



## Effect or organic amendments on soil enzymatic activities. Results of the “Bioindicators” French program

Wassila Riah, Nathalie Cheviron, Jean Trap, Steven Criquet, Clément Peltre, Sabine Houot, Marc Legras, Jean-François Vian, Antonio Bispo, Cécile Grand, et al.

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# ***Effect of organic amendments on soil enzymatic activities. Results of the French “Bioindicator program”***

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- ❖ Soil = a non-renewable resource
- ❖ Soil degradation = still accelerating

**Soil must be protected**

## Available Tools ?

- ❖ Tools for soil quality assessment
  - ➔ physical and chemical indicators commonly used
  - ➔ indicators based on biological parameters are still rare

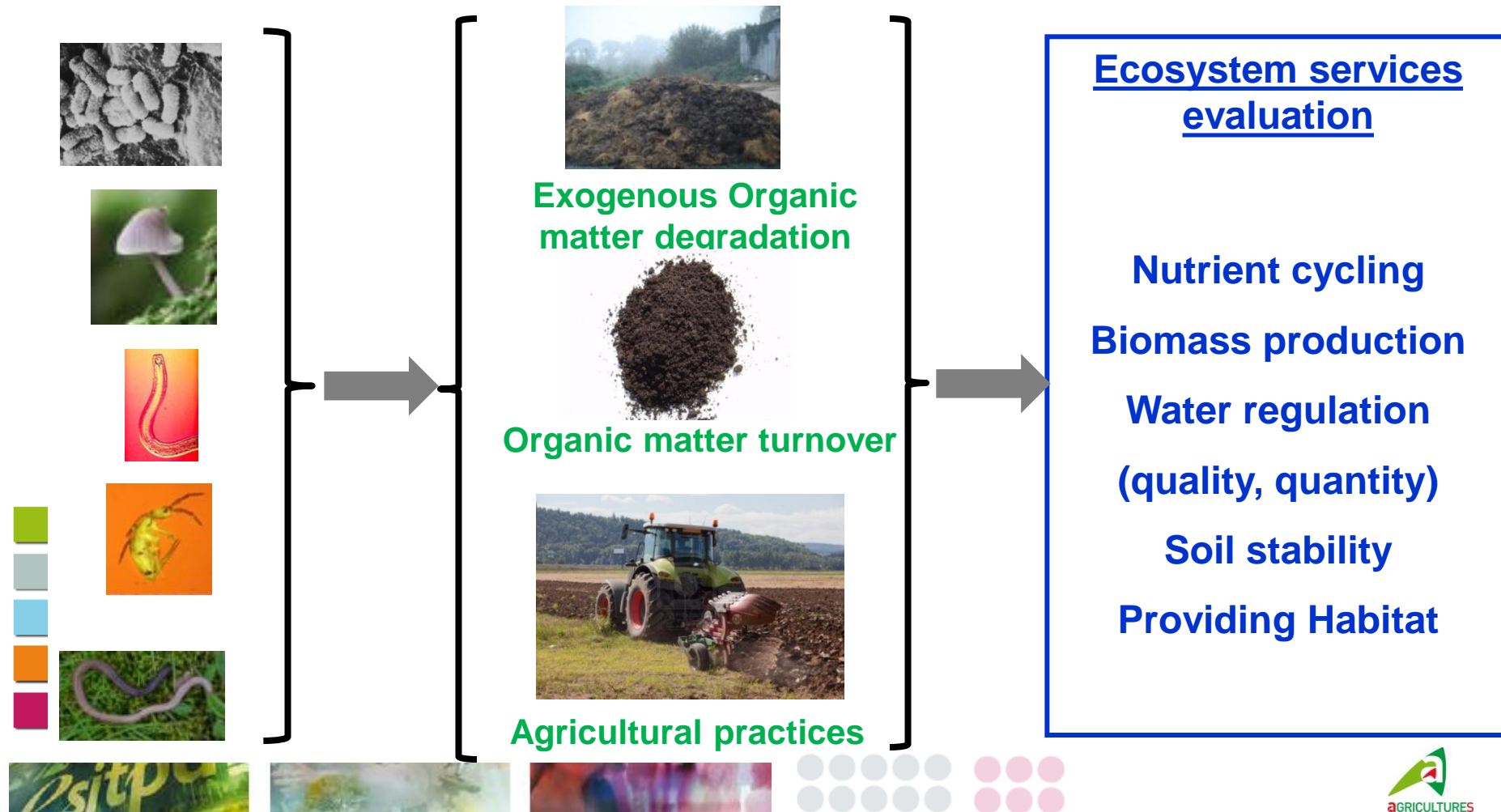
However, soil biota is known to respond to multiple impacts and to react quickly to natural and anthropic stresses

**needs of bioindicators**



# What are the needs in agricultural soils ?

Tools to measure biological state and **soil functions**  
and at least to evaluate agricultural soil **ecosystem services**



**ADEME** (French Agency for Environment and Energy Management) initiated a national research program to develop such indicators : **“Bioindicator Program”** (2006-2012).

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

## Objectives

- to **calibrate the bioindicators on the basis of referential values** in order to interpret the results
- to **provide relevant biological tools** (sensibility, accessibility, cost, ...) depending on the purpose :
  - soil monitoring
  - characterization
  - risk assessment
- to **transfer** the tools to stakeholders (policy makers, farmers, technical advisers...)



## 5 Strenghts of Bioindicator program

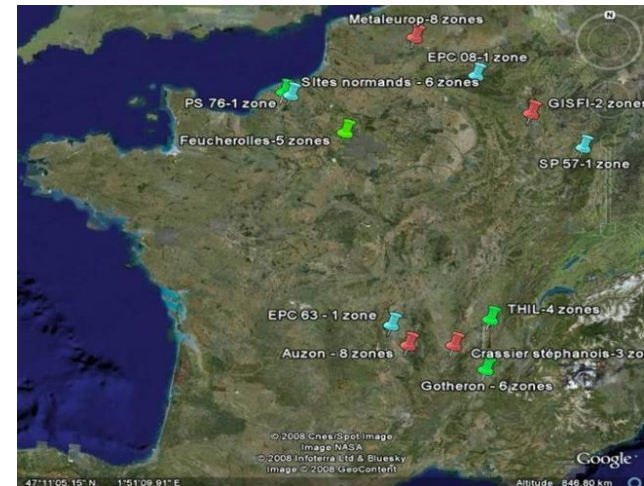
- Test and comparison of 47 bioindicators (from microflora to fauna and flora)

- Study large number of situations 13 situations (47 plots)

- Homogeneity of procedures and protocoles

- **Development of a data base** to manage a large amount of data (~200.000)

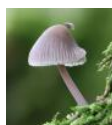
- **Development of a common data mining for data processing**



## 1) Microorganisms

### Parameters of quantity

- Global (MB, DNA)
- Non-global (Fungi, Bacteria)



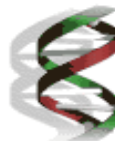
### Parameters of activity (examples)

#### Enzymatic Activities (14)

- Metabolical Functions (mineralization)

### Parameters of genetic structure

- PCR TTGE (Bacterial, Fungal)
- PCR Empreinte ARISA (Bacterial)



## 3) Flora

### Parameters -> individual effects

- Lipid Biomarkers (Oméga 3)
- Metal Bioaccumulation
- Free Amino acid content



## 2) Fauna

### Global Parameter

- Total Macrofauna

### Parameters -> Community level

- Earthworms
- Collembola
- Nematodes



### Parameters -> Individual level

- Molecular earthworm biomarkers  
(metallothionein coding gene expression,  
Cd sensitivity)
- Metal Bioaccumulation  
(snail, micromammal)



## What are soil enzymes?

Enzymes are proteins with specific catalytic properties



## Origin of Soil Enzyme Activity

1. Soil microorganisms - living and dead
2. Plant roots and crop residues
3. Soil animals

## an integrative Indicator...

## Why it is important?

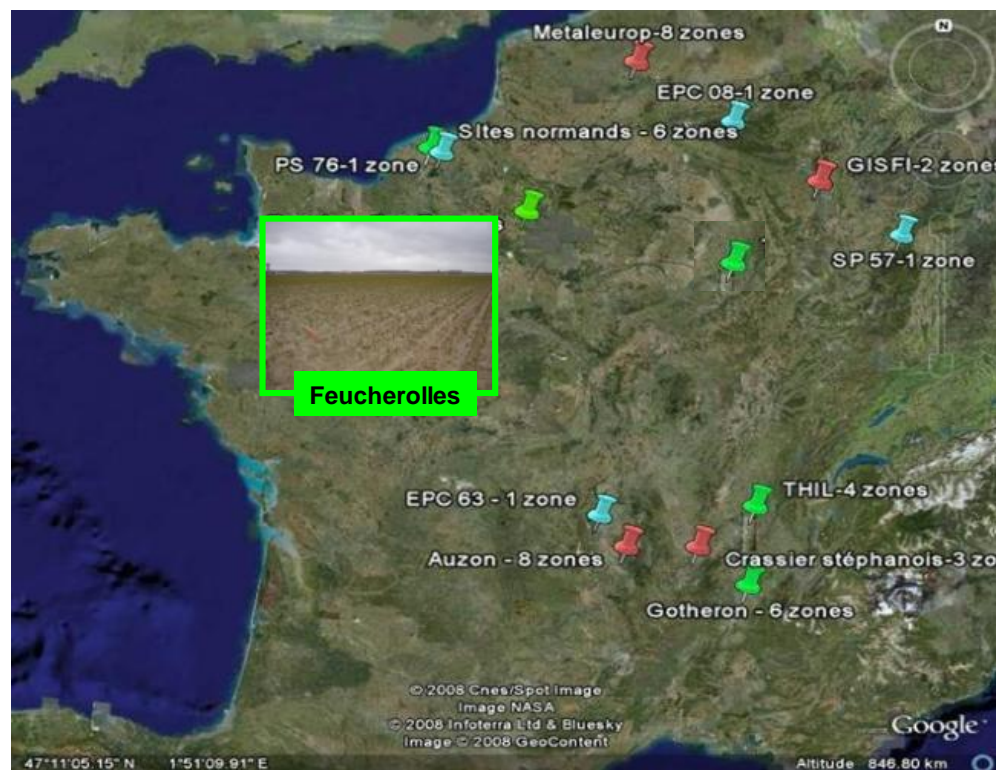
- Soil enzymes play an important role in organic matter decomposition and nutrient cycling
- Enzymes are recognized as early indicators of soil functioning changes due to modifications of soil management or agricultural practices
- **40 to 60% of enzyme activity can come from stabilized enzymes**
- **not necessarily highly correlated with microbial biomass or respiration**



Do enzyme activities reveal changes in the biological functioning of soils caused by organic amendment ?

Could soil enzyme be indicators of soil organic matter quality or organic products stability ?





QualiAgro

INRA  
SCIENCE & IMPACT

EGC

VEOLIA  
ENVIRONNEMENT

## QualiAgro-Feucherolles site *S. Houot (INRA Grignon)*

- Located at Feucherolles, in north-western France (50 km west from Paris)
- The climate is oceanic



- The soil is cultivated following a wheat–maize rotation
- At the date of sampling soils were cultivated with wheat
- The last inputs have been made 18 months ago





# Selected soil enzymatic activities

Fourteen enzyme activities involved in the different nutrient cycling were measured by the spectrophotometric methods

**Table 1:** E.C. numbers, specific substrates (concentrations and pH buffers) , references of protocols used to measure enzyme activities

Enzymes	Codes	E.C. number	Substrates (buffer pH and concentration)	References
<b><u>Global activities</u></b>				
Dehydrogenase	DEH	1.1.1.1	2,3,5 triphenyltetrazolium chloride ((pH 7; 0.12M)	Schaefer et al, 963
Fluorescein diacetate	FDA	209.877.6	Fluorescein diacetate (pH 7.6: 1000 $\mu\text{g.mL}^{-1}$ )	Adam & Duncan. 2001

NP: nitrophenyl

**Soil enzymes assays are based mostly on the quantification of the product released after adding known amount of substrate to the soil**



1. Homogenizing, sieving and weighing soil
2. Preparation of soil solution
3. Addition of a specific substrate and incubation
4. Stop the enzymatic reaction and measure absorbance with a spectrophotometer
5. Data analysis

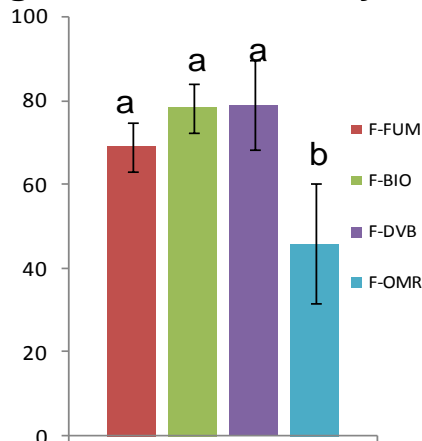
## Enzyme activity expression :

nmole of hydrolyzed or oxidized substrate / min / g of dried soil



## 1. Organic matter stability index and organic carbon content in Feucherolle site

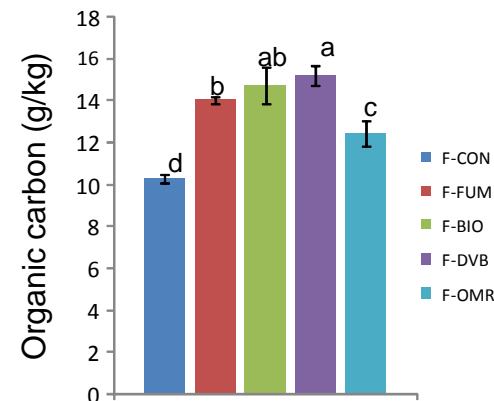
Organic matter stability index



➤ FUM, BIO and DVB exhibited the same OMSI

➤ The lowest OMSI was obtained for OMR

Organic carbon content



➤ all organic amendments drive to an increase in soil organic carbon content by comparison with the control

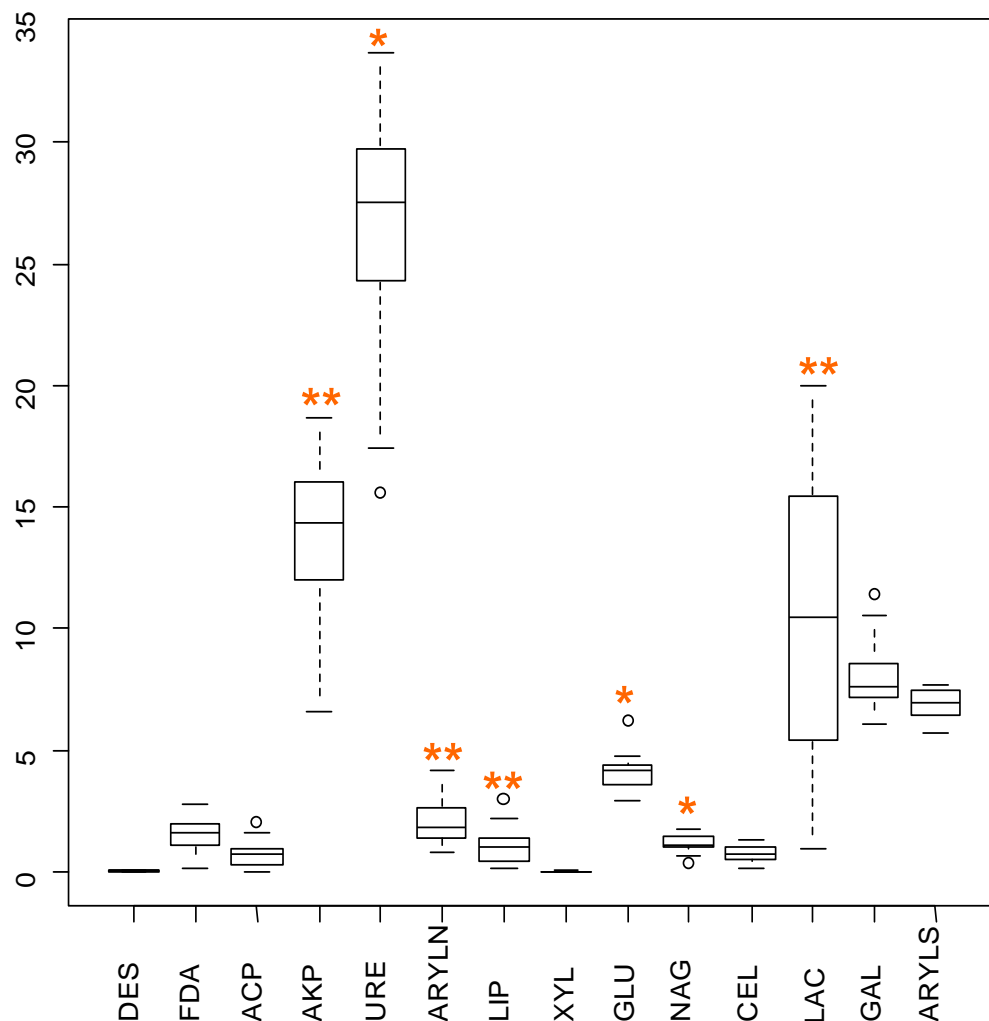
➤ FUM, BIO and DVB have the highest effect.

➤ OMR is intermediate between control and other organic treatments.

**Organic amendments influence soil organic carbon, in relation to their initial stability**



## 2. Variation of soil enzymatic activities in Feucherolle site



Lipase,  $\beta$ -glucosidase, N-acetylglucosaminidase, laccase, arylamidase, urease and alkaline phosphatase were **modified according** to the spreading of **organic amendments** onto the soil

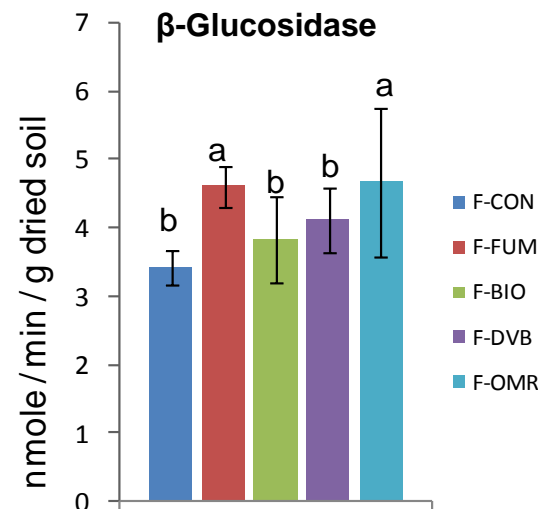
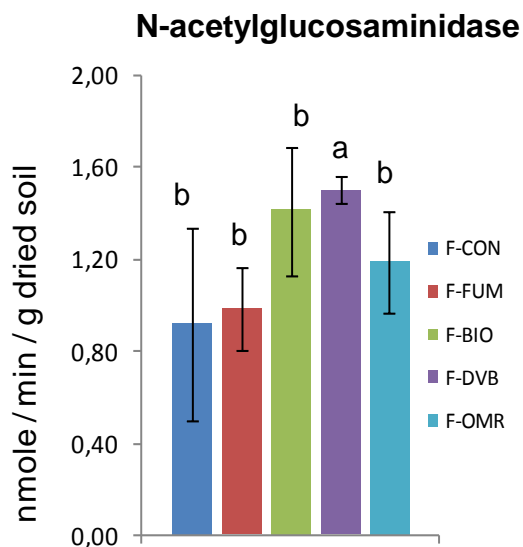
\*  $P < 0.05$

\*\*  $P < 0.01$

Synthesis of enzymatic activities response to organic amendment according to Kruskal-wallis test  $n=4$



## 3. a Variation patterns of enzymatic activities affected by organic amendments



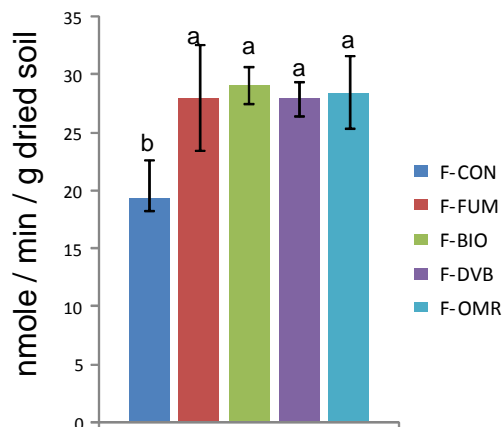
**18 months after organic amendment spreading :**

- **NAG and GLU enzyme activities did not reflect the soil organic matter content**
- **The effect of exogenous organic matter (EOM) on GLU and NAG activities was transient**
- **These enzyme activities are less suitable for monitoring long-term effect of OM**



## 3. b Variation patterns of enzymatic activities affected by organic amendments

Urease

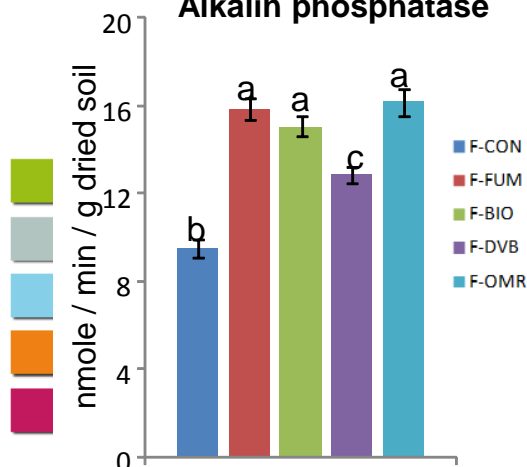


➤ Significant increase of URE activity in the soil regardless of addition of compost or manure

➤ No significant difference appears between organic treatment

**18 months after compost and manure spreading:  
URE and AKP activities were stimulated by organic amendements.**

Alkaline phosphatase



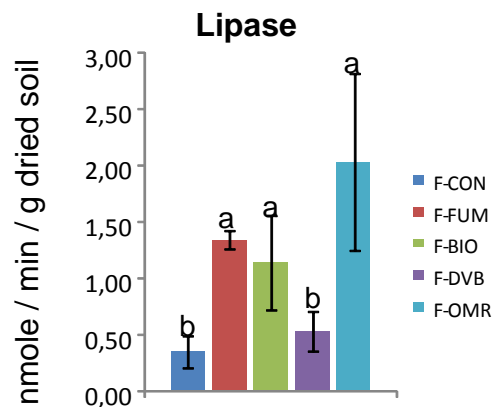
➤ All treatments increased AKP activity

➤ The increases in AKP activity was similar in the soils amended by OMR, BIO and FUM

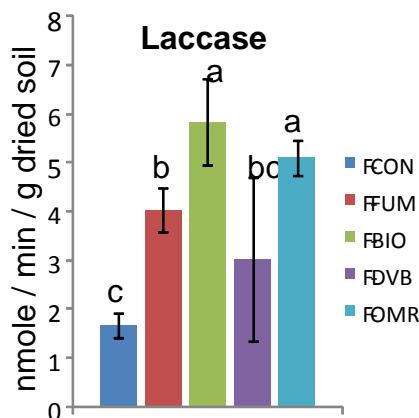
**Link with biomass stimulation due to an increase of organic C content in soils?**



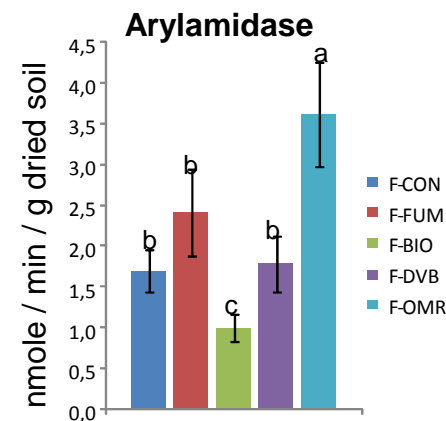
## 3.c Variation patterns of enzymatic activities affected by organic amendments



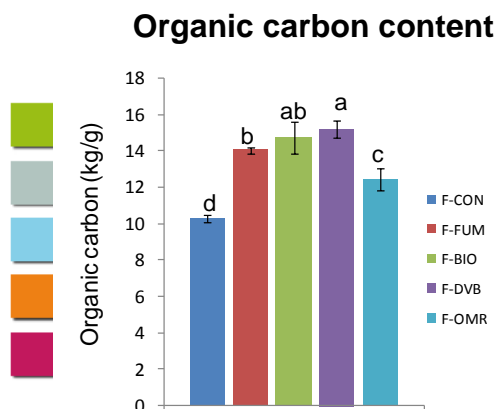
- All treatments increased LIP activity
- No significant difference between DVB compost and CON



- All treatments increased LAC activity
- The largest increases in LAC activity was observed in BIO



- BIO exhibited the lowest ARYLN activity
- OMR exhibited the highest ARYLN activities
- No difference in ARYLN activity between FUM, DVB and CON



➤ No relationship between levels of enzyme activities variation and organic C content patterns

More specific activities ?

Link to amendment chemical, physical and biochemical nature?

Link to soil organic carbon composition?



## Effect of organic amendment on soil enzymatic activities

- Organic amendments drive to an increase in soil organic carbon content, as exogenous organic matter added is stable.
- ACP, FDA, DEH, CEL, GAL, XYL and ARYLS activities were not affected by organic amendments, 18 months after the last input.
- Organic amendments seem to have transient effect on  $\beta$ -glucosidase, N-acetyl-glucosaminidase. **These enzyme activities are less suitable for monitoring long-term effect of OM**
- Lipase, laccase, arylamidase, urease and alkaline phosphatase activities were **modified** according to the spreading of organic amendments into soil
- Lipase, laccase, arylamidase activities may be linked to organic matter biochemical state or the composition of the soil organic carbon
- The municipal solid waste compost (F-OMR) plot that have the lowest OMSI and organic carbon content exhibited in average the highest enzymatic activity values (AKP, LIP, GLU, URE and ARYLN)



# Bioindicator program : A large research network



22 french research teams (i.e. 70 members)

