



HAL
open science

Towards an index of biological state of the soil as a new tool for ecotoxicological studies

Christian Mougin, Karine Laval, Salima Taibi, Daniel Tessier, Anne-Sophie Lemaire, Sylvie Barry

► To cite this version:

Christian Mougin, Karine Laval, Salima Taibi, Daniel Tessier, Anne-Sophie Lemaire, et al.. Towards an index of biological state of the soil as a new tool for ecotoxicological studies. 15th SETAC Europe Annual Meeting, May 2005, Lille, France. 2005. hal-01600208

HAL Id: hal-01600208

<https://hal.science/hal-01600208>

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

***Towards an index of biological state of the soil
as a new tool for ecotoxicological studies***

Christian Mougin, Karine Laval

Salima Taïbi, Daniel Tessier, Anne-Sophie Lemaire, Sylvie Barray



The context: ecotoxicology of the soils

Assess the fate and impact of pollutants on the soil ecosystem

- the cropped soils

(plant protection products, medicines, EDCs, ETM...)

Take into account the other perturbations of these ecosystems

- anthropic: practices, management, wastes
- natural: pedo-climatic

Question:

What should be an equilibrated state of the soil ecosystem (a situation of « reference ») useful to assess the real impact of pollutants ?

Our research program

« An index of biological state of the soils »



3 objectives:

- build an index for natural (meadow) and cropped soils
- study its variation in response to agronomic (practices at the field) or environmental (pollutions in microcosms) constraints
- validate it *in situ* using fields, polluted soils... exhibiting various physico-chemical and biological properties

Our hypotheses

- **The biological state of a soil can be assessed by a mathematical combination of variables, describing both the structural and functional diversity of the microbial communities**
- **The multiplicity of the determined variables allows the modulation of the bias related to each method**
- **Meadows and crops behave as systems of reference regarding polluted sites**

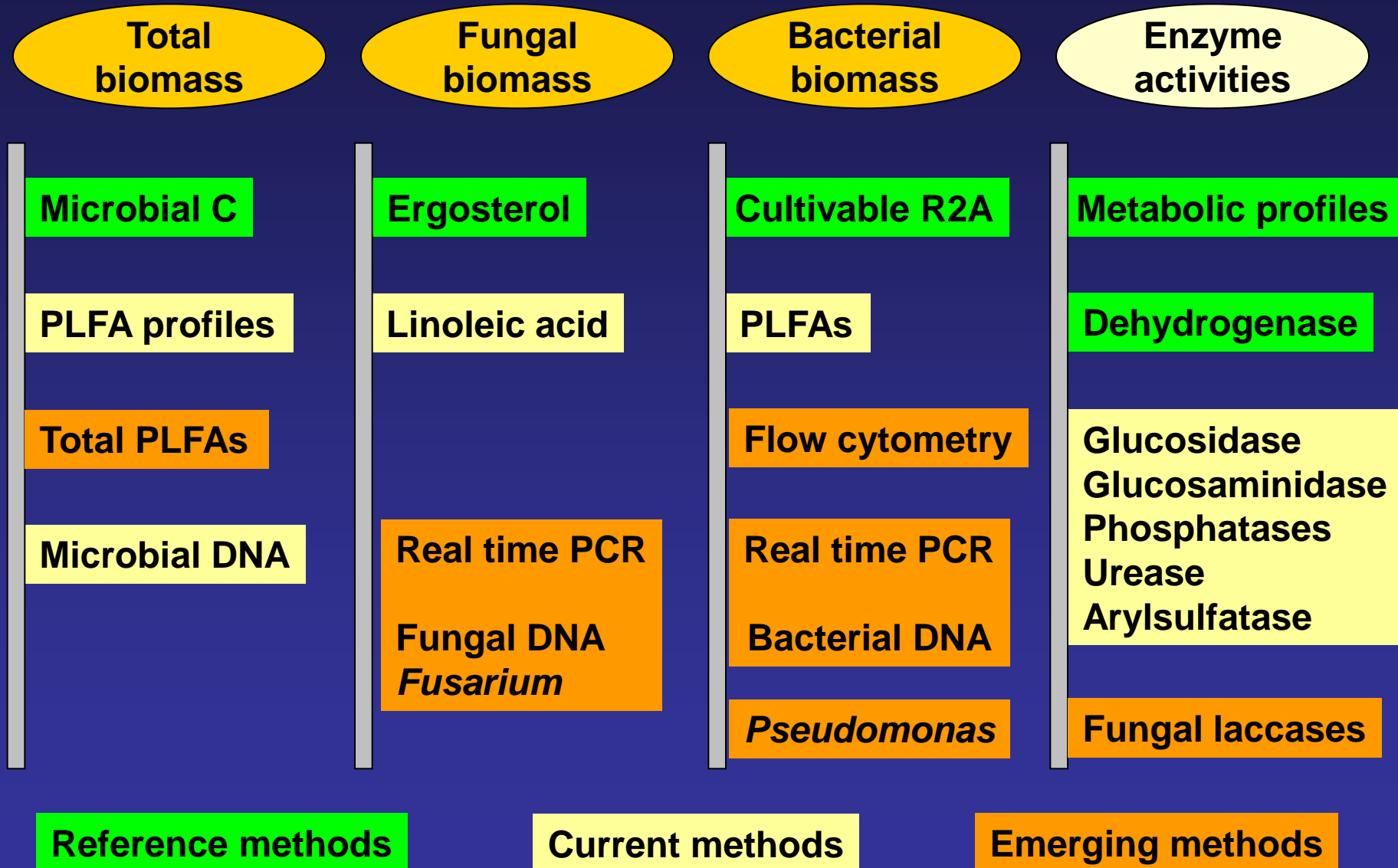
Our strategy

The description of the relationships between physico-chemical and biological variables in contrasted situations

Three steps linked by mathematical modelling:

- **measure of the variables in agronomic situations (year 1)
meadows and crops**
- **assessment of the variations due to pollution (year 2)
microcosms, organics, ETM**
- **validation *in situ* in fields, polluted soils... (year 3)**

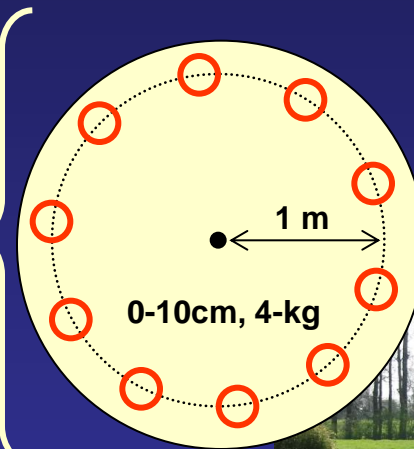
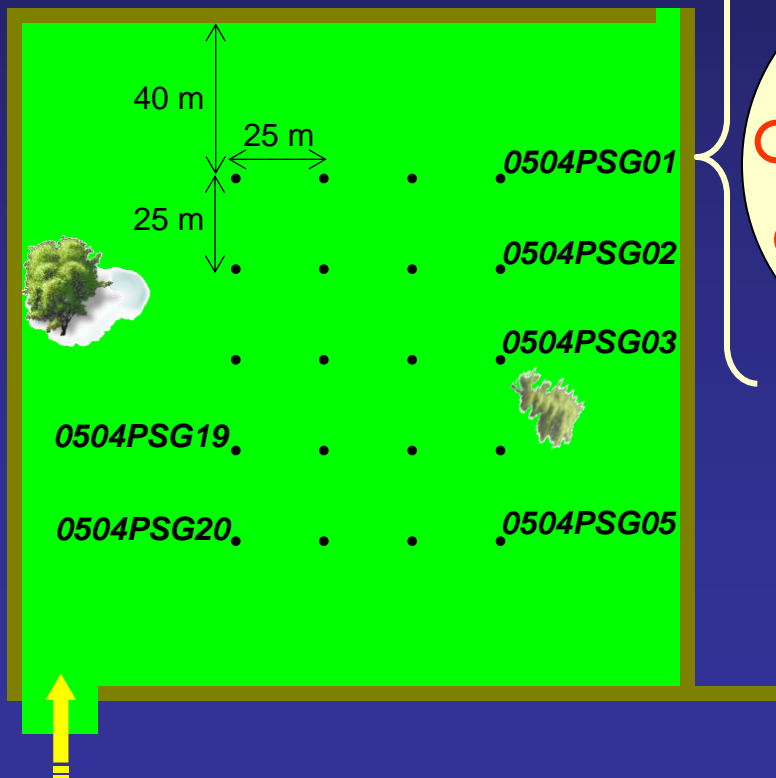
Variables describing the microbial communities



Measure of the variables at the field

- 2 sites: St Georges and Yvetot (near Rouen)
- 2 situations: meadows and crops
- 3 periods of sampling: april, june and september

St Georges, meadow

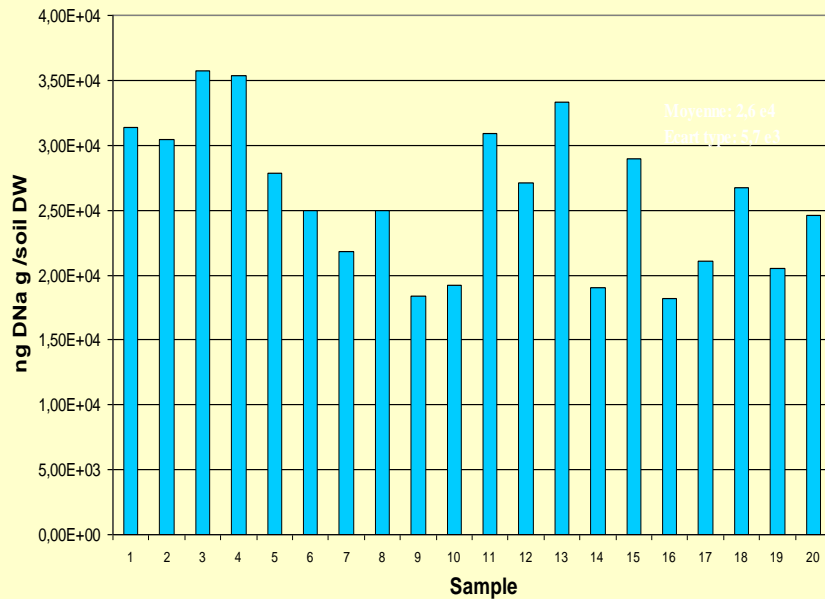


Sub-soil: chalk
Soil: silt layer + silex



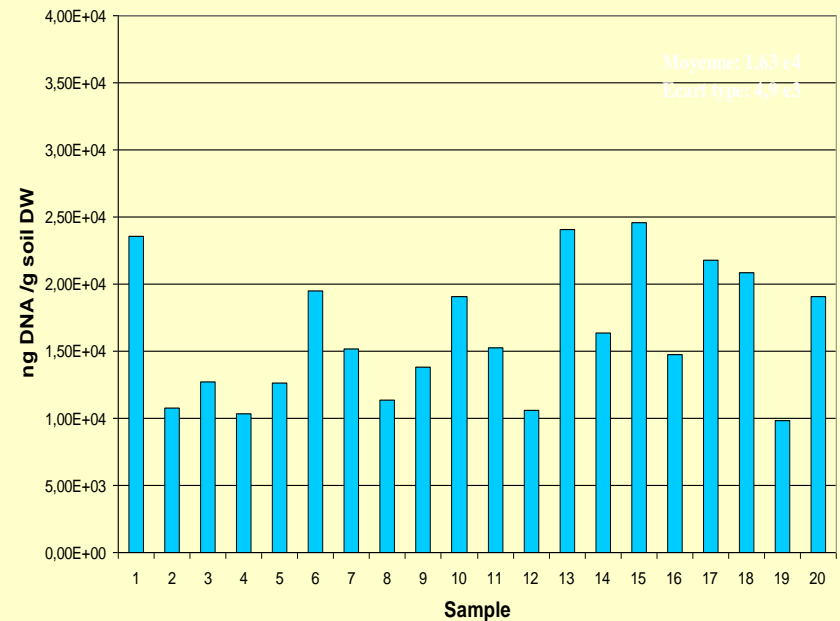
St Georges: total biomass, microbial DNA

Meadow



26.0 ± 5.7 µg/g

Crop: wheat



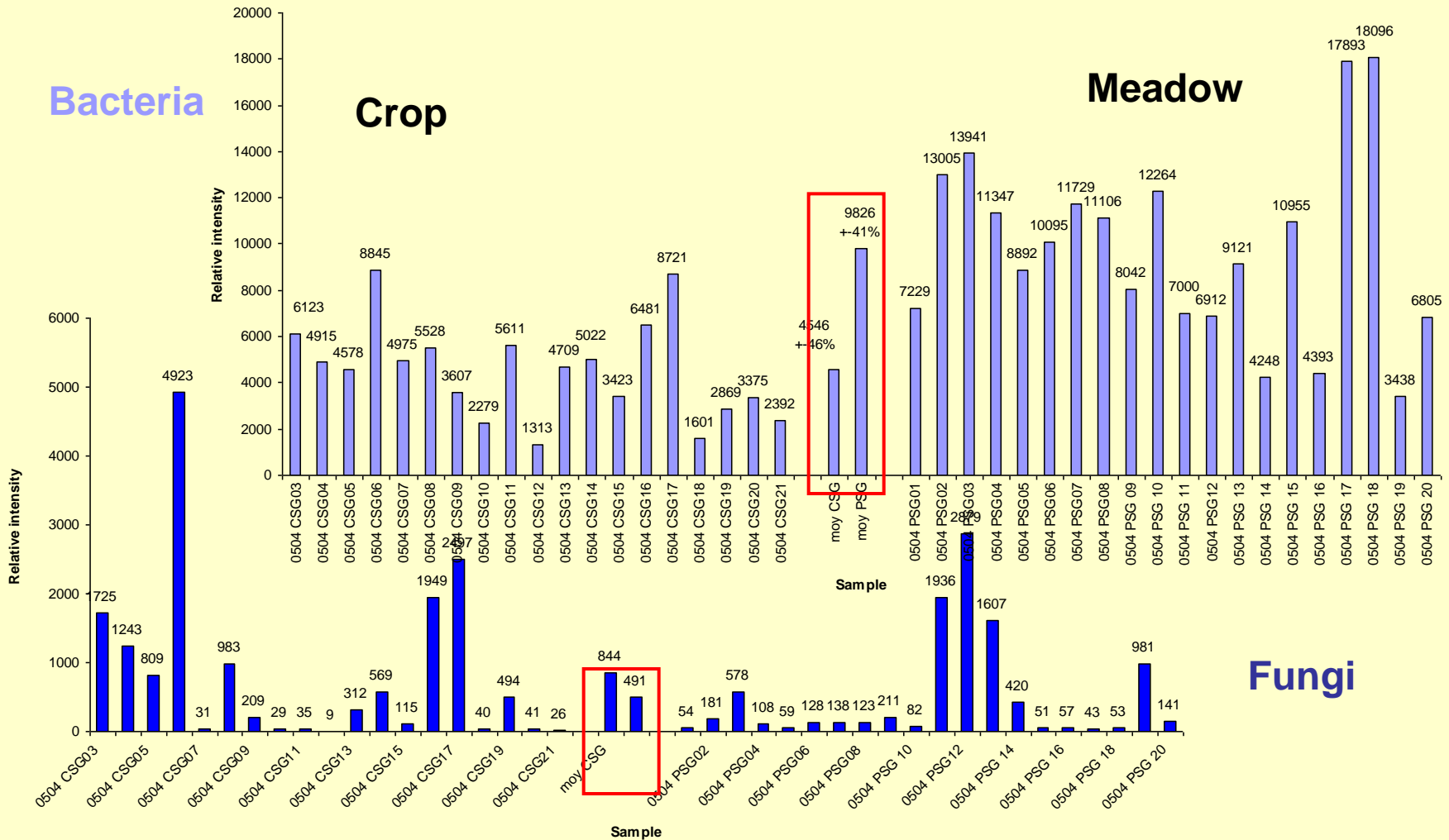
16.3 ± 4.9 µg/g

► higher total microbial biomass in the meadow, with a lower spatial variation

Structural diversity: partial results

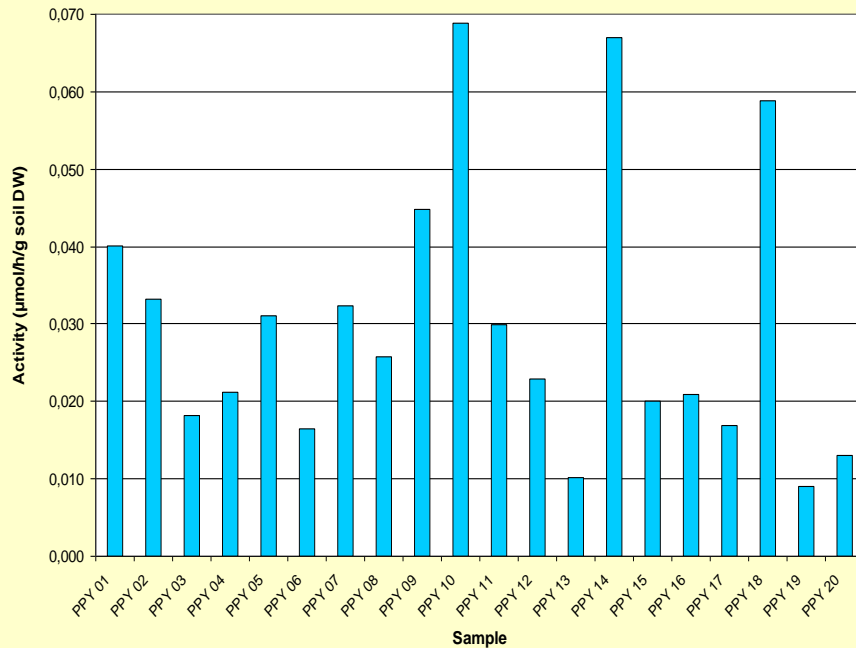
Variable	St Georges meadow	St Georges crop	Yvetot meadow	Yvetot crop
Microbial DNA ($\mu\text{g/g}$ soil DW)	26.0 \pm 5.7	16.3 \pm 4.9	36.0 \pm 4.9	15.6 \pm 2.1
Fungal DNA (ng/g soil DW)	201.0 \pm 139.0	18.7 \pm 8.5	118.0 \pm 41.8	33.4 \pm 8.4
Ergosterol ($\mu\text{g/g}$ soil DW)	12.2 \pm 3.3	3.4 \pm 0.6	4.7 \pm 1.9	1.6 \pm 0.5
<i>Fusarium</i> DNA (ng/g soil DW)	0.7 \pm 0.5	0.2 \pm 0.2	1.7 \pm 0.6	0.5 \pm 0.2
Cultivable bac. (bact/g soil DW)	4.10 ⁸	5.10 ⁸	1,5.10 ⁹	5.10 ⁸
Flow cytometry (bact/g soil DW)	5.0.10 ⁹ \pm 1.1.10 ⁹	2.8.10 ⁹ \pm 7.6.10 ⁸	1.2.10 ¹⁰ \pm 7.3.10 ⁹	3.0.10 ⁹ \pm 1.8.10 ⁹
<i>Pseudomonas</i> (bact/g soil DW)	7.10 ⁶	1.3.10 ⁷	2.6.10 ⁷	1.9.10 ⁷

St Georges: PLFA



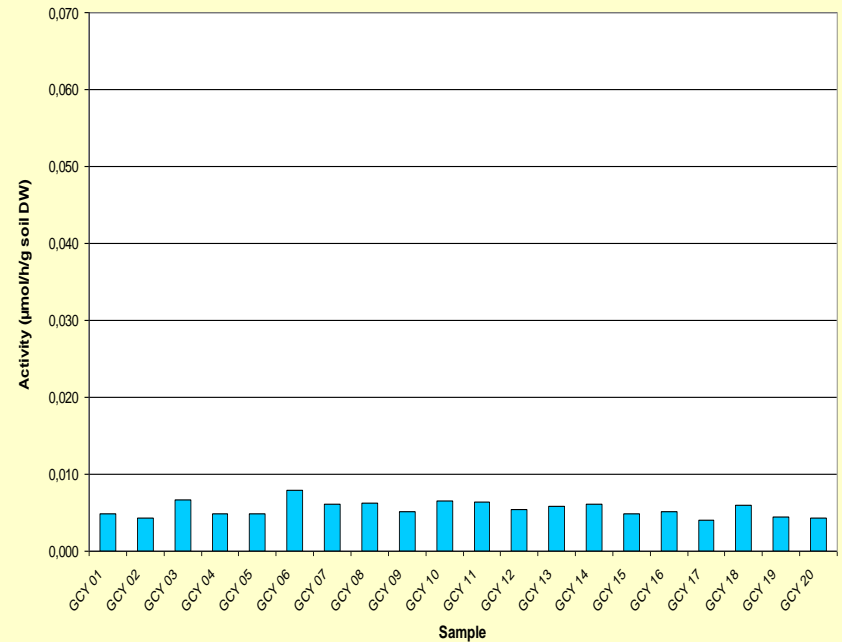
Yvetot: dehydrogenase

Meadow



30.0 ± 18.0 nmol/h/g soil DW

Crop: wheat



6.0 ± 1.0 nmol/h/g soil DW

► higher activity in the meadow, but with a higher spatial variation

Functional diversity: enzymes

Variable	St Georges meadow	St Georges crop	Yvetot meadow	Yvetot crop
Dehydrogenase (nmol/h/g soil)	11.0 ± 7.0	8.0 ± 5.0	30.0 ± 18.0	6.0 ± 1.0
Glucosidase (nmol/h/g soil)	266.1 ± 123.6	653.0 ± 162.6	670.0 ± 170.0	249.7 ± 76.2
N-acetyl gluco. (nmol/h/g soil)	486.2 ± 227.4	200.1 ± 81.1	212.0 ± 59.5	97.4 ± 72.4
Alk. phosphat. (nmol/h/g soil)	395.0 ± 90.3	506.5 ± 435.5	895.2 ± 642.8	747.8 ± 204.1
Aci. Phosphat. (nmol/h/g soil)	755.0 ± 354.3	640.0 ± 129.6	1368 ± 608	522.3 ± 321.9
Arylsulfatase (nmol/h/g soil)	91.0 ± 109.0	3.0 ± 48.0	444.0 ± 244.0	241.0 ± 10.0
Urease (nmol/h/g soi)	13.0 ± 4.0	10.0 ± 1.0	10.0 ± 4.0	8.0 ± 1.0

Conclusions

Structural diversity

- The mean values of the descriptors are higher in meadows than in crops
- Their spacial variations are lesser in crops than in meadows
- ▶ meadows seem to exhibit high amounts of fungal/bacterial biomass

Functional diversity

- Meadows strongly differ from crops
- The diversity of bacteria present in meadows is higher than this measured in crops
- ▶ meadows seem to exhibit the highest functional diversity
- ▶ Decreased selectivity of functional variables vs diversity

Perspectives

- **Variation of the descriptors with the season**
- **Profiles of soil enzymes**
- **Relationship with physico-chemical descriptors**
- **Impact of pollutant**
- **Database**
- **Mathematical treatment**
- **Development of new descriptors**

Flow cytometry

5 g + 45 ml NaCl



Vortex, centrifugation 1000 rpm 5mn



Supernatant, dilution in NaCl



10^{-1} et 10^{-2}



10^{-2} et 10^{-3}

Labeling with **SYBR Green I**
0.05 % final (488-525 nm)

Spreading on R2A medium: 100 μ L
Incubation

Cytometry (13 μ L)



Quantification of total bacteria

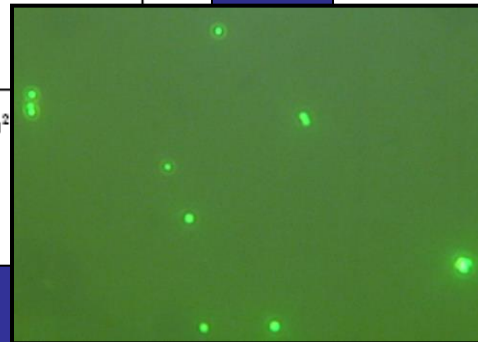
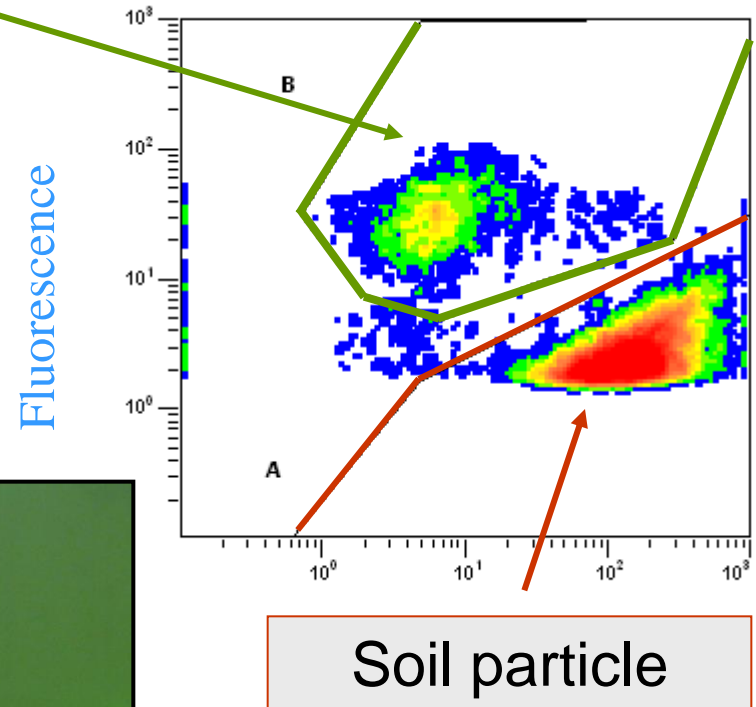
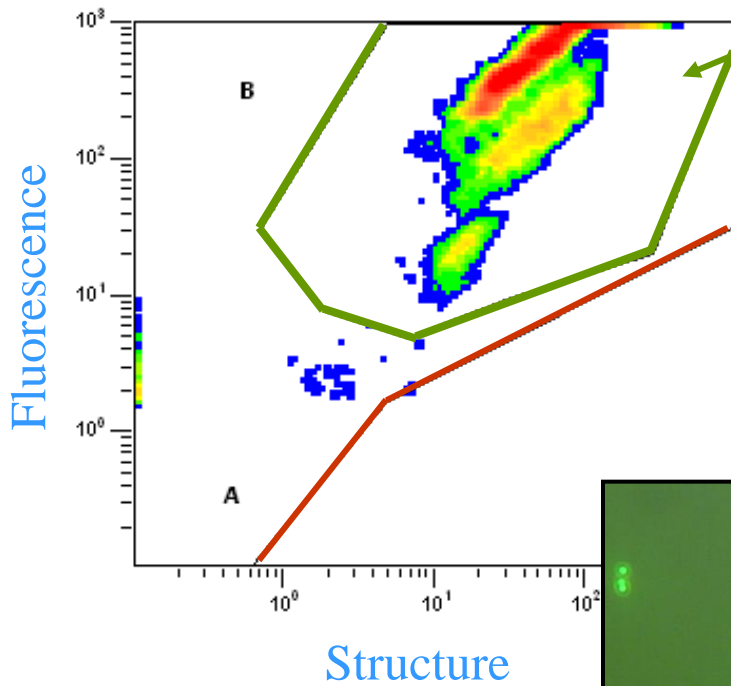
Enumeration of cultivable bacteria

Cytograms

Pure strain of
Pseudomonas from soil

Soil

Bacteria



Effect of xenobiotics on the production of laccase proteins

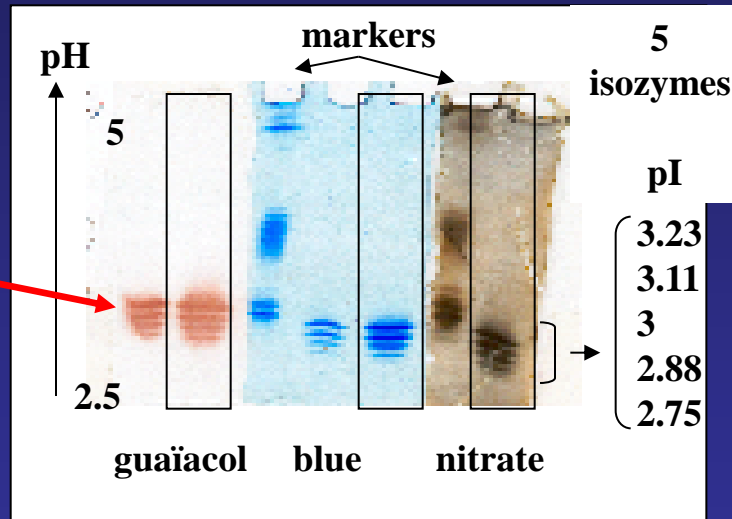
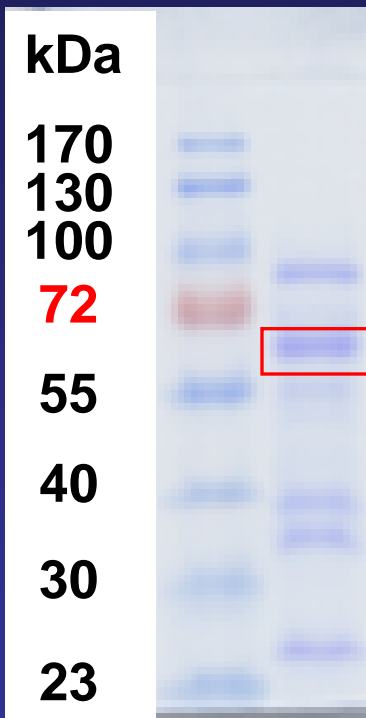
- Treatment of 4-day liquid cultures of *T. versicolor* with xenobiotics
- Separation of the extracellular fluid
- Precipitation of polysaccharidic compounds with 10% acetone
- Diafiltration of the protein solution (cut off 10kD, Amicon)
- Purification on Concanavalin A-Sepharose 4B column (Sigma-Aldrich) (elution with 0.2M Tris Hcl in the presence 0.5M NaCl and 0.2M o-methyl glucose)
- Separation by SDS-Page (12%)
- Identification of proteins by Maldi-TOF (Bruker)



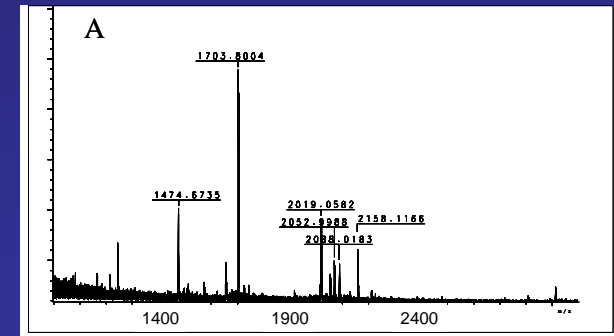
profiles of proteins

Separation and identification of lac A isoforms

1st migration mass → 2nd migration pI → Maldi-TOF



MW Da
58463
59776



► Towards a proteomic approach of soil diversity

Partnership

Soil biological descriptors

BioSol – ESITPA

M. Akpa, C. Bailleul, S. Desaire, C. Gangneux, I. Gattin,, K. Laval, M. Legras

Phytopharmacy – INRA

A. Brault, N. Demont, J. Dubroca, J.C. Dur, C. Jolival, C. Malosse, C. Mougine

LMDF – University of Rouen

S. Barray, N. Cariou, B. Pawlak, P. Plassart

Soil physico-chemical descriptors

Soil Science Unit – INRA

E. Huard, J.P. Leydecker, M. Pernes, D. Tessier, N. Wolf

Mathematical modelling and biomathematics

LAMSAD – ESITPA

S. Taibi

R. Salem – University of Rouen

A.S. Lemaire

Acknowledgements

All our students

Agence De l'Environnement et de la Maitrise de L'Energie
(grant n° 0475C0071)



www.esitpa.org/biosol

www-phyto.versailles.inra.fr