



Chemical contamination versus non chemical stressors: the case study of agricultural soils

Elaboration and validation of an index of the biological state of soils

Christian Mougin¹, Karine Laval², Marc Legras², Salima Taïbi³, Sylvie Barray⁴, Daniel Tessier¹

1 Unité PESSAC , INRA Centre de Versailles-Grignon, 78026 Versailles Cedex, France

2 Laboratoire BioSol , Esitpa - Ecole d'Ingénieurs en Agriculture, 13 Rue du Nord, 76000 Rouen, France

3 LAMSAD , Esitpa - Ecole d'Ingénieurs en Agriculture, 13 Rue du Nord, 76000 Rouen, France

4 Laboratoire LMDF , UFR des Sciences, Université de Rouen, 76821 Mont-Saint-Aignan Cedex, France



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 of quality 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



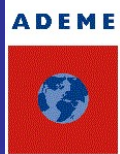
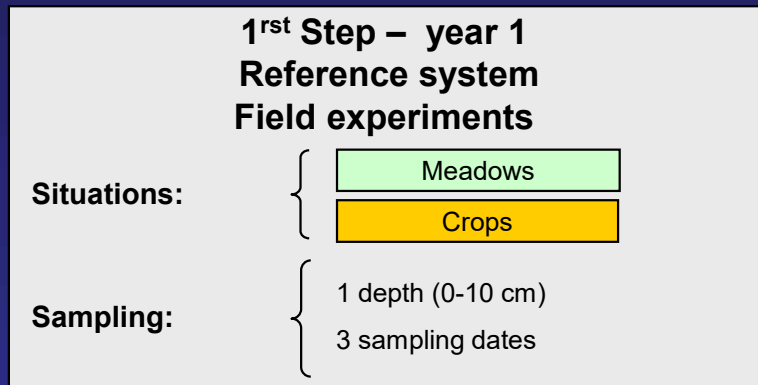
Our hypotheses

- 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
- To multiply the analytical methods using multidisciplinary approach (chemical, biological, biochemical,...) in order to modulate the inherent flaw of each method and thus to better describe the state of the soil.
- Meadows and crops are considered to be reference systems in contrast to polluted sites

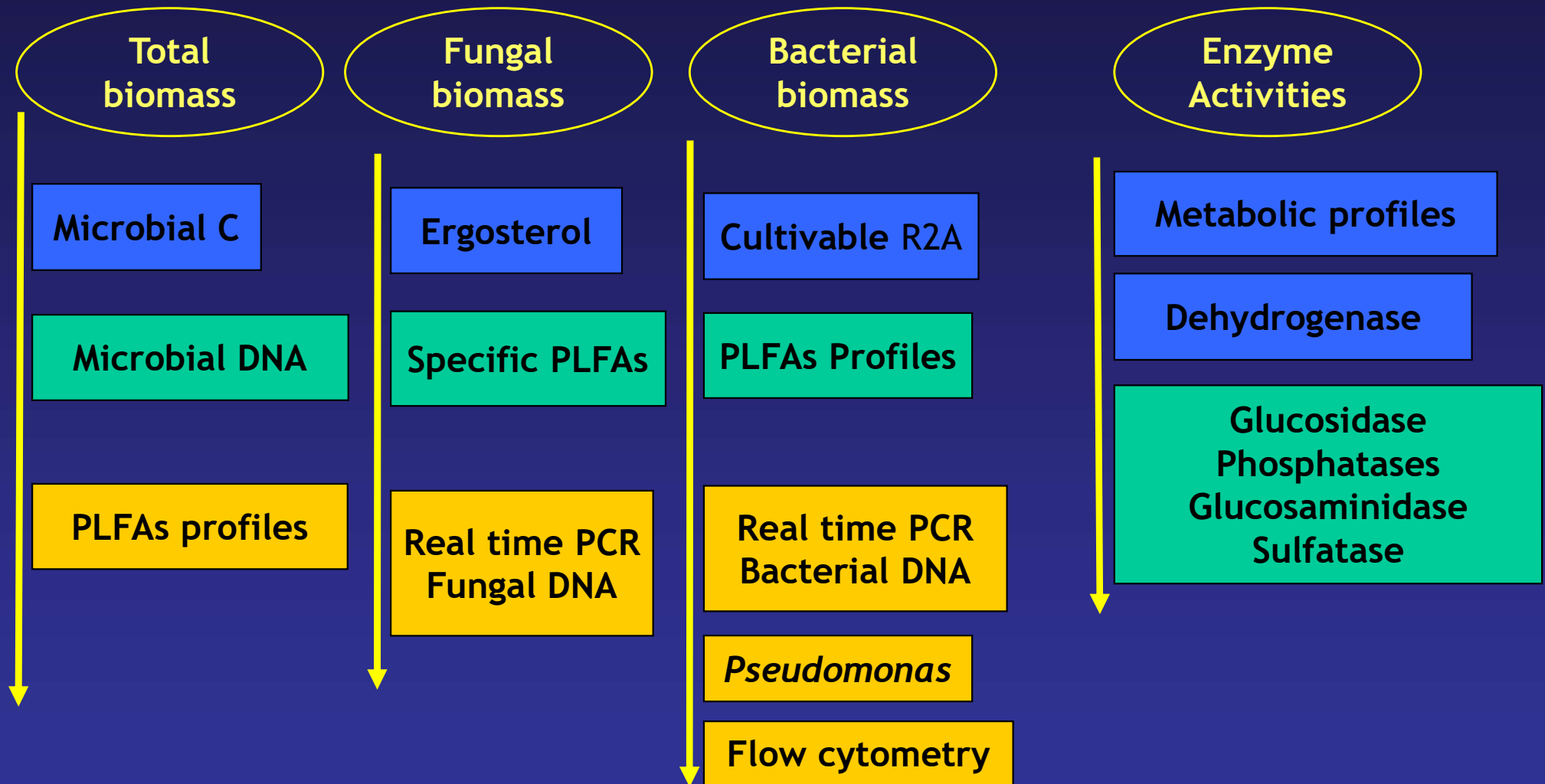


Our (experimental) strategy

Is to find, to characterize, to describe the relationships between physico-chemical and biological variables in contrasted situations



Variables describing the microbial communities

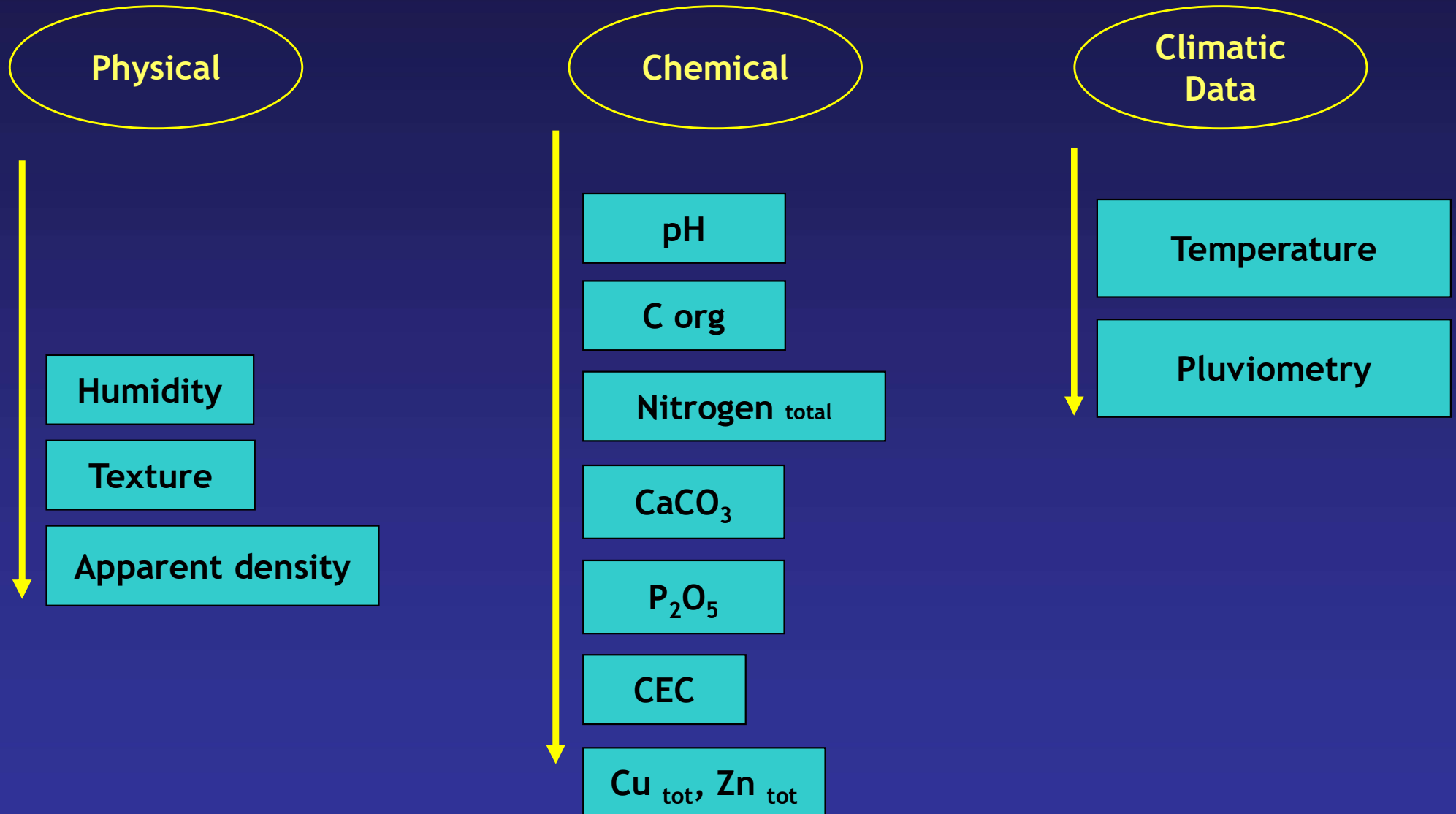


Reference methods

Developed methods

Emerging methods

Variables describing the soils and climate



1st Step: Measure of the variables at the field

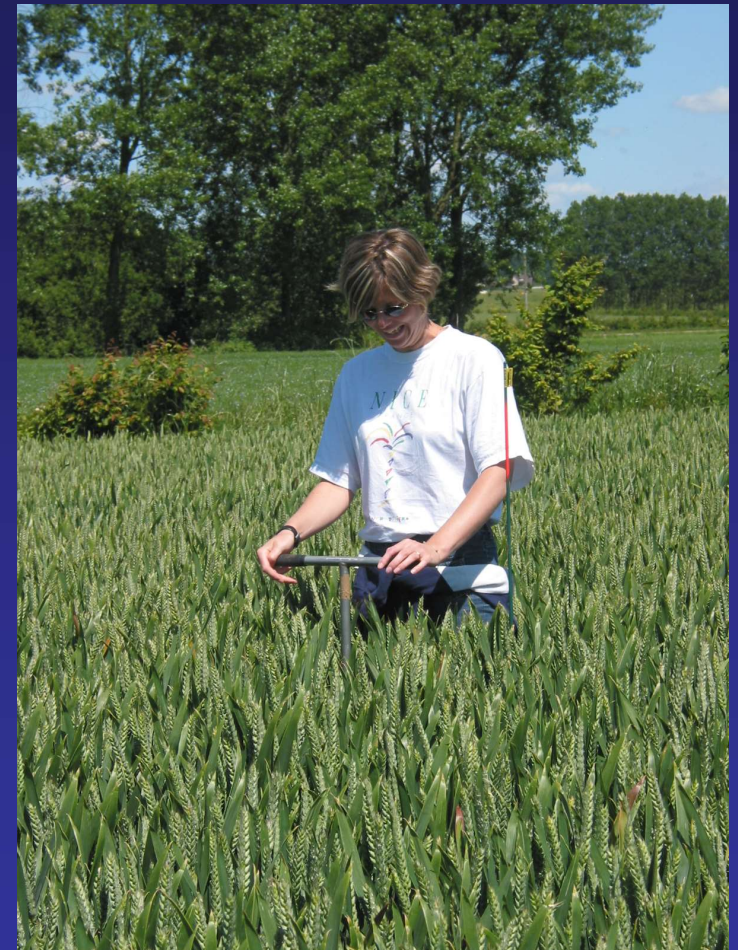
- 2 sites in Normandy : Yvetot - Saint Georges (near Rouen)



Sub-soil: chalk
Soil: silt layer + silex

1st Step: Measure of the variables at the field

- 2 sites in Normandy : Yvetot - Saint Georges (near Rouen)
- 2 situations: meadows and crops



1st Step: Measure of the variables at the field

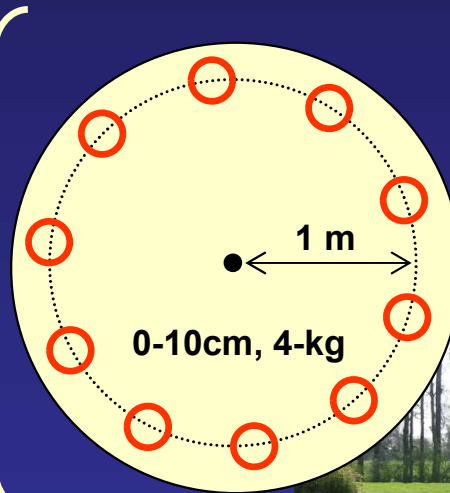
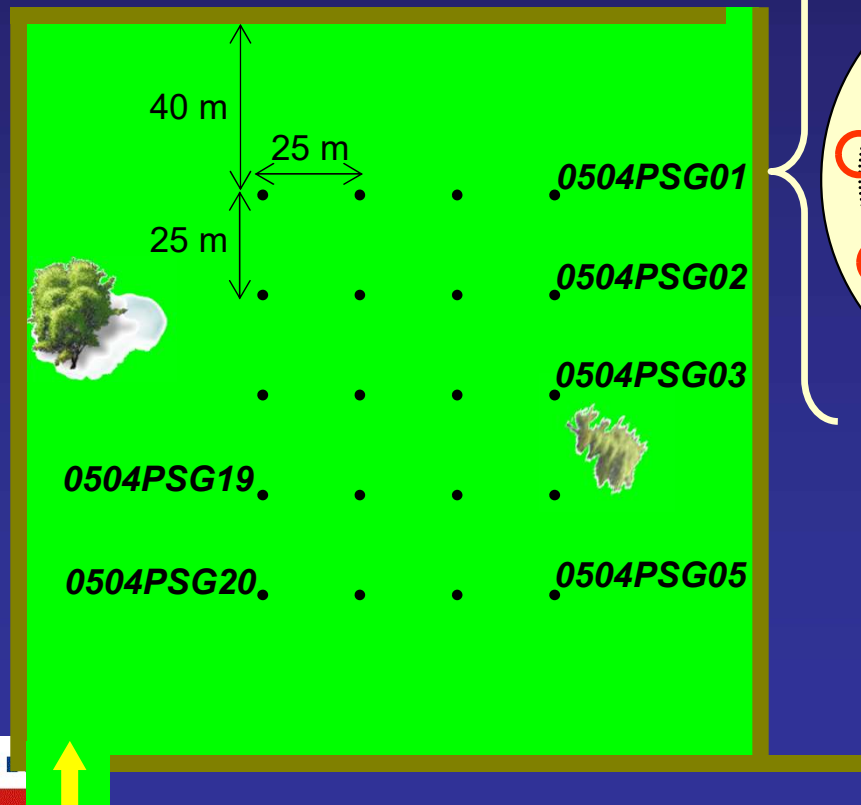
- 2 sites in Normandy : Yvetot - Saint Georges (near Rouen)
 - 2 situations: meadows and crops
 - 3 periods of sampling: April, June and September



1st Step: Measure of the variables at the field

- 2 sites in Normandy : Yvetot - Saint Georges (near Rouen)
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St Georges, meadow

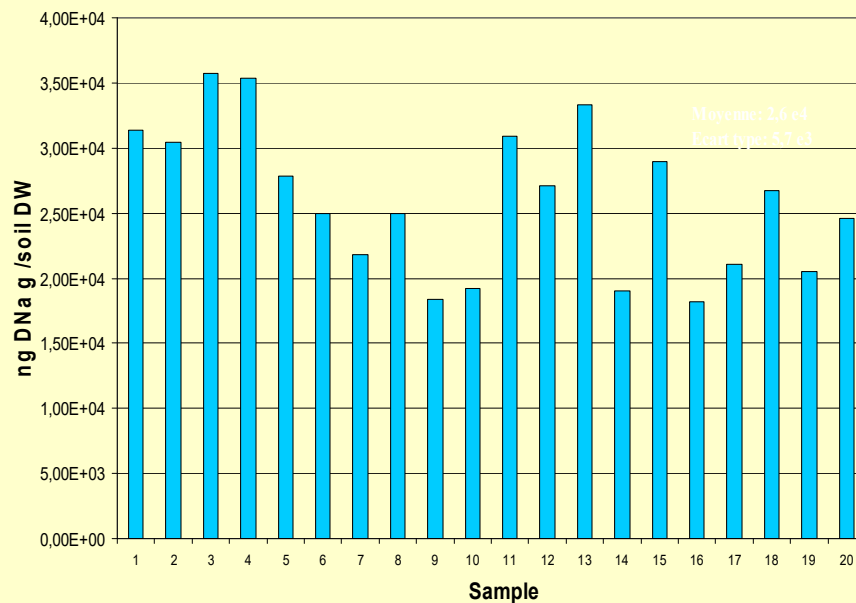


1st Step: Results 2005

Characterization of systems of reference

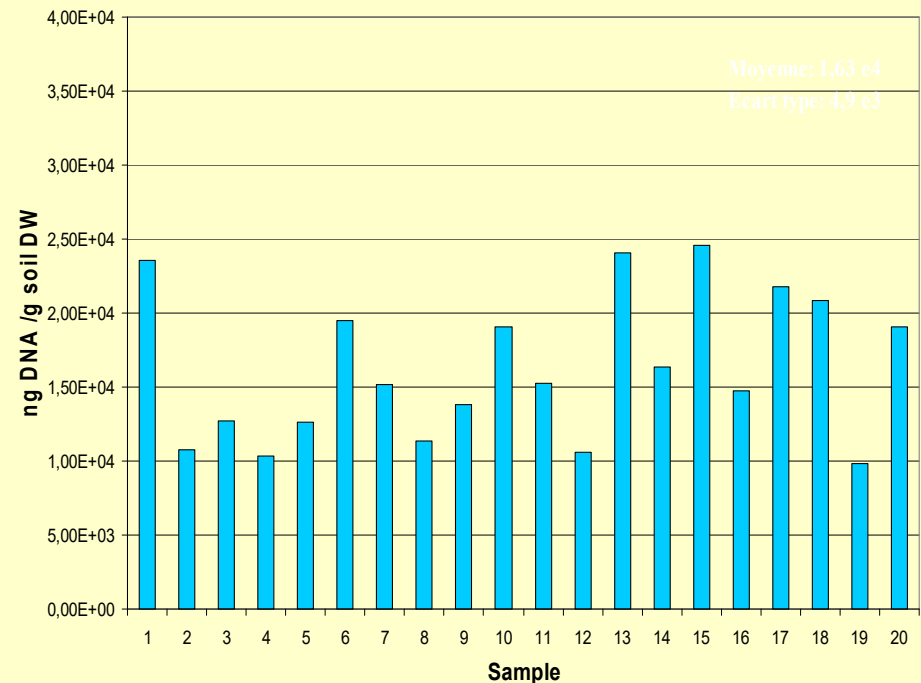


Meadow



26.0 ± 5.7 µg/g

Crop: wheat



16.3 ± 4.9 µg/g

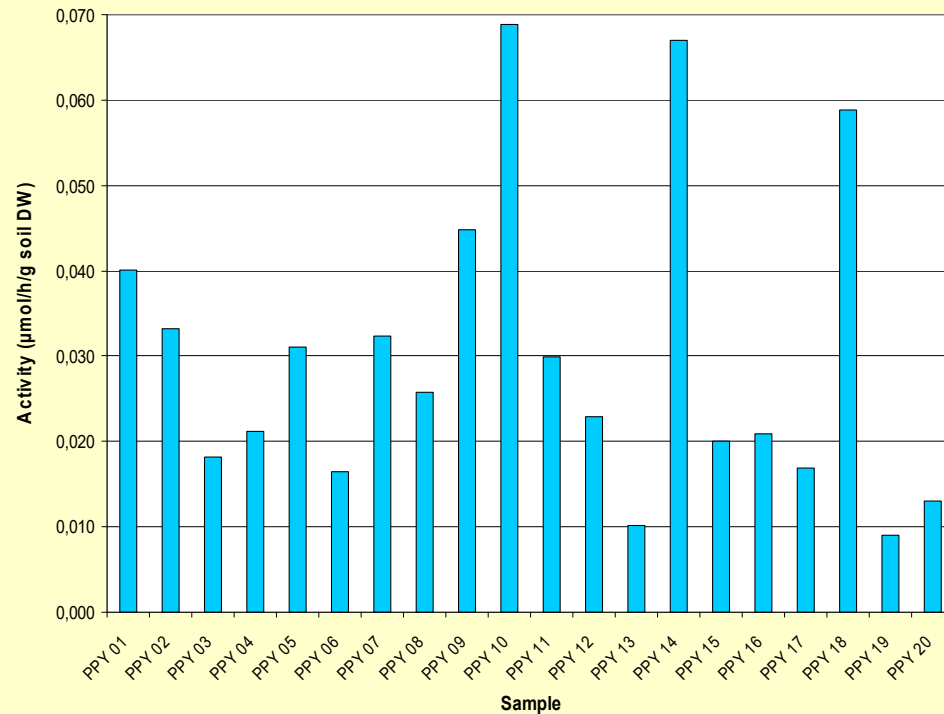
► higher total microbial biomass in the meadow, with a lower spatial variation



Activite: dehydrogenase

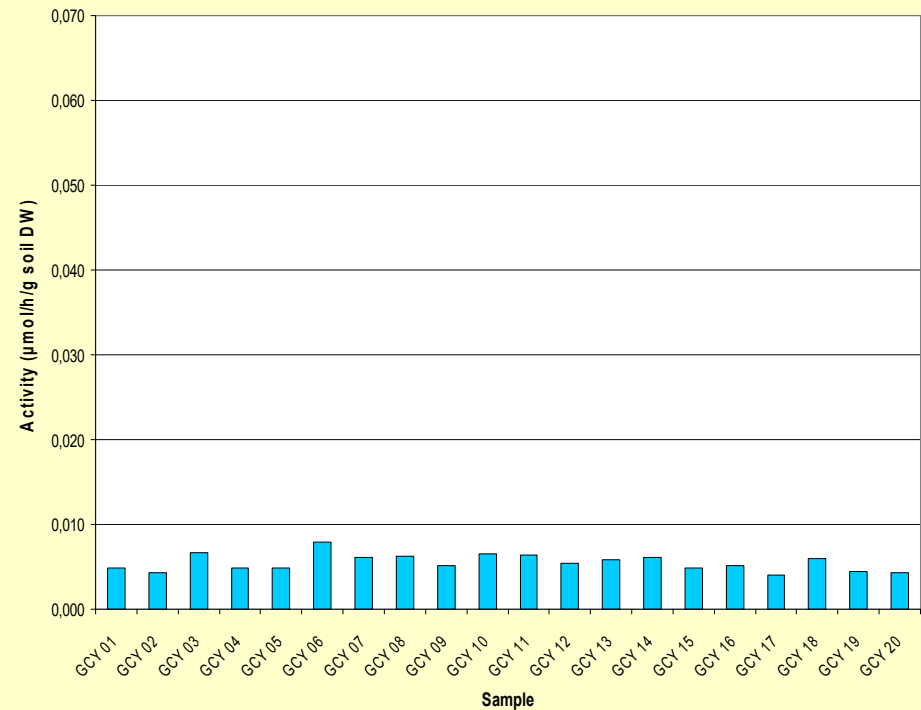
april - Yvetot

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

Biological Characteristics - Structural diversity

	Meadows	Variation coefficients		Crops
<u>Microbial C</u> ($\mu\text{g/g}$ soil DW)	300	40	60	75
<u>Microbial DNA</u> ($\mu\text{g/g}$ soil DW)	26	30	30	13
<u>Ergosterol</u> ($\mu\text{g/g}$ soil DW)	6,5	50	200	2,2
<u>Fungal DNA</u> ($\mu\text{g/g}$ soil DW)	6,7	100	50	1,3
<u>C16:1w5</u> ($\mu\text{g/kg}$ soil DW)	2,3	70	50	0,8
<u>Cultivable bac.</u> (10^7UFC/g soil DW)	1,6	80	50	1,5
<u>Bacterial DNA</u> ($\mu\text{g/g}$ soil DW)	1,7	30	30	0,7
<u>Flow cytometry</u> (10^9UFC/g soil DW)	1,0	80	70	0,4



Reference data - n= 120 samples
(annual average by situation for all sites)

Biological Characteristics - Functional diversity

	Meadows	Variation coefficients		Crops
<u>β-D-glucosidase</u> (nmol/h/g)	760	60	50	263
<u>Phosphatases acides</u> (nmol/h/g)	1400	40	50	460
<u>N-acétyl-β-D-glucosaminidase</u> (nmol/h/g)	277	60	80	102
<u>Uréases</u> (nmol/h/g)	12,5	40	40	13,8
<u>Phosphatases alcalines</u> (nmol/h/g)	480	100	80	388



Reference data - n= 120 samples
(annual average by situation for all sites)

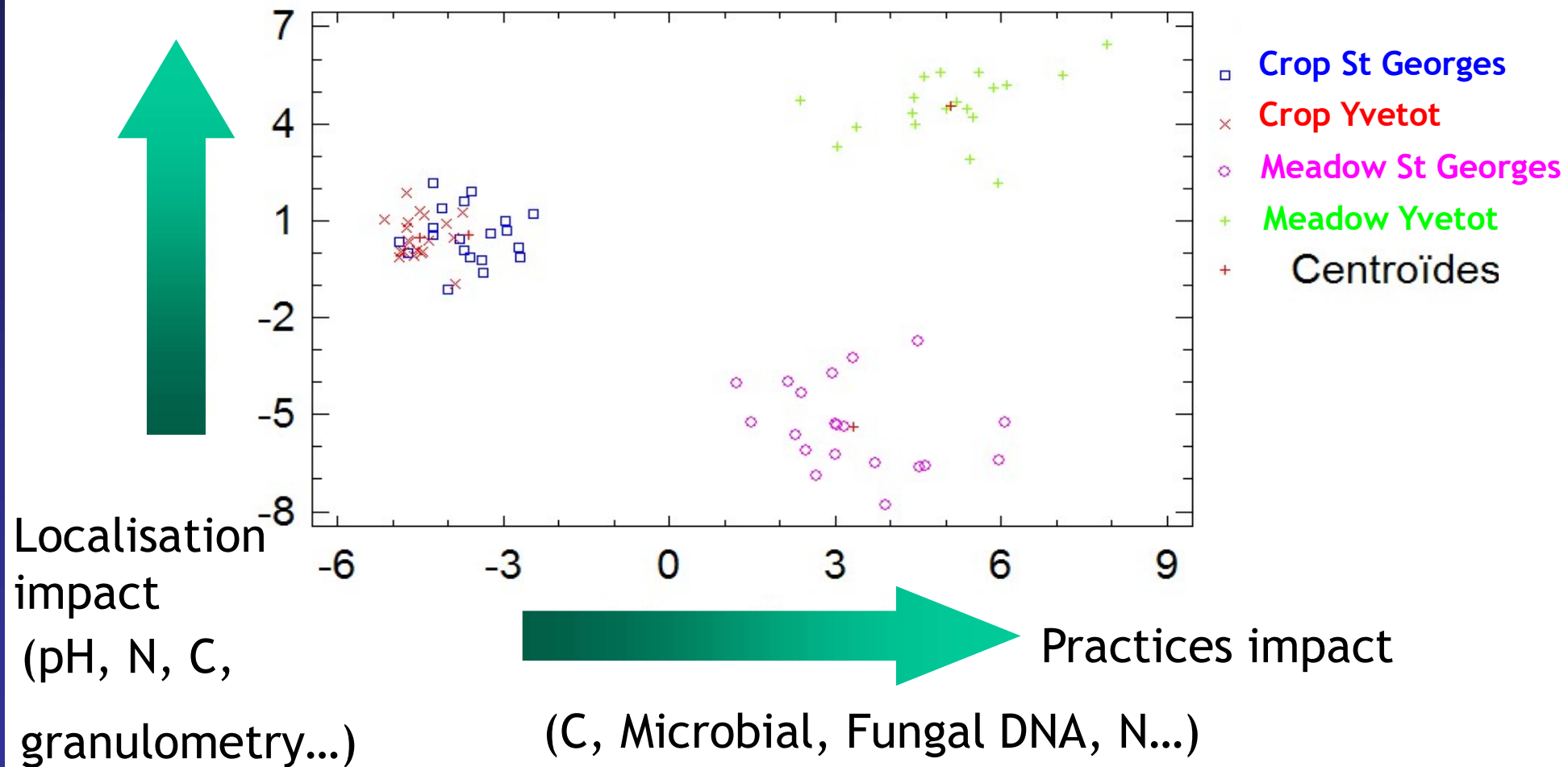
Physicochemical characteristics

	Meadows	Variation coefficients		Crops
<u>Humidity</u> (%)	26	30	28	21
<u>pH</u>	5,6	10	5	6,8
<u>C org</u> (ppm soil DW)	30	18	17	11,5
<u>Nitrogen tot</u> (ppm soil DW)	2,6	14	20	1,2
<u>CEC</u> (ppm soil DW)	8,7	25	18	7,8
<u>Apparent Density</u> ()	1,24	10	5	1,45



Reference data - n= 120 samples
(annual average by situation for all sites)

PCA of results (2005)



2nd Step: Results 2006

Copper Impact on microbial communities Microcosms Experiments



Microcosms Creation



Crop plot of Yvetot

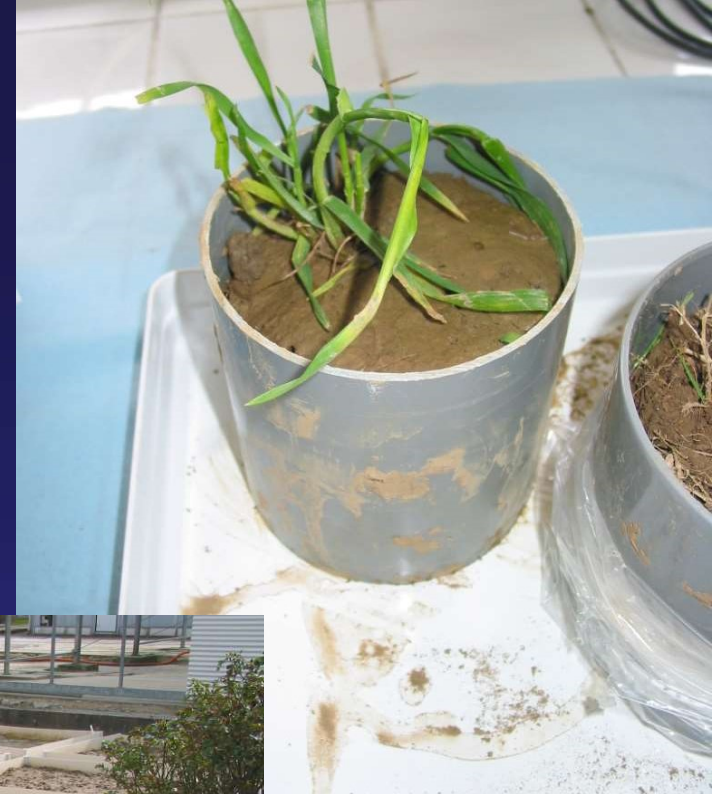
The microcosm samples have been taken from the same plots as the previous year.

ADEME



Microcosms Creation

Re-settled in soil



They are re-settled on soil in Versailles (near Paris) and the climatic conditions are also checked



Microcosms Creation

Re-settled in soil

Copper inoculation (T_0)

They are inoculated with copper in two concentrations corresponding to agronomic reality, an agronomic concentration ($CuA = 2ppm$) and a concentration a hundred times agronomic ($CuB = 200ppm$) which can correspond to concentrations found in the surface of vineyard soils.



T_0 : April 04, 2006 - Versailles



Microcosms Creation

Re-settled in soil

Copper inoculation (T_0)

Collected for analysis (T_1 , T_2 , T_3)

The microcosms are then collected, destructured, homogenized and analysed for the same descriptors on three occasions.

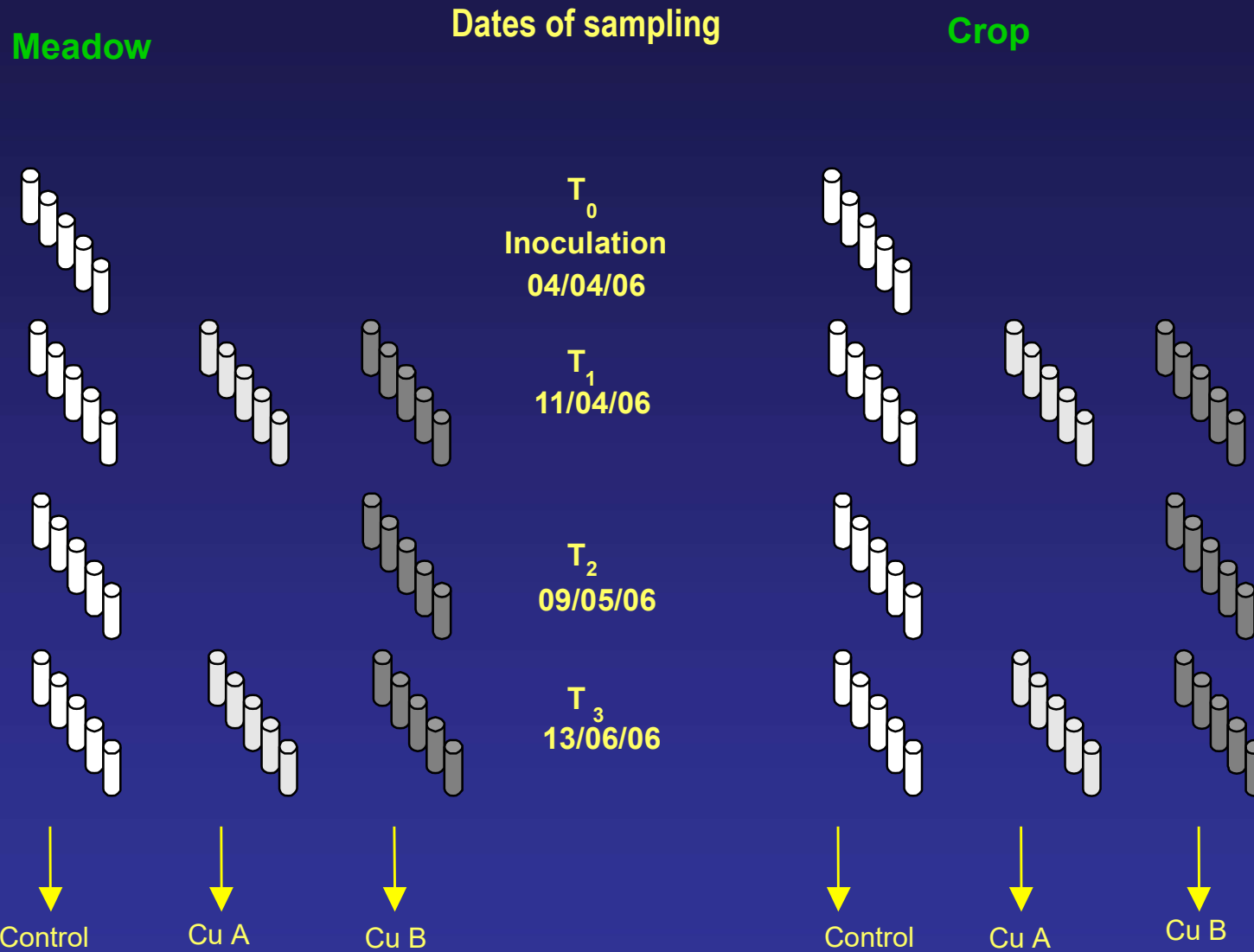
T_1 : April 11, 2006

T_2 : May 09, 2006

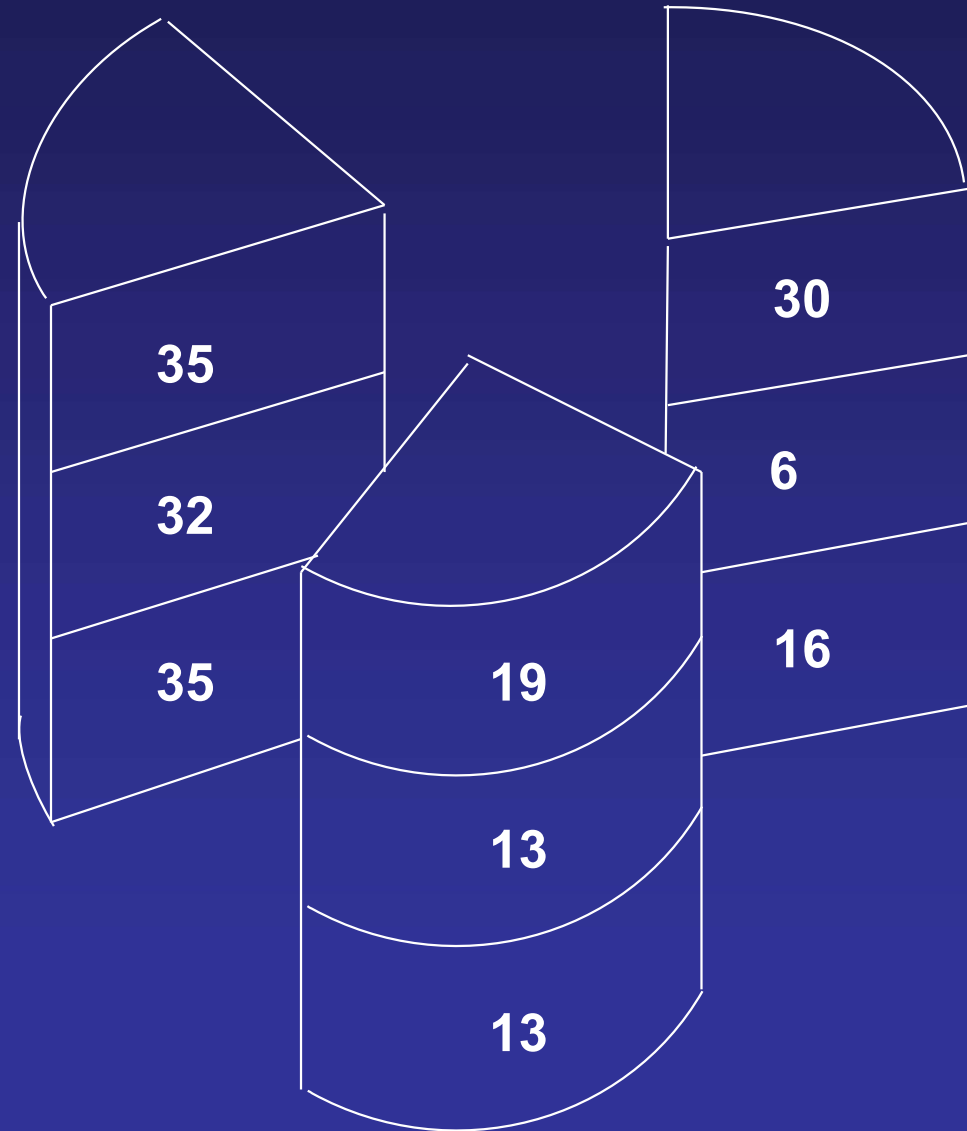
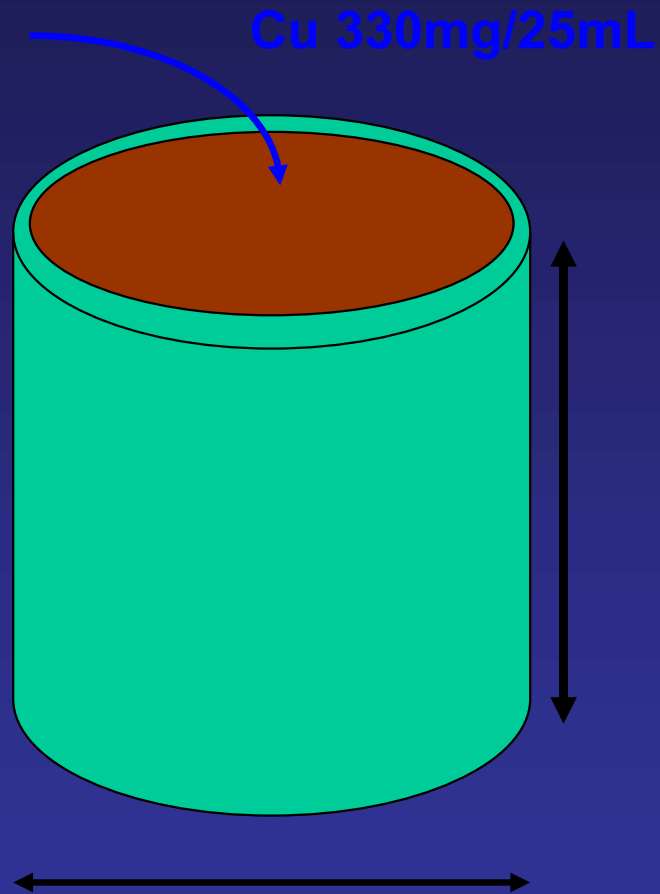
T_3 : June 13, 2006



Set up Experiments 2006



Impact de la mise en microcosme sur les variables descriptives de l'état du sol.

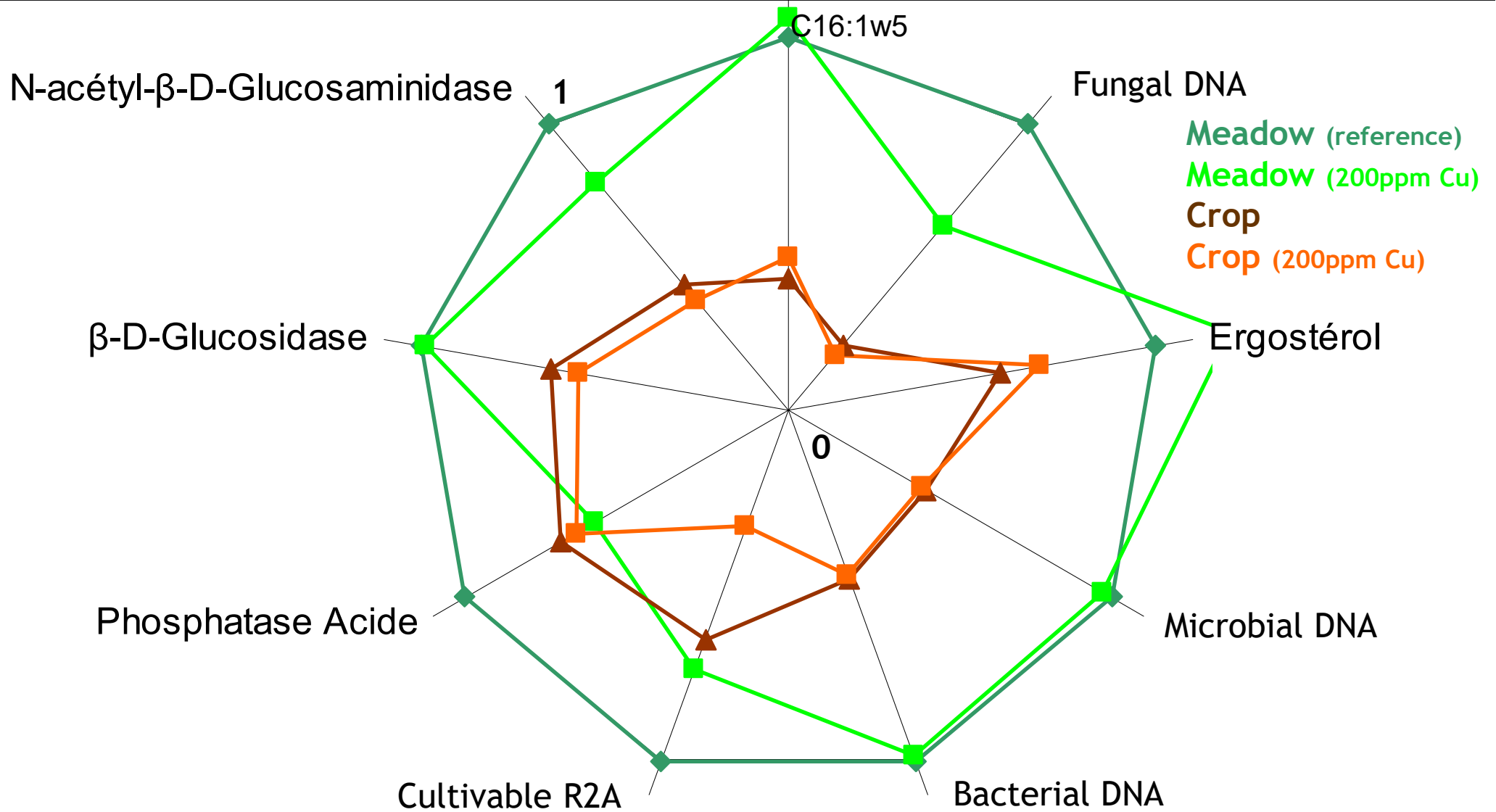


Impact de la mise en microcosme sur les variables descriptives de l'état du sol.

Comparaison des échantillons terrains et des échantillons témoins microcosmes après 1 semaine d'équilibre

- L'analyse mathématique des données montre pour la totalité des variables analysées une identité entre les valeurs des échantillons terrains et celles des échantillons témoins (T_0 après 1 semaine d'équilibre)
- L'approche en microcosmes avec sol non remanié ne semble pas perturber l'écosystème à T_0 . Ceci permet de valider notre approche méthodologique initiale.

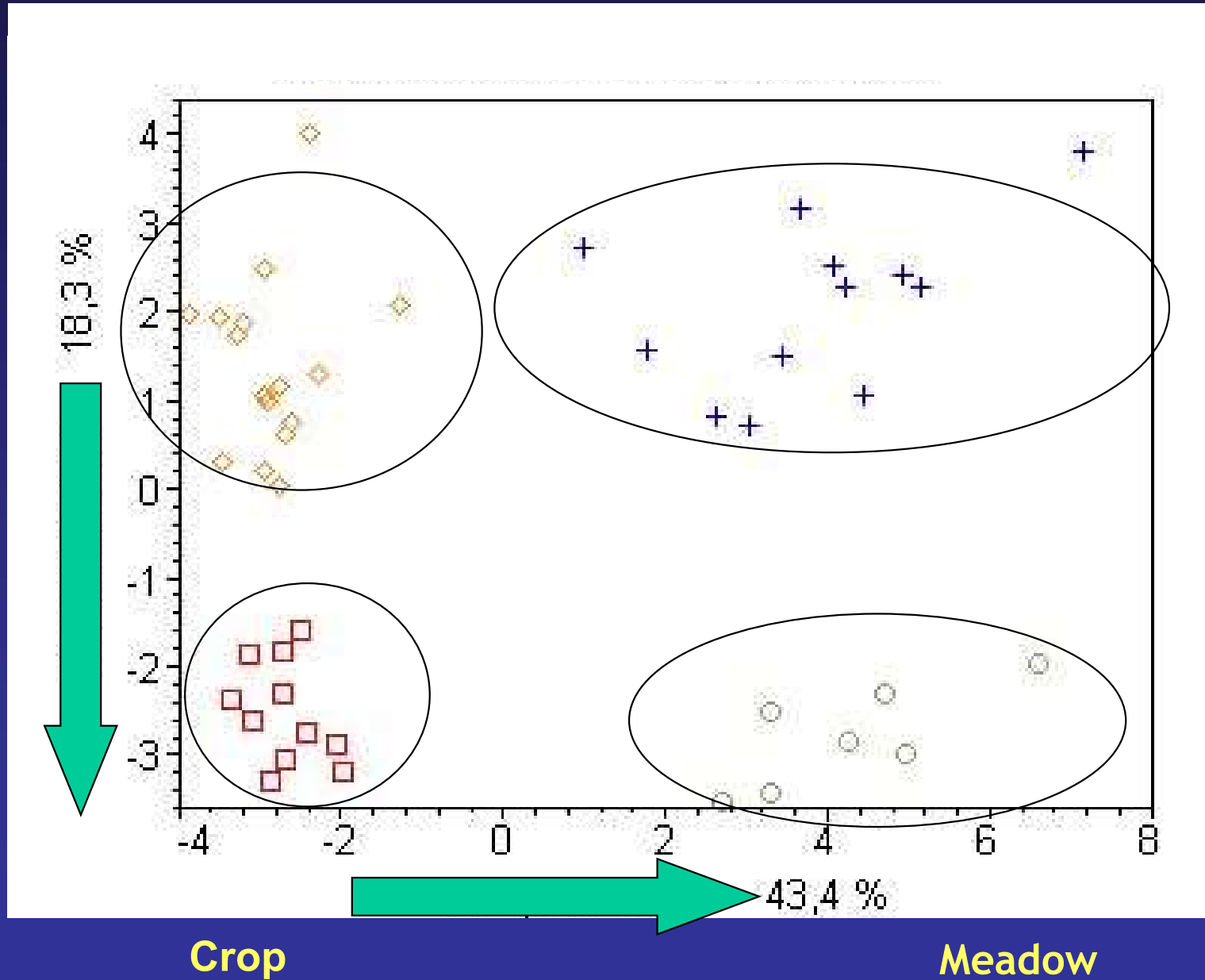
Evaluation of the copper impact on the descriptors of the microbial communities



PCA of results 2006

April

May



Conclusions 2005-2006

- **Validation de l'approche « microcosme » utilisée dans cette étude**
- **Le système de culture est déterminant dans les différences observées**

We have established a hierarchy of the impacts of anthropic activities on the descriptive variables of microbial communities :

- 1. Practice**
- 2. Site**
- 3. Date**
- 4. Copper**



Hypothèses et perspectives

- - **Did the copper reach its target ?**
- - **Does the copper toxicity result from the deterioration of the habitat (physical protection of soil)?**
- - **Is the toxicity of copper linked to the presence of a microbiological activities due to the provision of carbon?**
- - **Has the copper induced modification in the structure of communities without having measurable impact on the microbial biomass and the resulting enzymatic activities?**

Acknowledgements

All our students

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www.esitpa.org/biosol

www-phyto.versailles.inra.fr



Partnership

Soil biological descriptors

BioSol – Esitpa

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

PESSAC – INRA

M. Belkacem, A. Brault, J. Lebrun, C. Mougou, I. Touton

LMDF – University of Rouen

S. Barray, N. Cariou, P. Plassart

Soil physico-chemical descriptors

PESSAC – INRA

J.C. Dur, E. Huard, D. Tessier

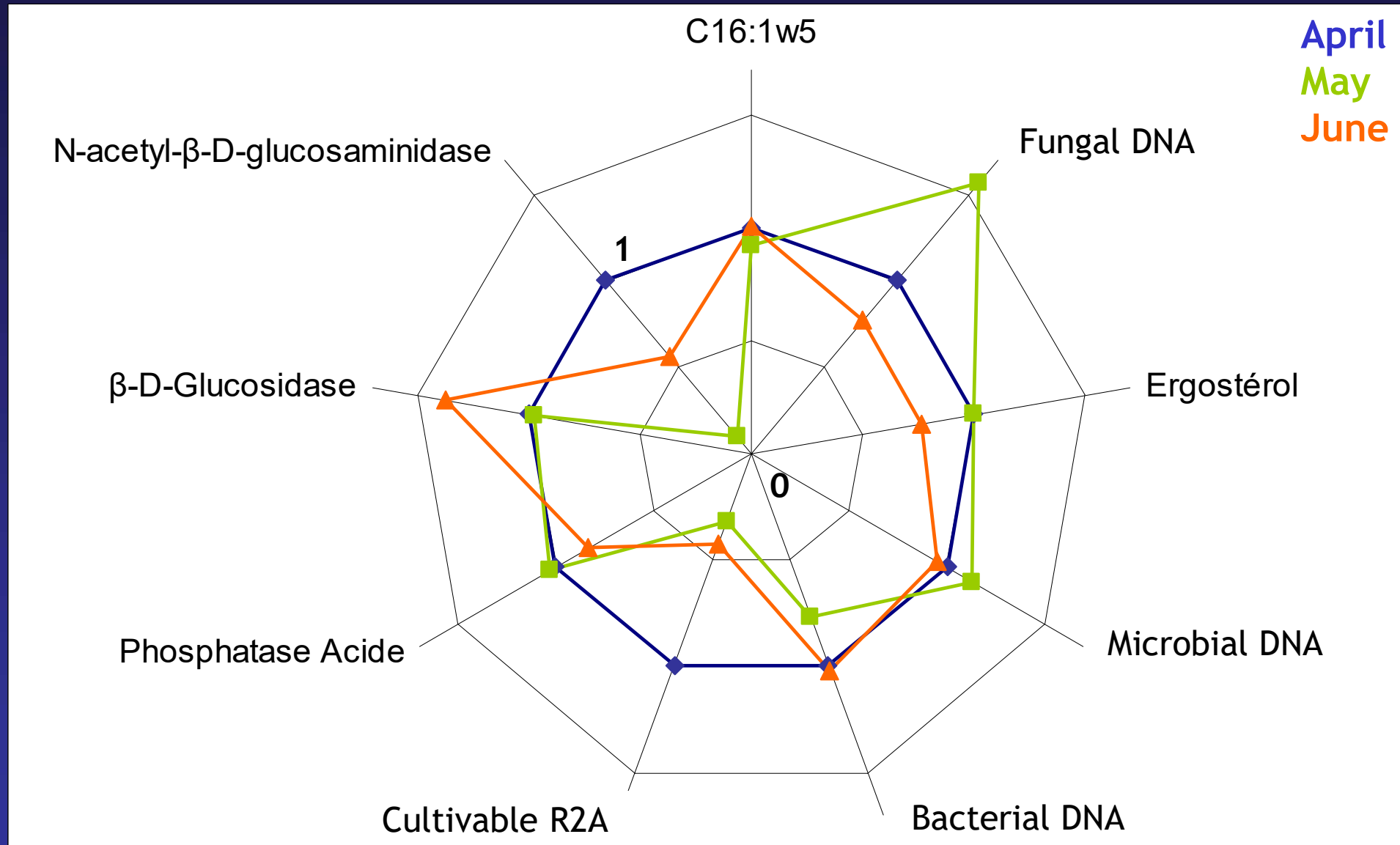
Mathematical modelling and biomathematics

LAMSAD – Esitpa

P. Lepelletier, S. Taibi



Temporal evolution of the descriptors of the microbial communities



Results in Crop

