCl2 measures

(de Vaufleury et al., 2012)



2.1 – Tools for transfer assessment (Bioavailability)

Plants (CMT) & Snails (SET)

➔ Consistence between CMT and SET

➔ Different responses depending on the biological tool

Synthesis

➔ Biological tools give new informations compared to physico-chemical analysis (MTE CaCl2)

➔ Biological tools are Complementary to classical tools

➔ Complementarity between biological tools (Plants, snails)

(de Vaufleury et al., 2012)

Results

1 – referential values

2 – Transfer and risk assessment tools

2.1 Transfer tool

2.2 Risk assessment tool

2.2 – Tools for risk assessment

Bioindicators - bioaccumulation

Snail exposure 28 days

ICP MS

Quantity of Metal in snail (C_{esc})

Comparison with CIRef

QA = (C_{esc}/CIRef)

SET : Addition of metal transfer Excess

SET = Σ(QA-1)

Sample of a pool of different plant species

ICP MS

Quantity of Metal in plant (Cpl)

Comparison with reference values

IGmétal= (Cpl/NC)

CMT : Addition of metal transfer Excess

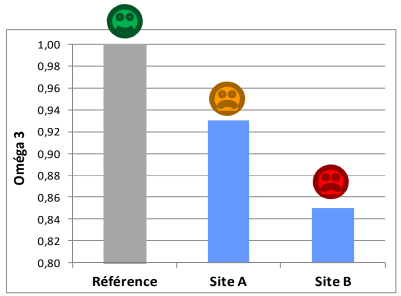
CMT= Σ(IGMétal)

Biomarkers - chronic effect

Sample of lieves from different species

Gaz chromatograph

Measurment Index **Oméga3**



Results

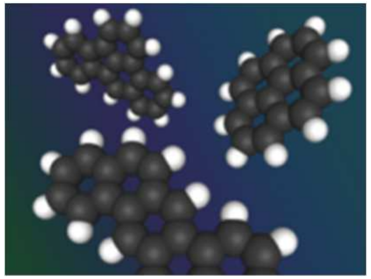
1 – referential values

2 – transfer and risk assessment tools

3 –sensivity of indicators

3.1 to organic contamination (PAH -Polycyclic Aromatic Hydrocarbons)

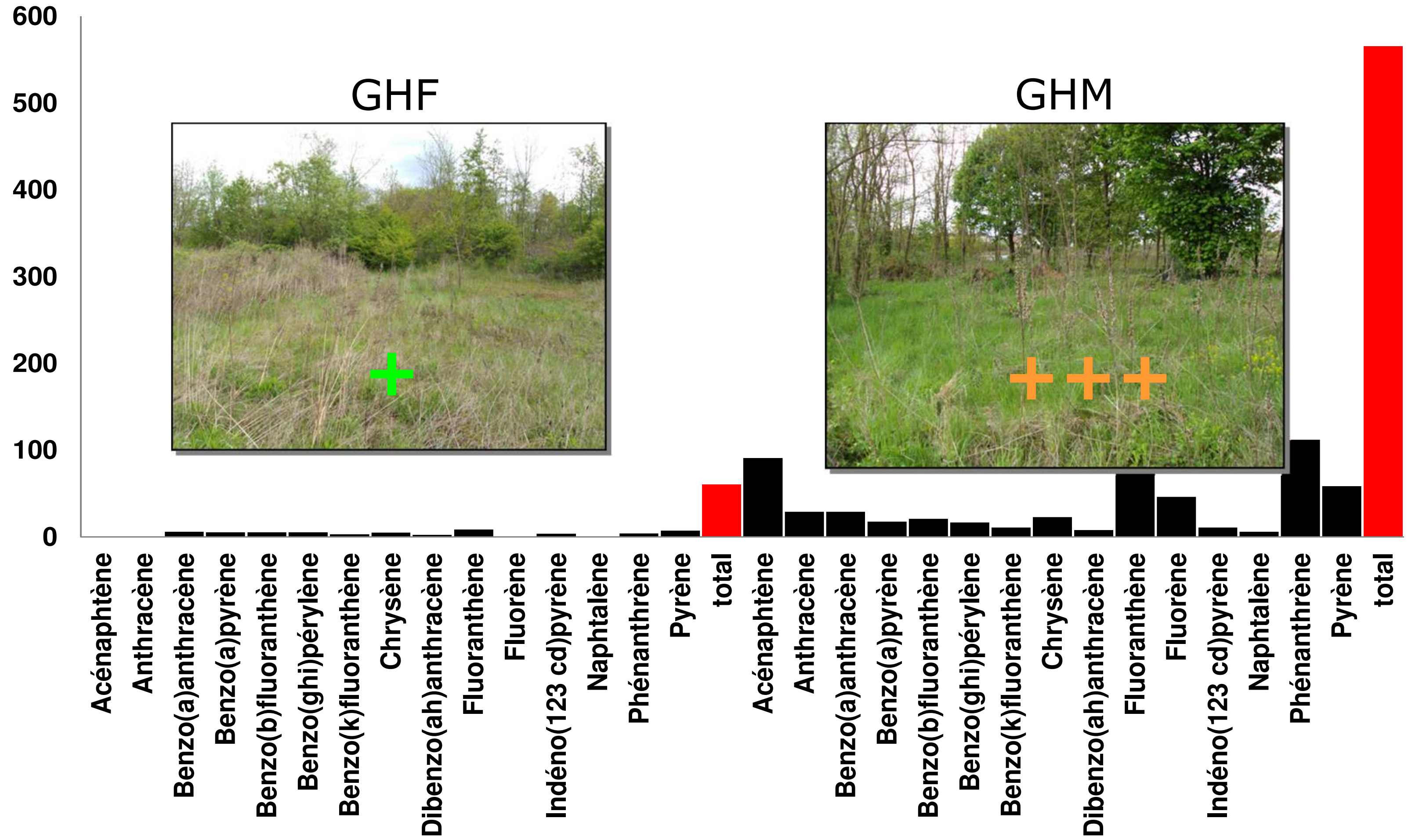
3.2 to metallic contamination



3.1 – Sensitivity to organic contamination

Study site : GISFI Homécourt-PAH

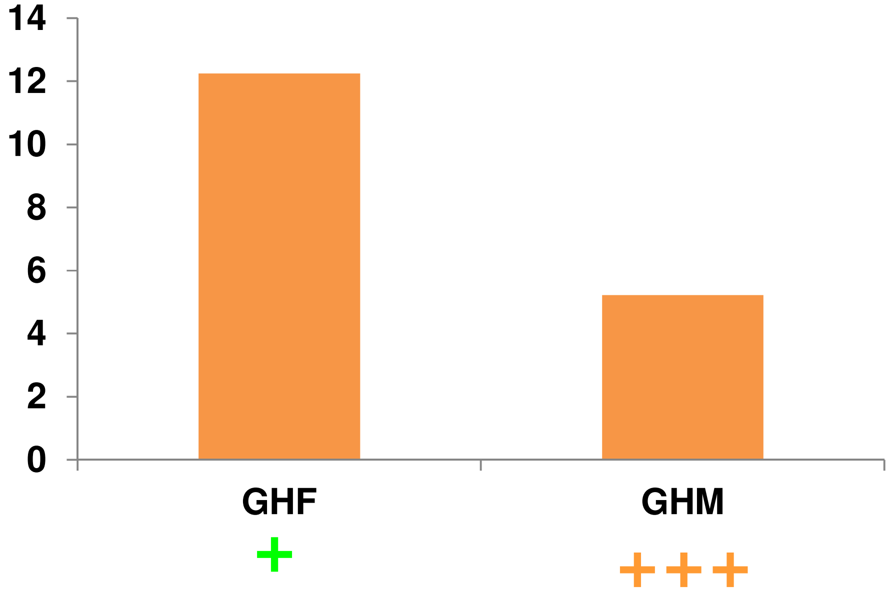
(Polycyclic Aromatic Hydrocarbons)



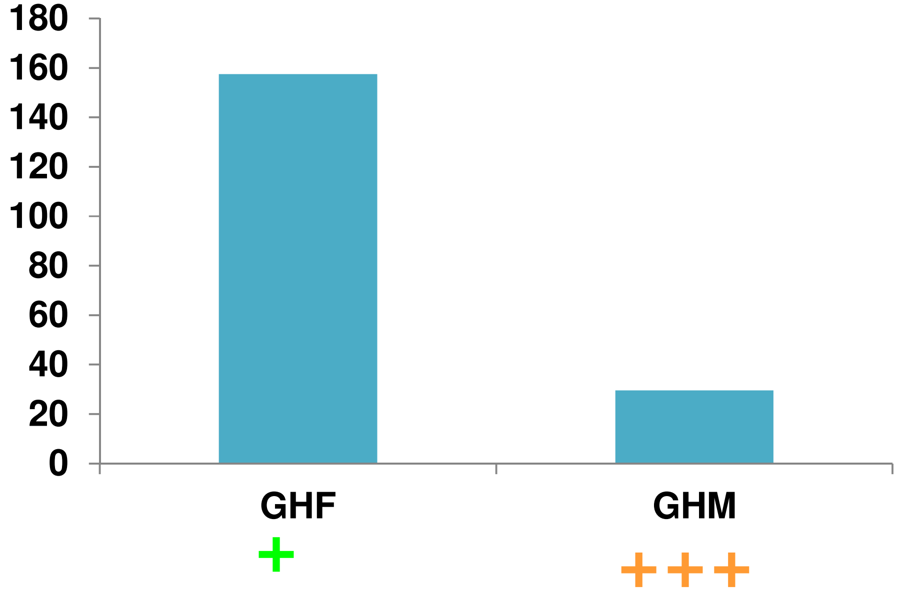
(Begueristain et al., 2009a; Grand et al., 2012a)

Results (Examples) : Microbiology

Enzymatic activité : β-glucosidase
(carbone cycle)



PLFA totaux
Microorganism abundance
(bacteria and fungi)

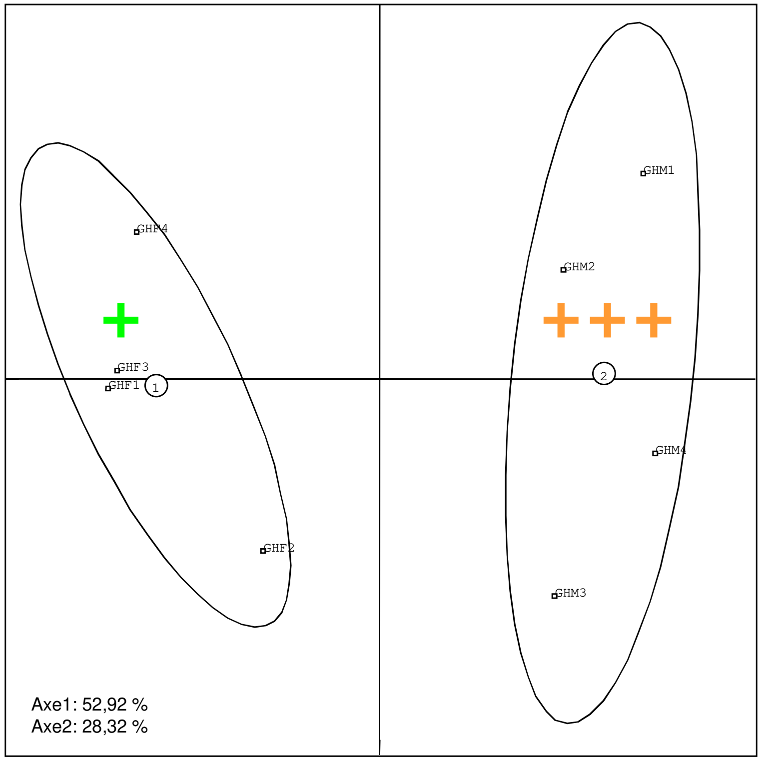


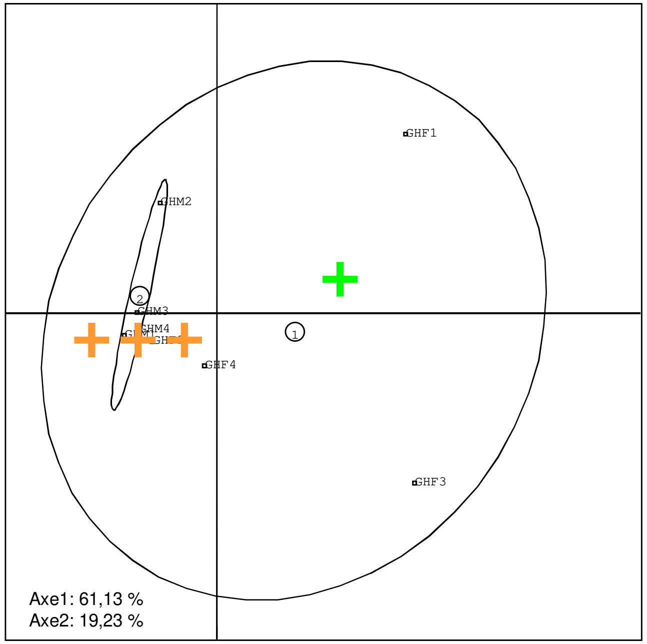
Negative impact of organic pollution on microorganims activities and density

(Laval et al., 2009; Grand et al., 2012a)

Results (examples) : Microbiology

Genetical structure (PCR- TTGE)





Bacteria structure communities are strongly different

Fungi structure communities are not different

(Beguiristain et al., 2009b; Grand et al., 2012a)

Results

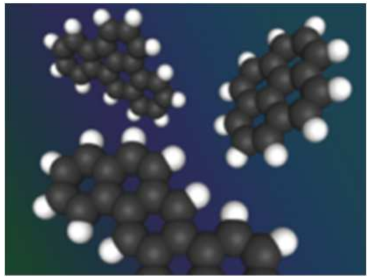
1 – referential values

2 – transfer and risk assessment tools

3 –sensivity of indicators

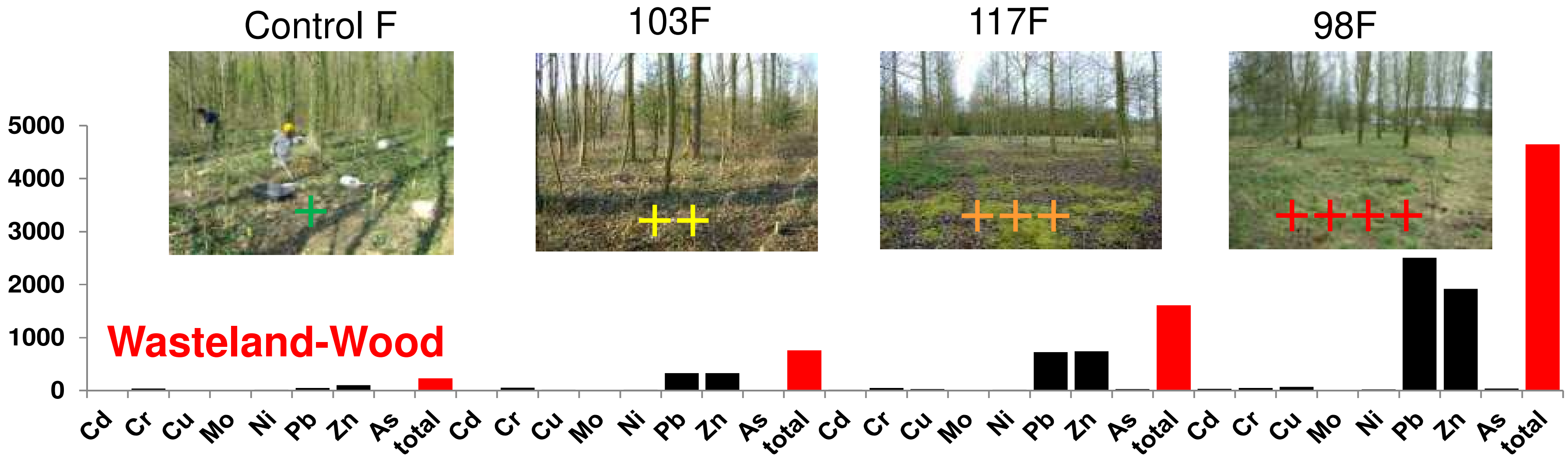
3.1 to organic contamination (PAH -Polycyclic Aromatic Hydrocarbons)

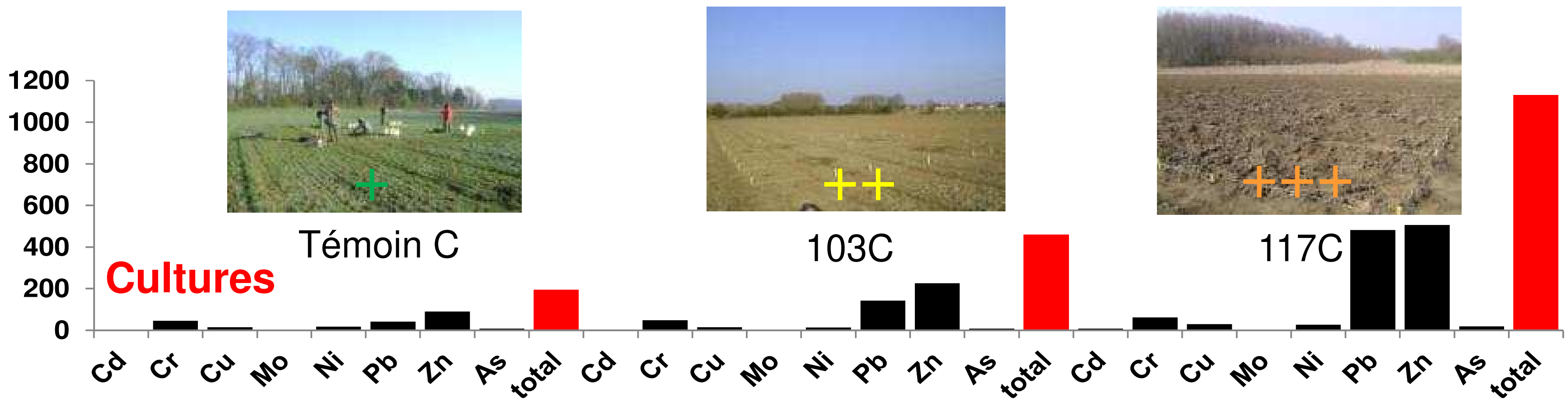
3.2 to metallic contamination



3.2 – Sensitivity to metallic contamination

Study site : Metaleurop Site

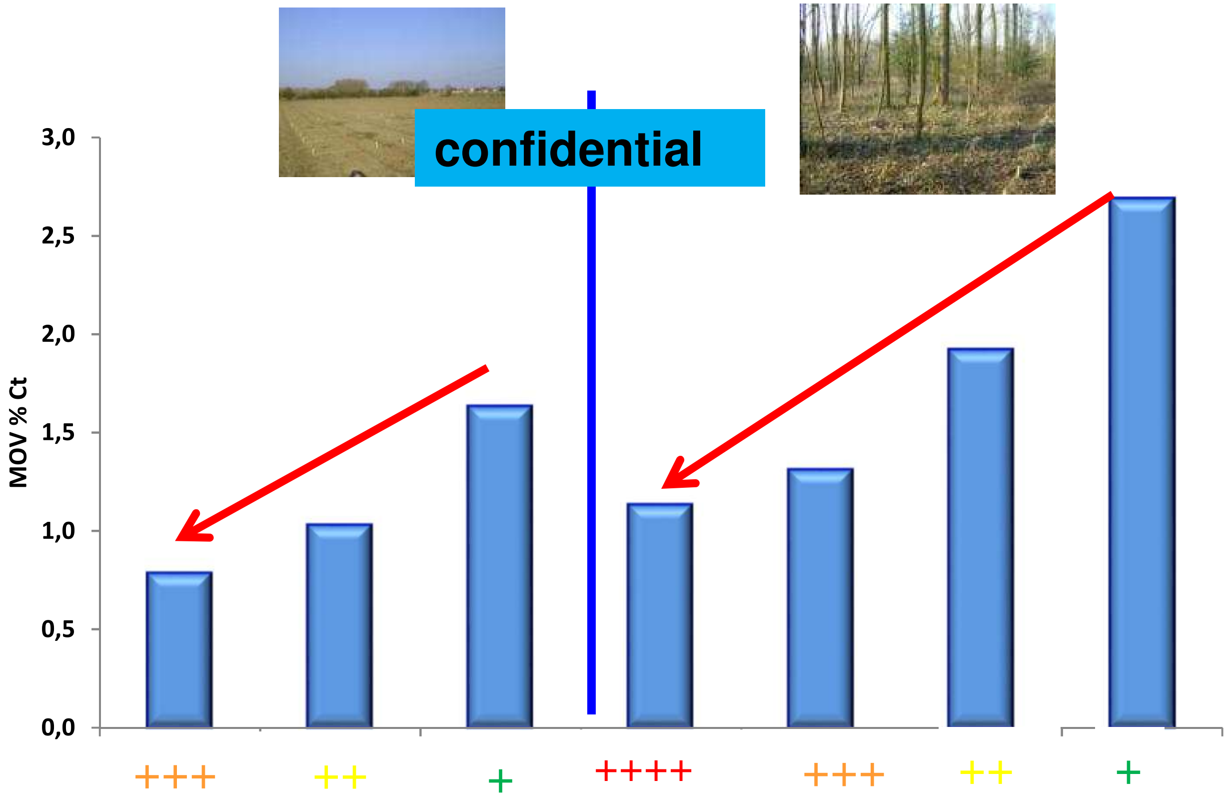




(Douay et al., 2009; Grand et al., 2012a)

3.2 – Sensitivity to metallic contamination

Results (example) : Microbial biomass



Microbial biomass decreases with increase of contaminant

(from Chausod et al., 2009)

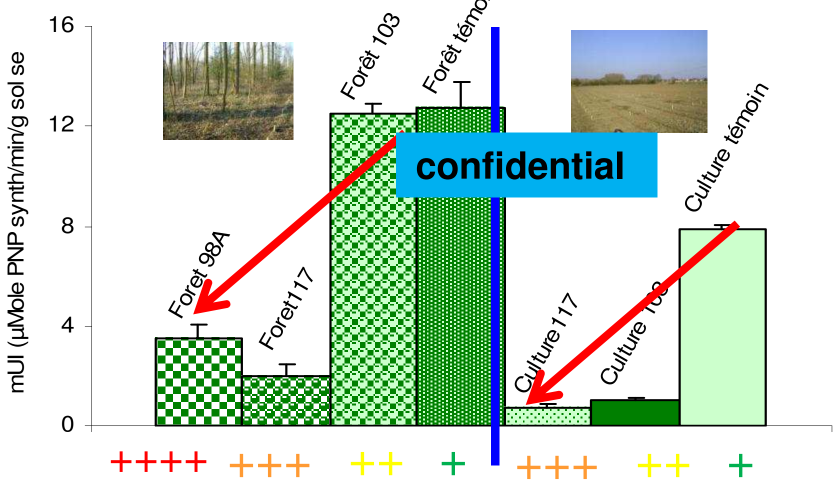
3.2 – Sensitivity to metallic contamination

Metaleurop Site

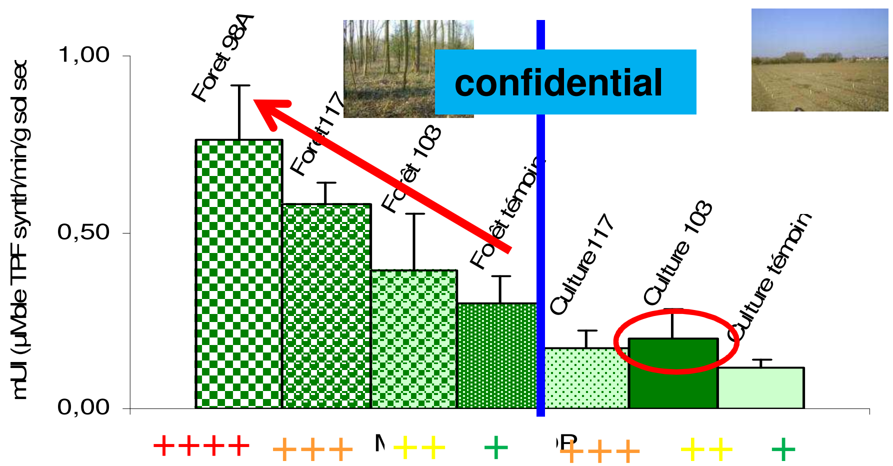
BIOindicateurs ADEME PeSac *(from Cheviron et al., 2009)*

Result (Ex) : Enzymatic Activities

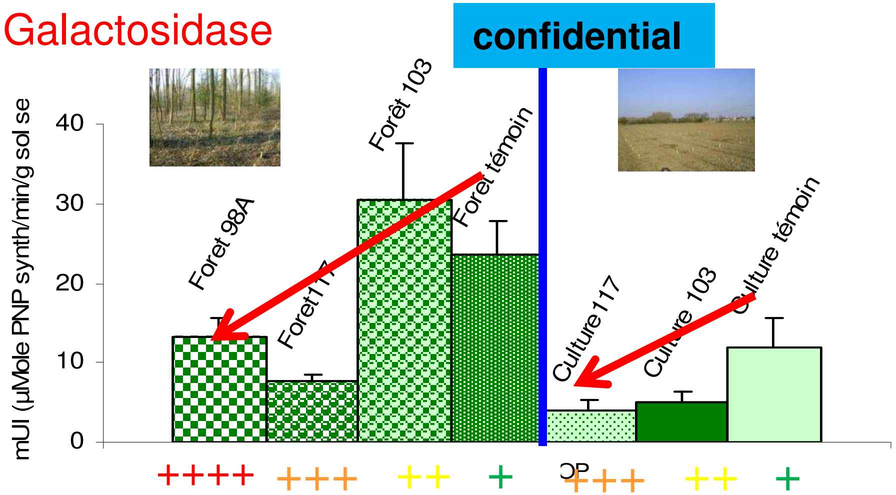
Sulfatase



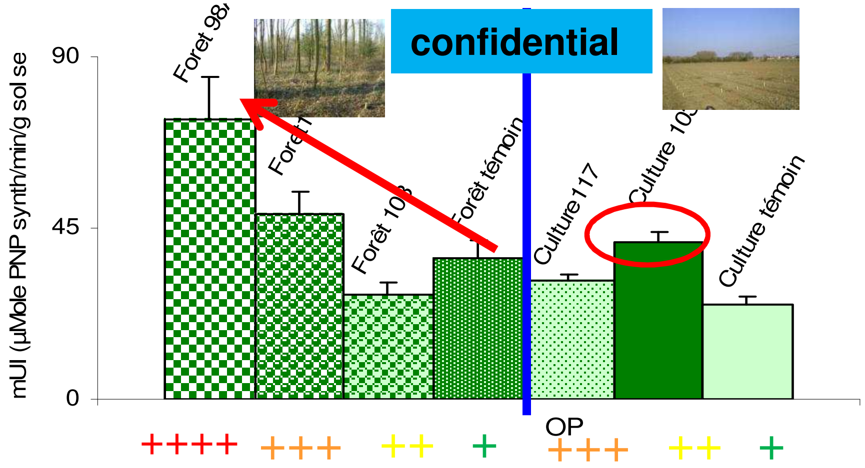
Hydrogenase



Galactosidase



Urease



Sulfatase and Galactosidase decrease with increase of contaminant

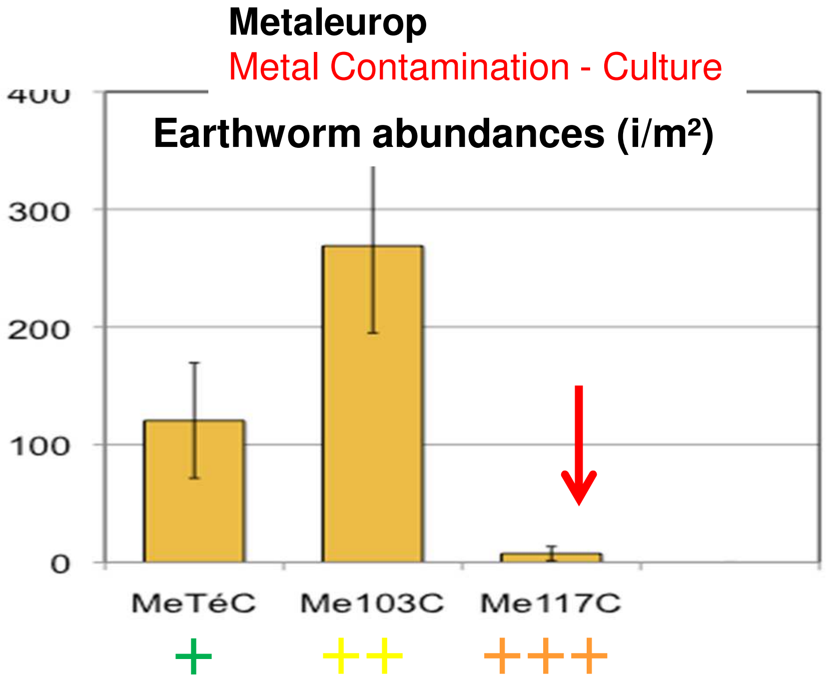
Hydrogenase and urease increase with increase of contaminant under forest more interactions under cultures

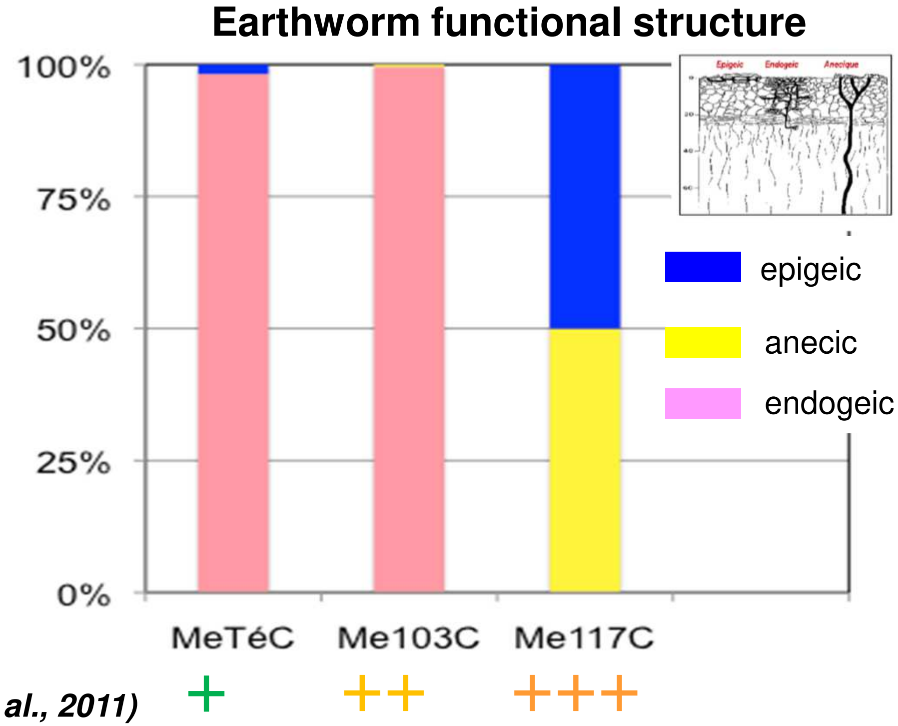
3.2 – Sensitivity to metallic contamination

Metaleurop Site

Results Fauna : earthworm

Scoring → Relevance of earthworm abundance and diversity





(From Pérès et al., 2011)

Earthworm abundance decreases but not related to contamination gradient

- Under low contamination : favourable conditions for earthworm developpment
- Under high contamination : strong impact on abundance

Earthworm functional structures

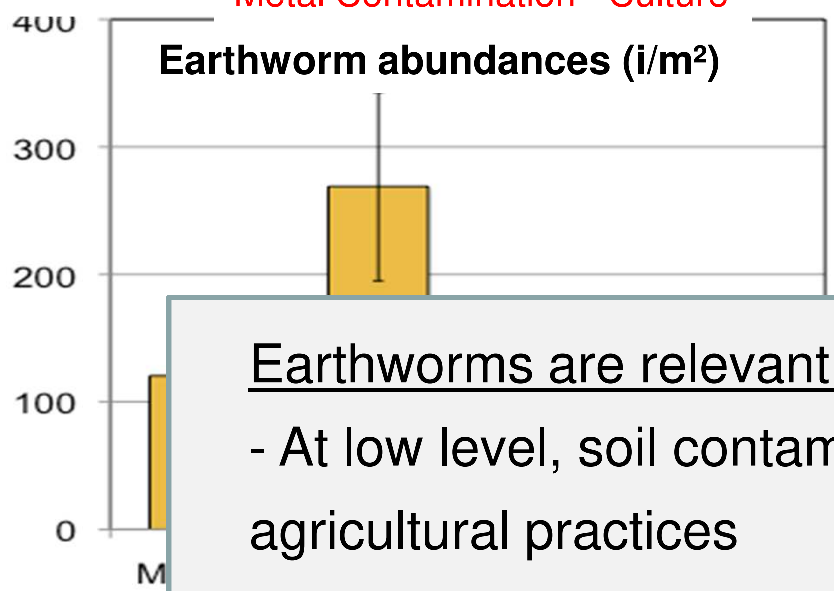
- Under low contamination : no impact of contamination
- Under high contamination : disappearance of endogeic species (which create granular structure)

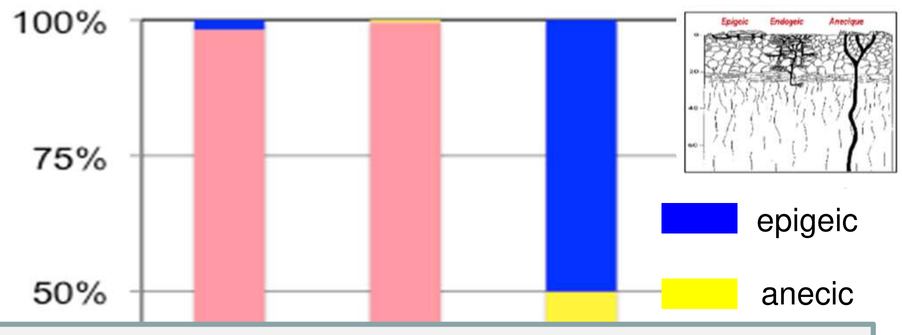
3.2 – Sensitivity to metallic contamination

Metaleurop Site

Fauna - earthworm

Scoring → Relevance of earthworm abundance and diversity





Earthwor

- Under l
- Under h

Earthwor

-Under lo
- Under h

ure)

<u>Earthworms are relevant indicators of perturbations</u>

- At low level, soil contamination can be balanced by agricultural practices

-Under high contamination level, earthworm community is strongly alterated

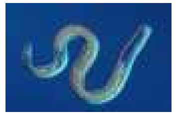
➔ degradation of soil functions (soil structuration, contaminant degradation)

➔ Earthworms are functional indicators

3.2 – Sensitivity to metallic contamination

Metaleurop Site

Example: Fauna - Nematofauna community

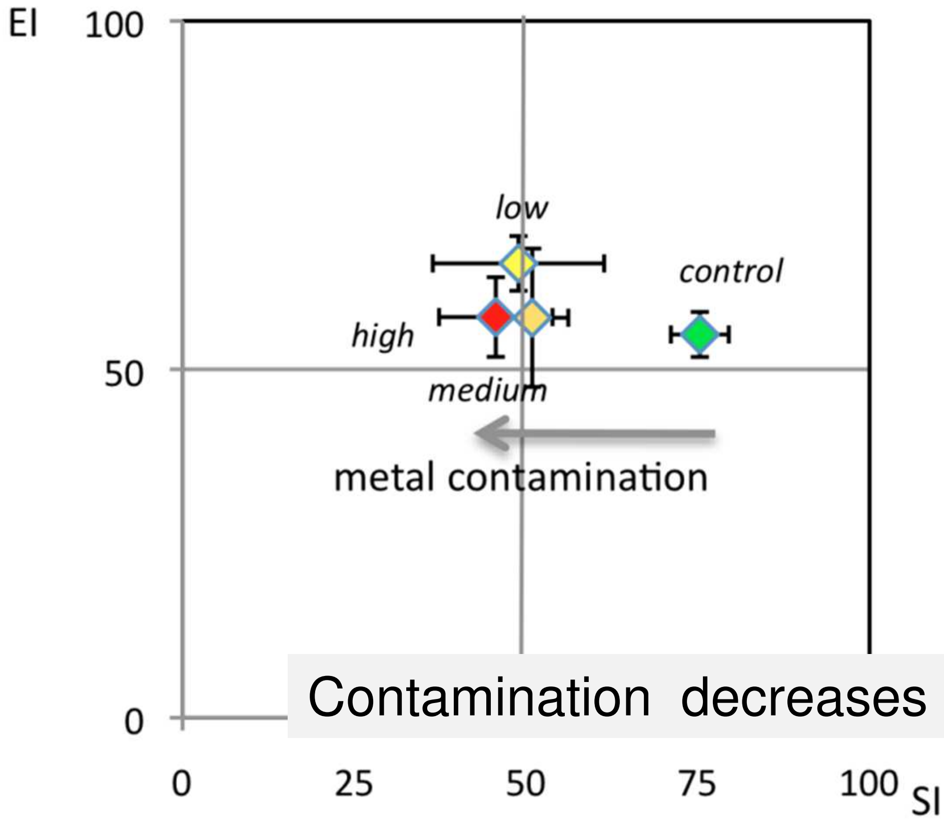


Scoring → Relevance of nematofauna abundance, and diversity

- SI: Structure Index (high presence of predators and omnivorous; stable community)
- EI: Enrichment Index (presence of decomposors; ressource availibility, nutrient flux)

Metaleurop

Metal Contamination - Woodland



Contamination decreases the community stability

Control

- SI > 50

→Stable community, food chain well developped

- EI > 50

→ Good ressource availability, flux of nutriment

Contaminated plots

- SI = 50

→Lower Stability of nematofauna community

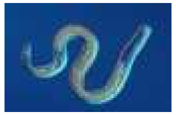
- EI > 50

→ Good ressource availability, flux of nutriment

From Villenave 2011

3.2 – Sensitivity to metallic contamination and HAP

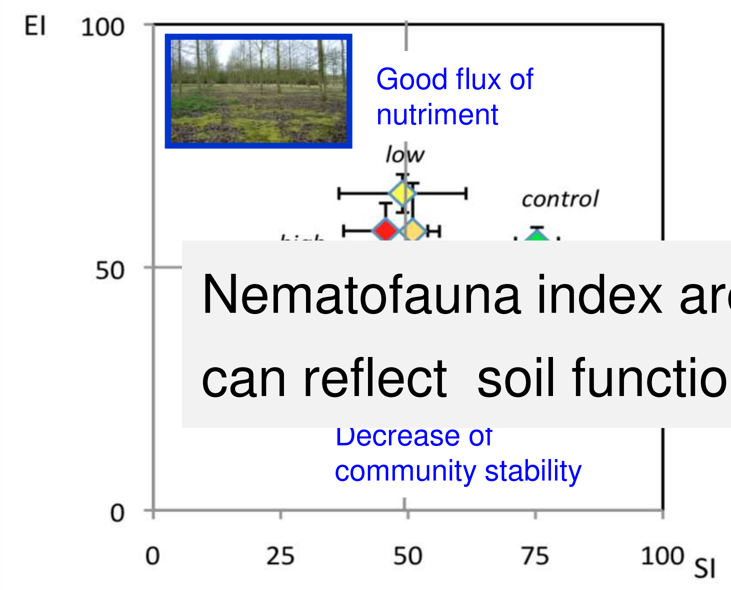
Example: Fauna - Nematofauna community



- SI: Structure Index (high presence of predator and omnivores; stable community)
- EI: Enrichment Index (presence of decomposor; ressource availibility, nutrient flux)

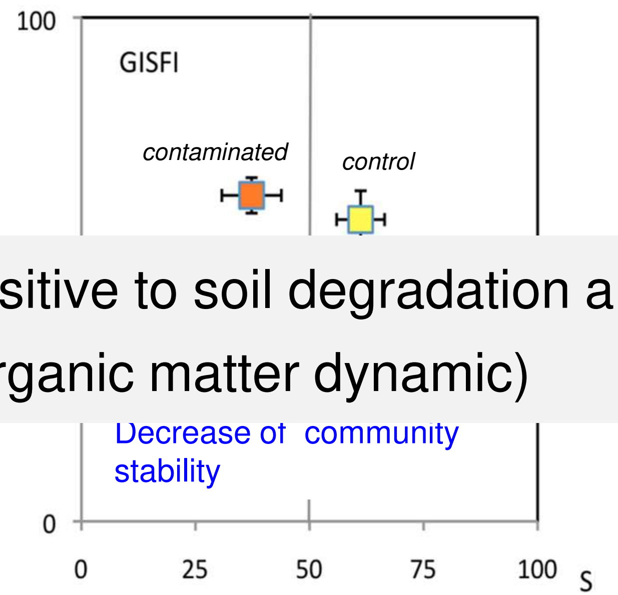
Metaleurop

Metal Contamination - Woodland



GIS-FI (Homécourt)

PAH – Industrial wastelands



Nematofauna index are sensitive to soil degradation and can reflect soil functions (organic matter dynamic)

- Contaminated soil (metal and HAP) → decrease of community stability (Structure indexSI)

From Villenave 2011

Results

1 – referential values

2 – transfer and risk assessment tools

3 –sensivity of indicators

4 – Synthesis

Synthesis :
which bioindicator for which need under contaminated soil?

DIAGNOSTIC

Besoins	Famille	Paramètres
Bioaccumulation	Flore	• Bioaccumulation in plants
	Faune	• Bioaccumulation snails • Bioaccumulation micromammals
Effect linked to contamination (organic and inorganic)	Microbiology	• Bacteria and fungi abundance • Bacteria and fungi diversity • Enzymatic activities: laccases, β-glucosidase, arylsulfatase
	Faune	• Earthowrm abundance and diversity • Nematode abundance and diversity • macrofauna abundance and diversity
	Flore	• Test Oméga 3

(Grand et al., 2012b)

BIOindicateurs ADEME

Synthesis :
which bioindicator for which need under contaminated soil?

Management of contaminated sites

Besoins	Famille	Paramètres
Biological state	Microbiology	• Bacteria and fungi abundance • Bacteria and fungi diversity • Enzymatic Activities: β-glucosidase, arylamidase, phosphatase alcaline • Carbon and Nitrogen mineralisation
	Fauna	• Earthowrm abundance and diversity • Nematode abundance and diversity • Macrofauna abundance and diversity • Microarthropods abundance and diversity

(Grand et al., 2012b)

Bioindicator program - Transfer to stakeholders : Website = an interface with acces to different tools

https://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur

- **Technical document** for all bioindicators → can be downloaded
  - Description of the indicator
  - Description of the sampling and laboratory methods
  - Results and application
- **Range values** for all bioindicator
- **Tool for selecting** bioindicator

Bioindicator program - Transfer to stakeholders :

➢ Website = Technical documents

TOOL WORKSHEET NO.2

Omega-3 fatty acid

M. Le Guédard, J.-J. Bessoule / LEB Aquitaine Trantert / ADERA ;
UMR CNRS 5200, Vilenave d'Ornon
Contact : **marina.leguedard@u-bordeaux2.fr**

DESCRIPTION OF THE INDICATOR

Name of the indicator: Omega-3 fatty acid: plant fatty acids, diagnostic and surveillance tool for soil contamination.

Figure 1

Ecological role of the organism under test: In the terrestrial ecosystem, plants are primary producers because they are completely independent from the rest of biosphere for their carbon and energy supplies. They create their own organic matter through photosynthesis only by using light and mineral matter (water, CO_2 and mineral salts, Fig 1). Photosynthesis reaction enables plant growth and results in the release of oxygen, essential to our lives.

In addition to their role as oxygen producers, plants also bring organic matter, used as a necessary source of energy and carbon for other trophic levels, thus forming the first link in the food chain. They are also used as shelters and food by fauna. Moreover, they bring elements required by soil bacteria that participate in the improvement of soil fertility. Therefore, they ensure the sustainability of terrestrial ecosystems. Furthermore, through their root systems, plants aerate, build and protect the soil by limiting erosion and crusting.

Type of indicator: The Omega-3 fatty acid indicator is a biochemical biomarker that highlights the exposure of plants to various soil contaminants (metals and organic). More precisely, this indicator shows the state of degradation of chloroplastic lipids exposed to soil contaminants. In fact, chloroplasts (Fig 2) contained in higher plants' leaf cells ensure chloroplastic membrane integrity and play a very important role in photosynthetic activity. Linolenic acid (C18:3) is a fatty acid mostly associated with chloroplastic lipids. Chloroplasts contain up to 90% of this Omega-3 fatty acid. Lipid degradation driven by the presence of contaminant(s) is evaluated by measuring the fatty acid content of plants' leaves and by calculating the Omega-3 fatty acid indicator, reported as the ratio between C18:3 content and other 18 carbon atoms-fatty acids. This indicator decreases in the presence of contaminants (Fig 3).

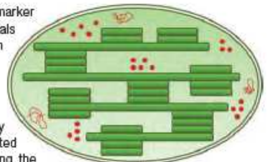


Figure 2

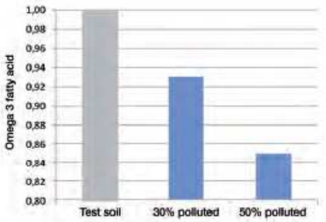


Figure 3

The Omega-3 fatty acid indicator can be used at the laboratory (AFNOR XP-X31 233 standard) or on the field. It allows for a global appreciation of the state of health of the ecosystem, an evaluation integrated over time and space of phytoavailable pollutants as well as an early detection of contaminants' effects. Measured effects on plants are even observed with contaminants present at "sub-acute" doses, which alter neither plant germination nor plant growth, and which even do not lead to any particular "visual" phenotype (chlorosis, yellowing of the leaves…).

INTERPRETATION OF RESULTS

Need for a local reference system:

The Omega-3 fatty acid indicator varies between 0 and 1, 1 showing the modality with the fewest effects. Data received are interpreted in relation to a reference situation chosen for the studied site. If, in the best-case scenario, a test modality has been identified, then data is compared to the test modality with a relative value of 1. This indicator allows us to rank modalities.

Based on our experience, the relative values determined for a modality are interpreted as follows: In relation to the modality with a relative value of 1:

- 1 > Relative value > 0.93: No effect of modality
- 0.93 ≥ Relative value ≥ 0.85: Average effect of modality
- Relative value > 0.85: Strong effect of modality

Based on data collected in the BIO2 program (Fig 9), the Omega 3-fatty acid indicator has a variation range of 0.89 to 1 on agricultural sites and of 0.74 to 1 on contaminated sites. Such results show that the Omega 3-fatty acid indicator is sensitive to specific cultivation practices (ex: tillage system) and pollution (metal and organic).

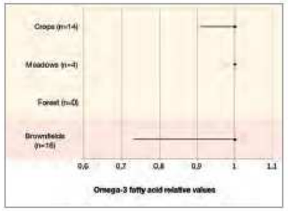


Figure 9

EXEMPLE OF APPLICATION

Auzon site: 7 modalities under study on this former phytosanitary manufacturing plant

Location	Modalities	Distribution Parameters	As	Pb	Sb
Outside the site	CtW	Median MAD	123^{a} 3	60^{a} 1	12^{a} 1
	CtG	Median MAD	115^{a} 8	52^{ab} 2	7^{a} 1
Within the Site	CoW	Median MAD	339^{b} 23	104^{a} 4	38^{b} 7
	CoWW	Median MAD	3285^{a} 1490	4575^{a} 2800	$3930^{d,e}$ 2827
	CoWa	Median MAD	$1087^{d,e}$ 530	1834^{a} 1448	2222^{d} 1864
	CoWH	Median MAD	661^{c} 216	282^{c} 145	176^{c} 100

Table 1 : Total contents (mg/kg) in As, Pb and Sb in soils of different modalities. On the same column, the letters indicate significant differences between modalities ($p<0.05$, Tukey's test).

As expected, total content in As, Pb and SB is significantly higher on soils of modalities located within the former manufacturing plant's walls than on soils of modalities located outside the site. Based on these analyses, within the site, it seems that modalities CoWW and CoWa are highly contaminated in As, Pb and Sb, while modalities CoW and CoWH are moderately contaminated.

As for the Omega-3 fatty acid indicator, relative values are significantly lower on modalities located within (in red, Fig 10) the former manufacturing plant's walls than on those located outside (in green). This indicator confirms the results of physico-chemical analyses as it distinguishes between contaminated and non-contaminated modalities. However, the ranking of different modalities in relation to the measured level of contamination with total content in As, Pb and Sb or the Omega 3-fatty acid indicator is not exactly the same.

Bioindicator program - Transfer to stakeholders :

➢ **Website = Range values for all bioindicators**

→ **Acces to all public to aggregate data**

Values reflectiong the variation of bilogical parameters depending on soil, land uses ….

Rappel des caractéristiques de sol choisies :

- Usage du sol :
- Niveau de contamination :
- Texture :
- pH :
- Carbone organique :

Faune : Lombriciens

Ces valeurs ont été établies à partir de **188** points de mesure.

Unité : Abondances : nombre d'individus par m2 / Biomasses : g par m2

	MIN	MAX	MOY	STD
Abondance totale	0	636	117	113
Biomasse totale	0.00	423.53	56.34	62.56
Abondance Epigés	0	191	14	28
Abondance	0	224	49	56

Biocindicator program - Transfer to stakeholders :

- **Website = Tool for selecting the relevant bioindicator**

- Introduction
  - Definition of bioindicator/ biomarker
  - Why do we need bioindicators ?
  - Interest of soil organisms (weight, diversity, functions)
  - Different criteria used for different bioindicators
- What are the need in contaminated soil ?
- Which tools do we have in contaminated soil and for which purpose ?

  Diagnostic (Risk assessment / soil quality), management (monitoring)

  French National program Bioindicator
  - Objectives, site and sampling design presentation
  - Results
  - Synthesis of indicators depending on the purpose
- **Case studies in Italie**
- Conclusion

European Context – Carpiano Programme

COMMISSIONE EUROPEA
Centro Comune di Ricerca

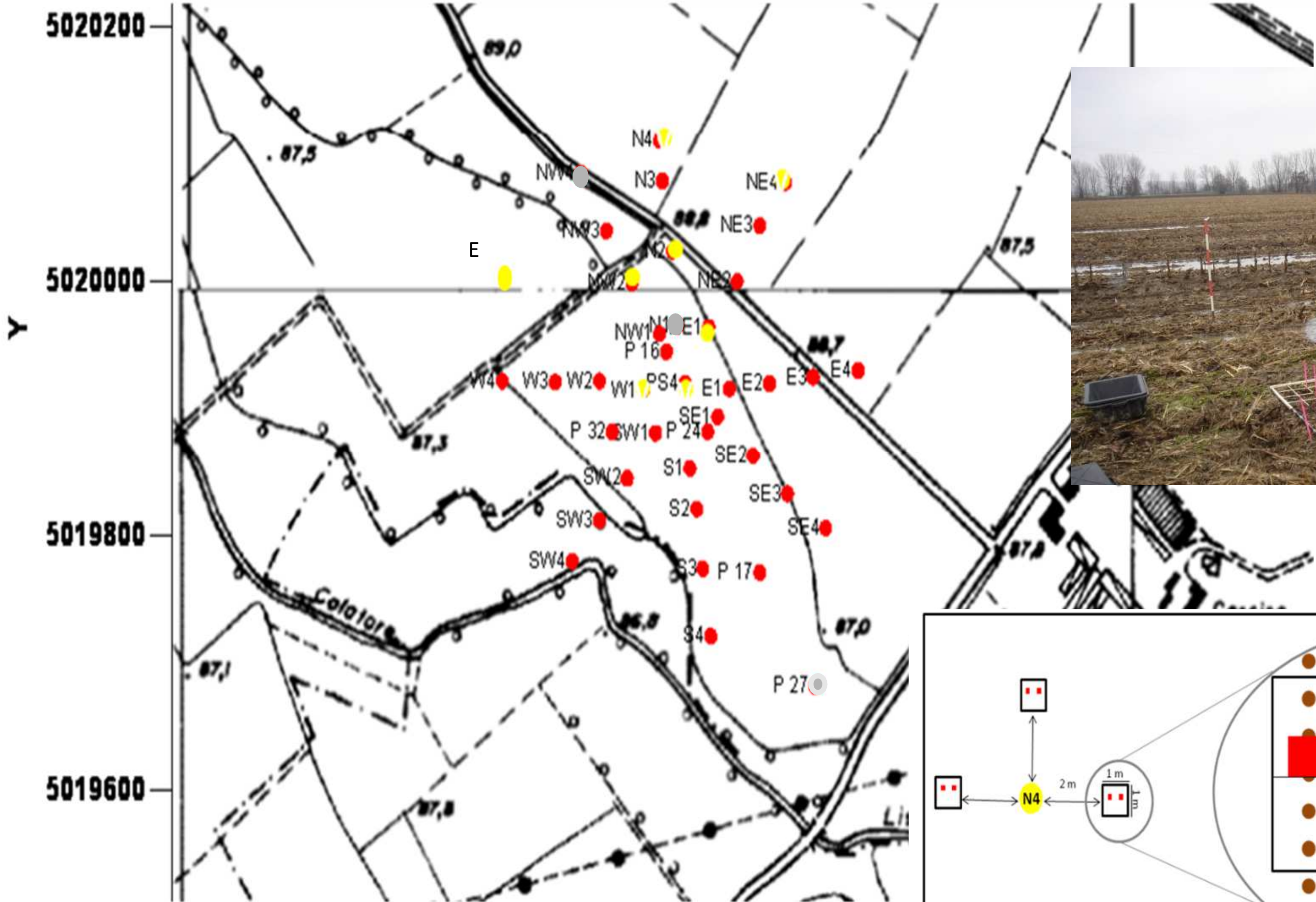
RegioneLombardia

Istituto dell'Ambiente e della Sostenibilità

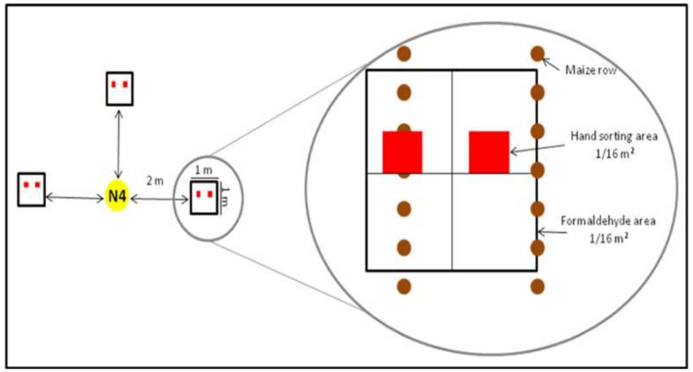
Progetto di monitoraggio ambientale di un'area contaminata nelle Province di Pavia e di Milano

European Context – M&M Carpiano project



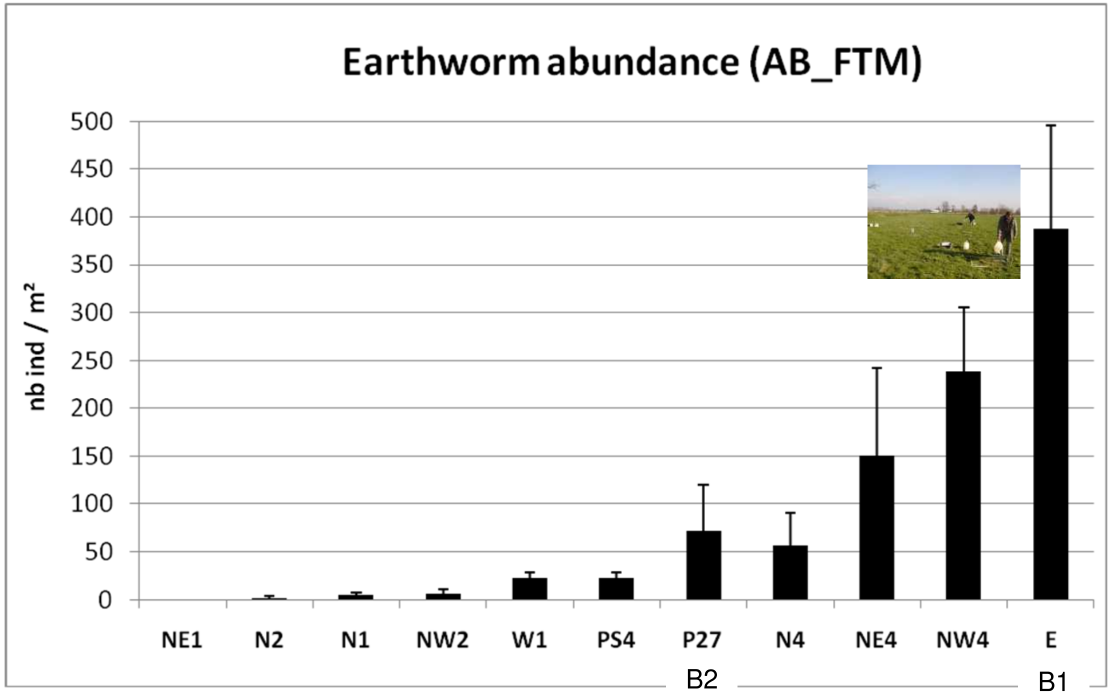
Sampling design (11 points)



3 replicates surounding the points

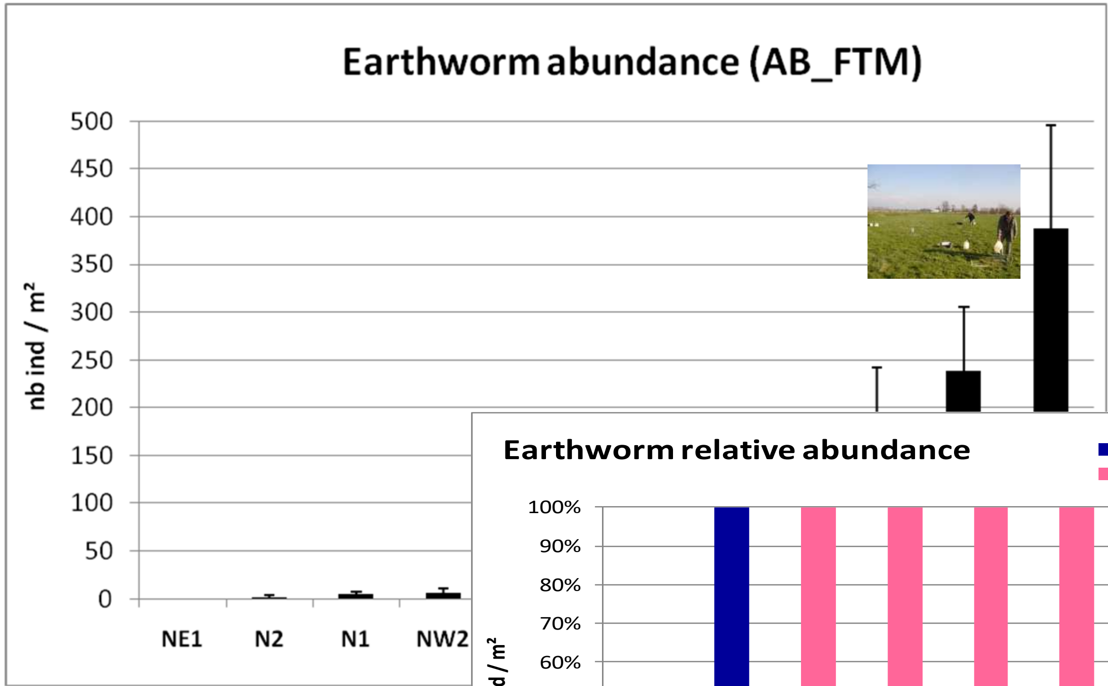
European Context – M&M and Results Carpiano

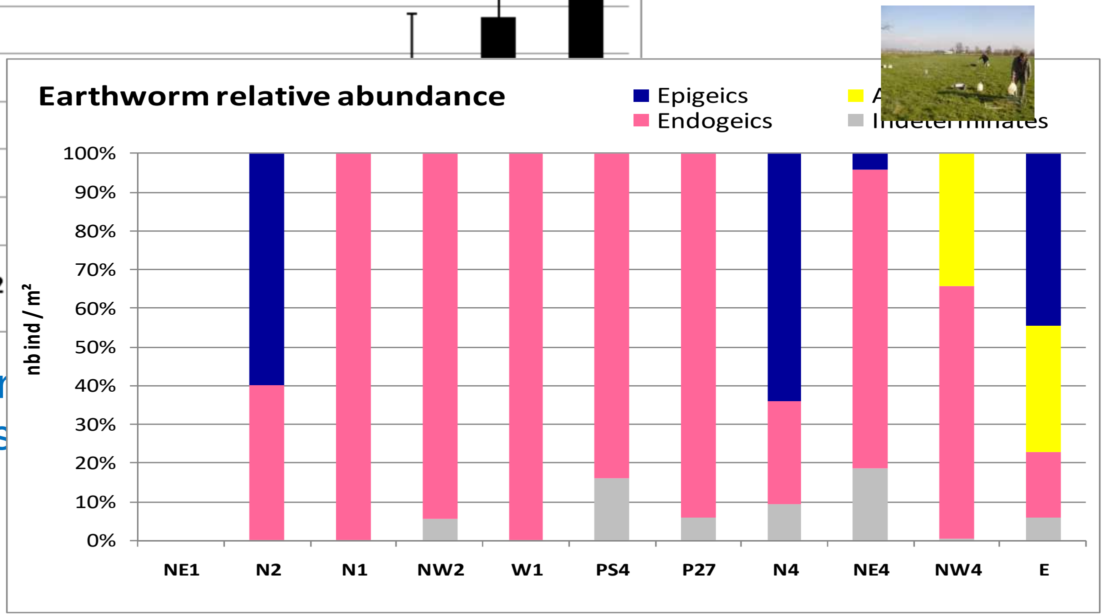


➔ Low values due to agricultural practices X contamination X soil conditions (wet)

➔ Abundances (biomass) increase with pollution decrease

European Context – M&M and Results Carpiano





➔ Low values due to agr

➔ Abundances (biomass

➔ soil pollution strongly impact on earthworm community

➔ Interest in use of ecological groups

- Introduction
  - Definition of bioindicator/ biomarker
  - Why do we need bioindicators ?
  - Interest of soil organisms (weight, diversity, functions)
  - Different criteria used for different bioindicators
- What are the need in contaminated soil ?
- Which tools do we have in contaminated soil and for which purpose ?

  Diagnostic (Risk assessment / soil quality), management (monitoring)

  French National program Bioindicator
  - Objectives, site and sampling design presentation
  - Results
  - Synthesis of indicators depending on the purpose
- Case studies in Italie
- **<u>Conclusion</u>**

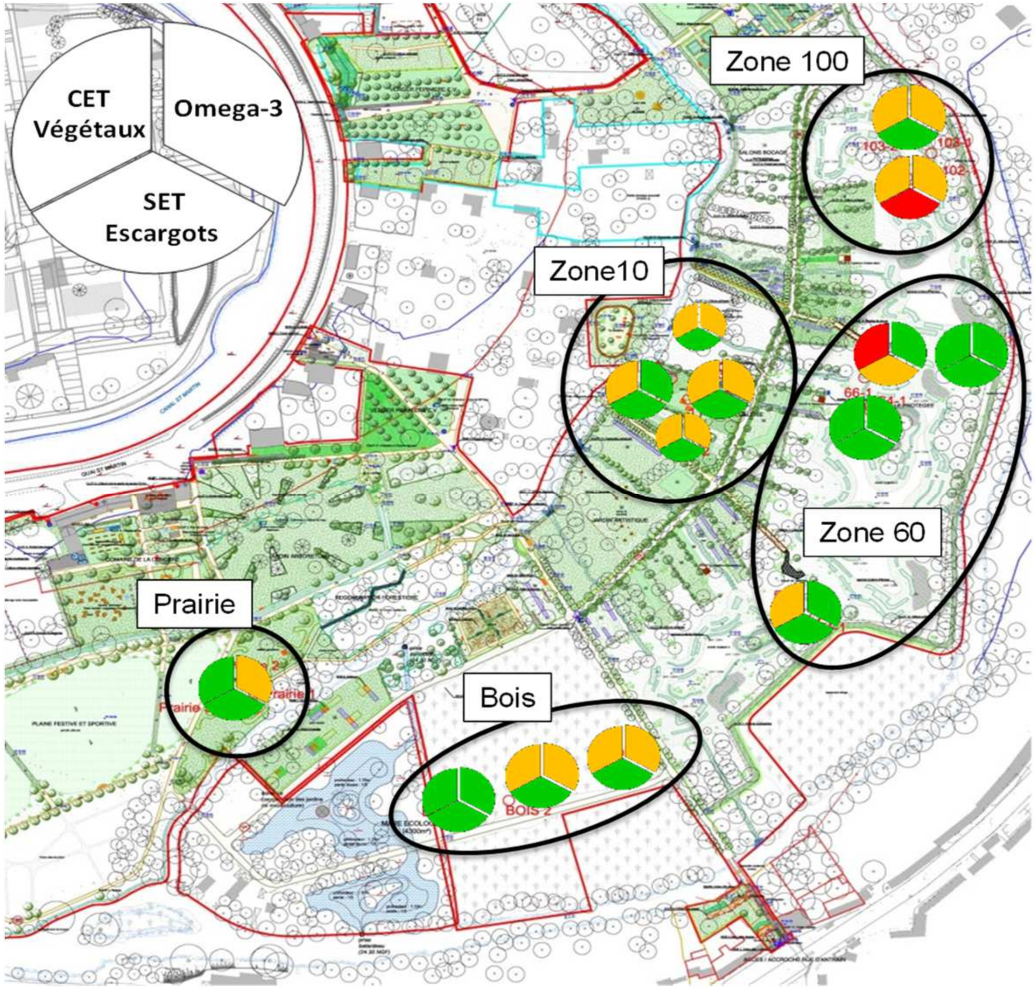
Lessons and perspectives

- **Soils are not dead … and solutions exist to improve their biological states**
- **Bioindicators are complementary to physico-chimical analysis (they do not replace them)**
- **Tools are mature and available (AFNOR, ISO)**

- **Only one parameter is never enough to provide a good view of biological state and functioning → need multicriteria approach**

Prairie Saint Martin Projet → Urban Park management on contaminated soil

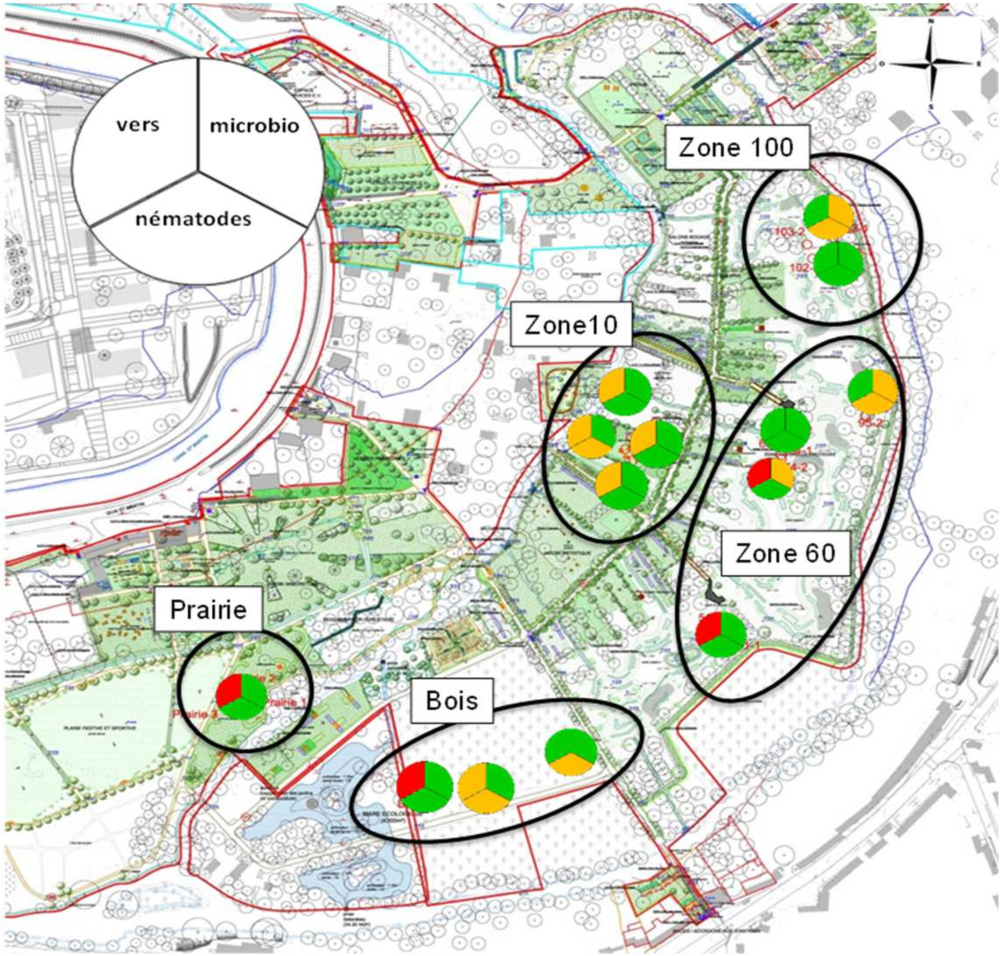
❑ Diagnostic of metal transfer – bioaccumulation - risk assessment



Pérès et al., 2015

Prairie Saint Martin Projet → Urban Park management on contaminated soil

❑ Diagnostic of soil biological quality



Pérès et al., 2015

Lessons and perspectives

- **Soils are not dead … and solutions exist to improve their biological states**
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Perspectives

- **Laboratories exist but we need to increase their developpment**

Biochem

SEMSE

ELISOL ENVIRONNEMENT

GenoBiome

OPVT

Laboratoire Analyses Microbiologiques Sols

        

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Thank you for your attention