

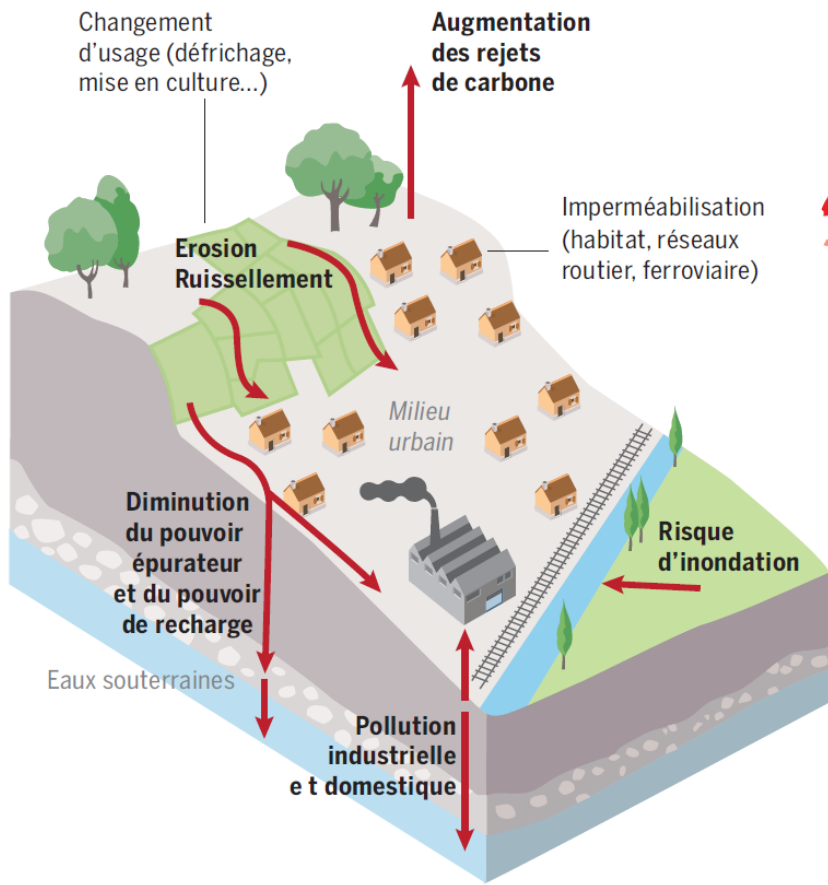
# S O S

## Béton, érosion, pollution minent les sols français

Fruit de dix ans de travail, le premier bilan de l'état pédologique du territoire invite à une gestion plus durable

### Des milieux fragilisés

PERTURBATIONS PAR L'ARTIFICIALISATION DES SOLS

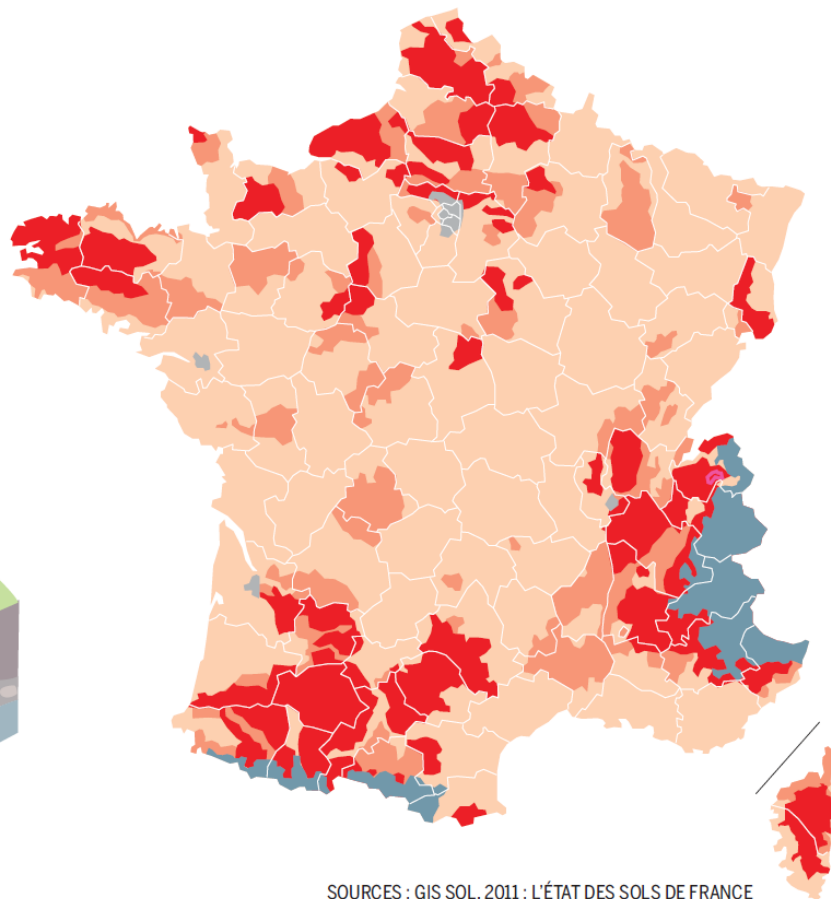


RISQUE D'ÉROSION DES SOLS

**Fort** **Moyen** **Faible**

Zones urbanisées

Haute montagne



SOURCES : GIS SOL, 2011 ; L'ÉTAT DES SOLS DE FRANCE

# ***Le mode de gestion des intrants organiques des sols à l'échelle de la parcelle contrôle la distribution, la biodisponibilité et l'impact du Cu à $\mu$ -échelle***

**Jean M.F. Martins, Aline Navel, Lionel Ranjard, Alain Manceau,  
Pierre Alain Marron, Isabelle Lamy, Pierre Faure, Lorenzo Spadini.**



Remédiation :  
restauration de la fertilité des sols  
par des amendements organiques



**Project funded by the French national EC2CO program**



# CONTEXT

**Copper** : oligo-element naturally present in soils (~2-50 ppm)

## Vineyard soils:

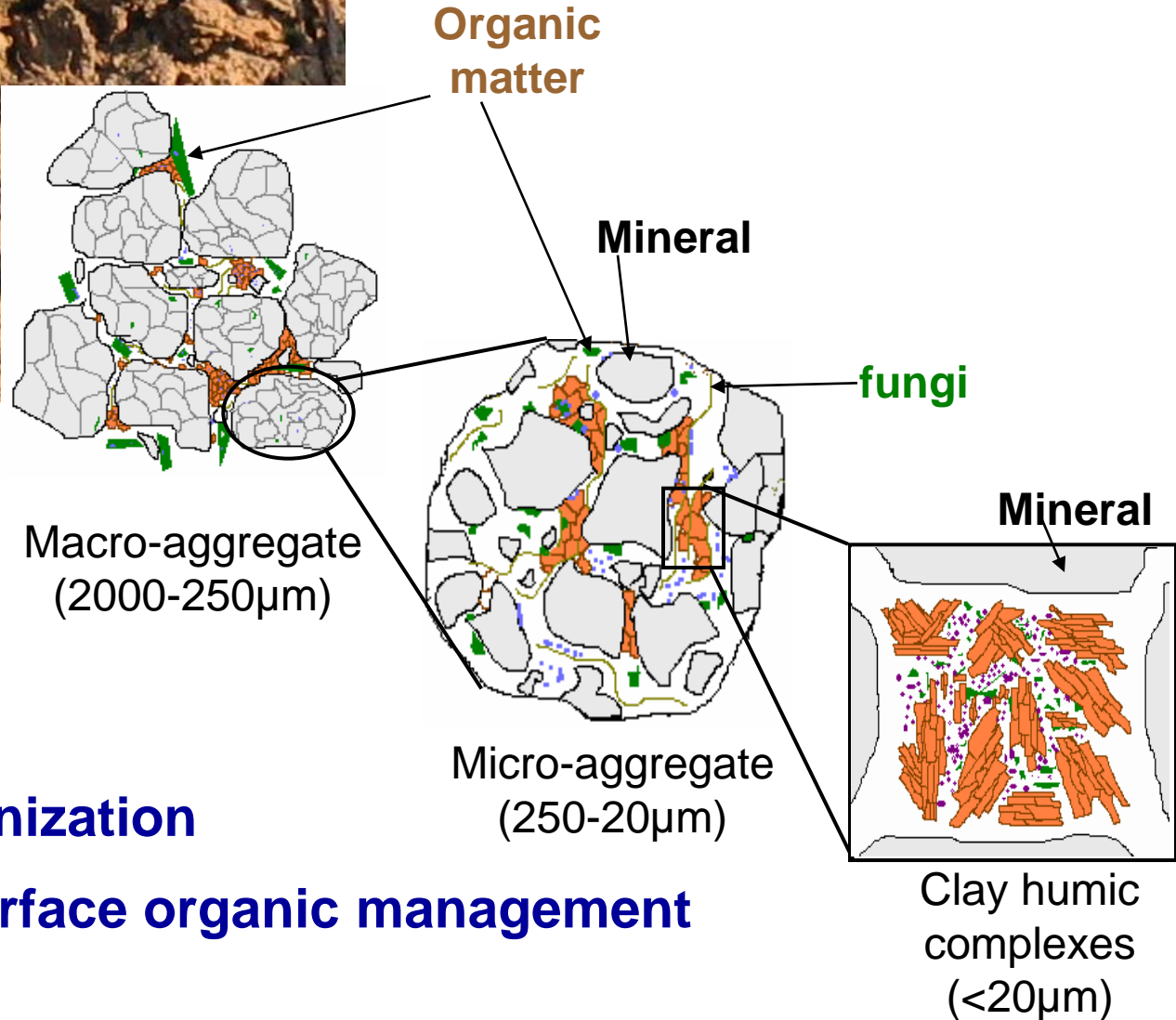
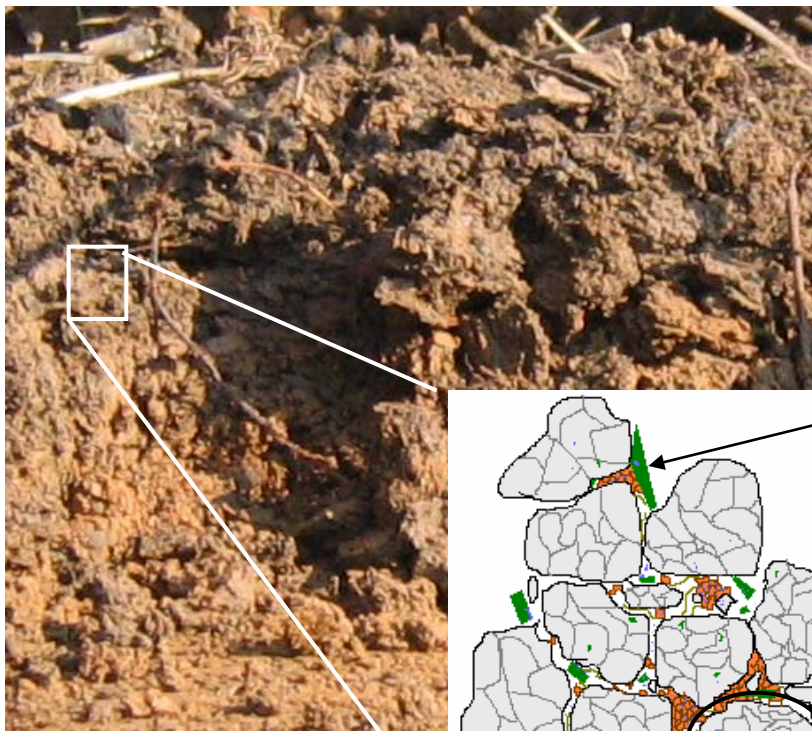
- \* **Copper** is largely used in France as **pesticide** (Bordeaux mixture)
- \* **Copper** strongly **accumulates** > 1000 ppm
- \* **Toxic** to soil organisms at such high concentration



**Biogeochemical factors that control copper impact on soils are poorly understood, especially in vineyard managed soils**

# Natural soils are spatially structured heterogeneous systems

→ present an aggregate-based organization

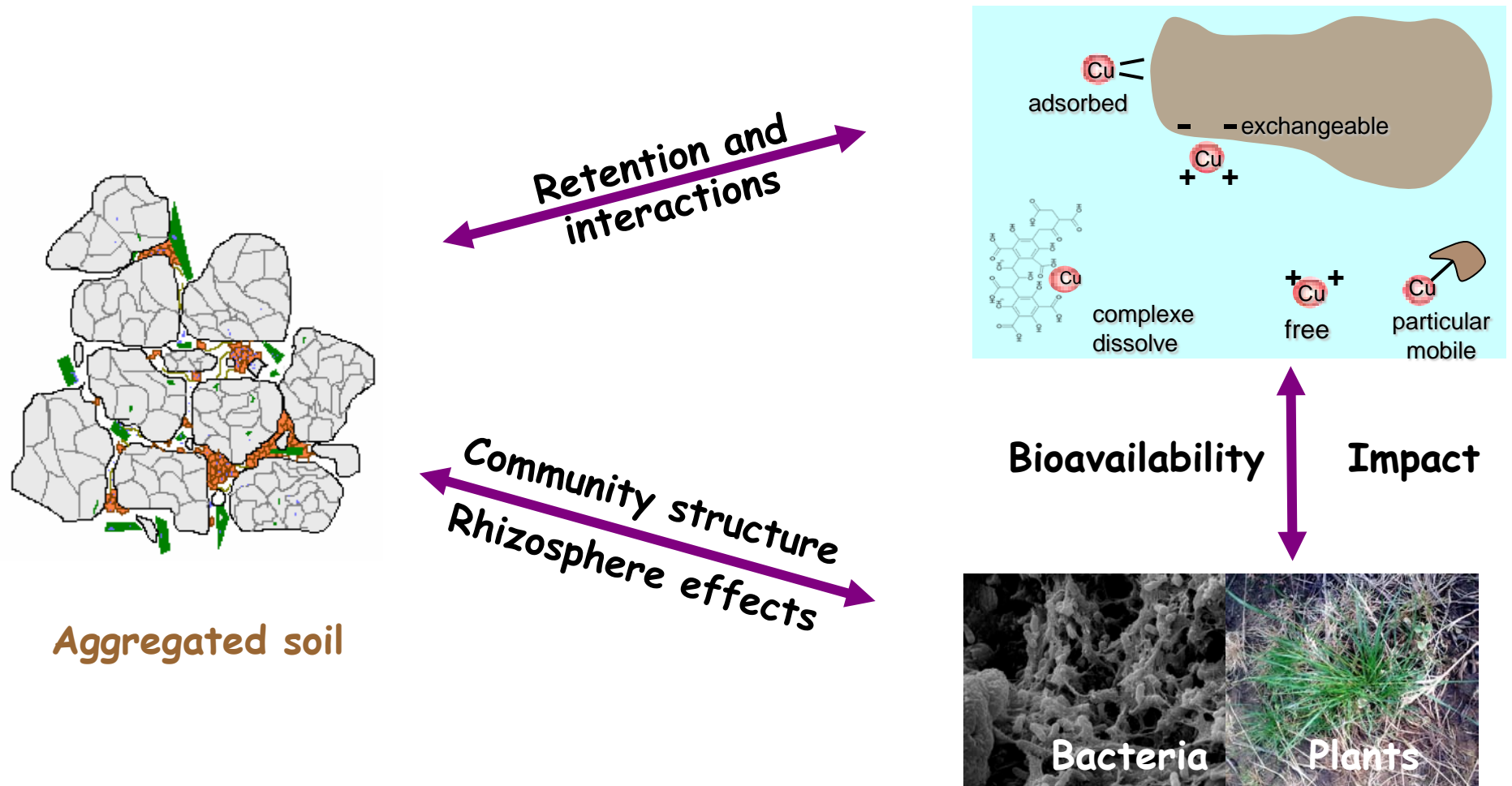


**Soil spatial organization**

**is affected by surface organic management**

# Objectives :

To investigate the relation between the field scale management of organic matter of a vineyard soil and the distribution and speciation of copper at the micro-aggregate scale and its bioavailability to bacteria and plants.



## The field site

### Vineyard soil - Clessé (Burgundy, France)



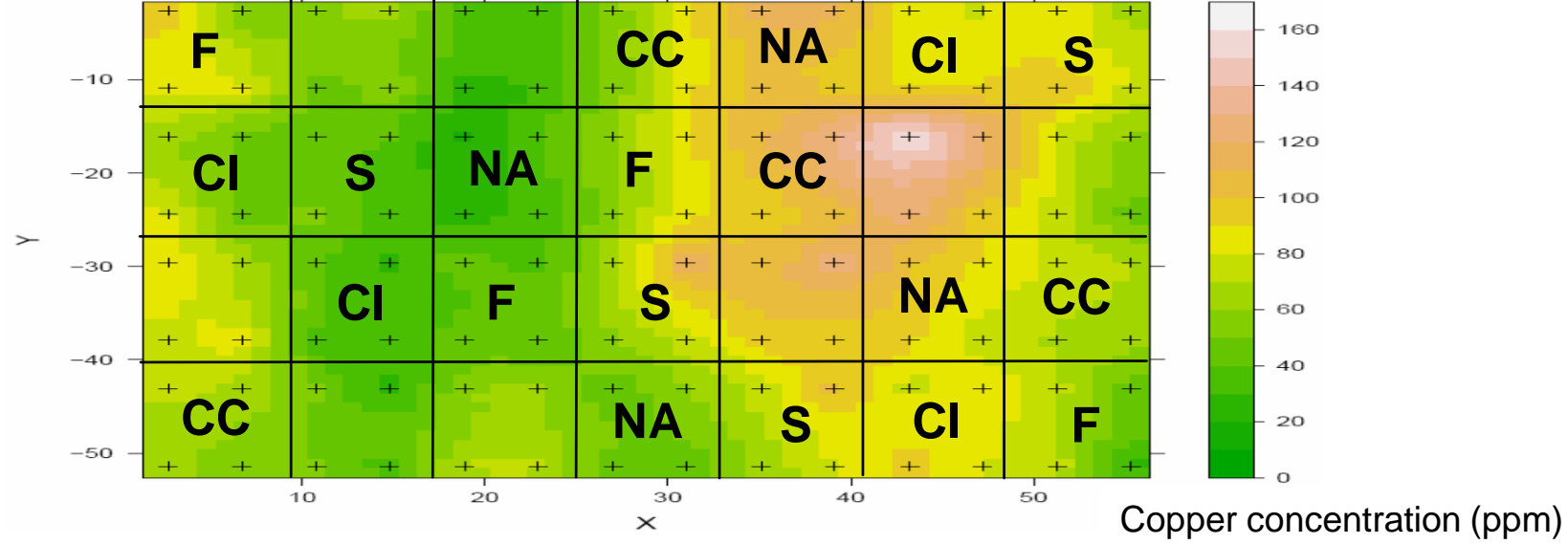
1 soil

→ 5 organic status :

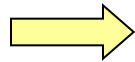
Soil amended/vegetated during 20 years:

- Non Amended Control soil (NA)
- Straw amendment (S)
- Conifer Compost amendent (CC)
- Fescue vegetation (F)
- Clover vegetation (CI)





Sampling of 50 kg of the 5 surface soils



Soil pre-drying and sieving

Soil analysis



# All collected soil samples → same analytical characterization

1  
↓

Soil  
fractionation

2  
↓

Soils and size fractions analysis  
(OC, N, Cu, Major elements...)

3  
↓

Copper speciation  
and availability

**Cu retained in the solid matrix**

**Cu released  
to soil solution**

**Cu bioavailable  
to bacteria and plants**

**Cu-Biosensor: *Pseudomonas fluorescens* DF57 [Cu]<sub>Bio</sub>**  
(Tom-Peterson et al. 2001)

**Cu-accumulation by the  
ryegrass *Lolium perenne***

**[Cu]<sub>Tot.</sub>**  
ICP-OES after extractions (*aqua regalis*)

**Solid speciation :**  
EXAFS Spectroscopy

**Ca-exchangeable Cu**

**[Cu<sub>ex</sub>]<sub>tot</sub> : ICP-AES**

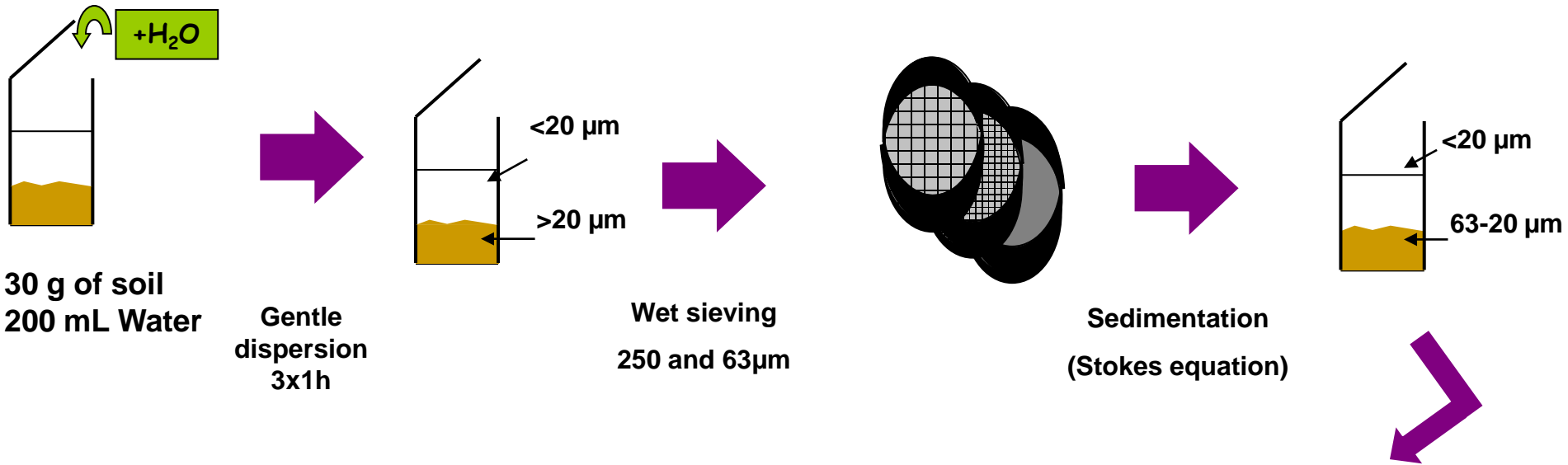
**Total copper**

**Mobile copper**

**Toxic copper**

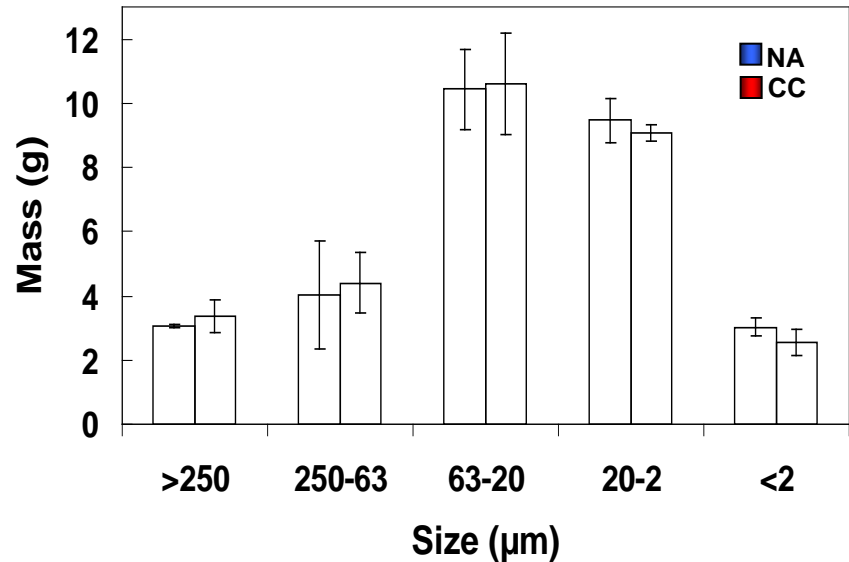


# Non destructive physical fractionation of soils using Ladd's procedure adapted by Monrozier et al. (1991)



**5 size-fractions (NA and CC soils):  
Specific functional compartments**

**→ Loamy soil**



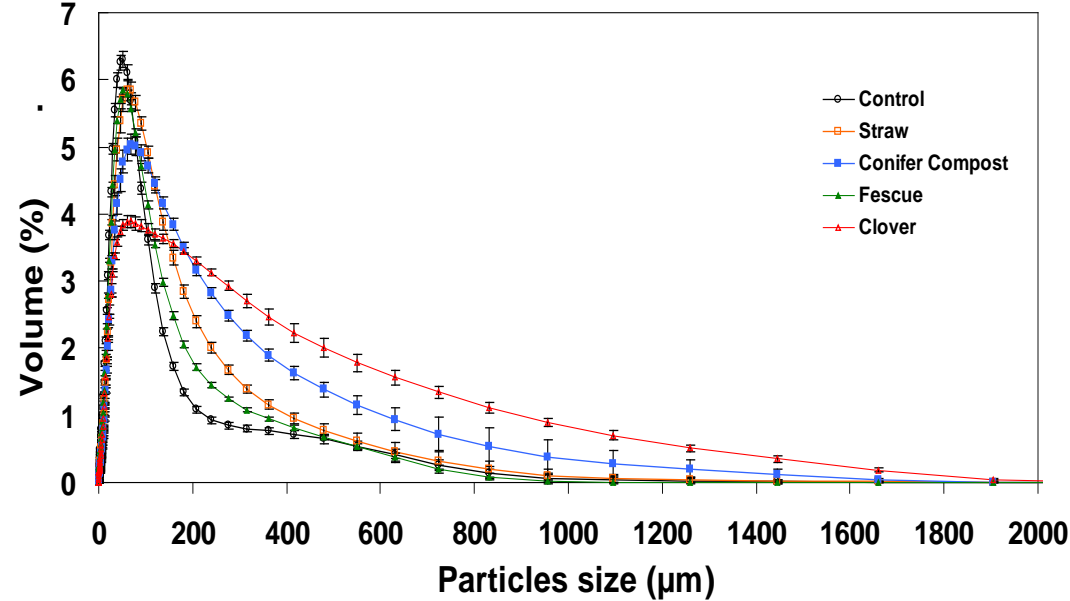
# Main bio-physical-chemical properties of the five soils

	Texture (%)			Bulk Density g.cm <sup>-3</sup>	Tot. Org. C g <sub>C</sub> .kg <sup>-1</sup> <sub>ds</sub>	Tot. Org. N g <sub>C</sub> .kg <sup>-1</sup> <sub>ds</sub>	pH <sub>w</sub>	Total Cu mg <sub>Cu</sub> .kg <sup>-1</sup> <sub>ds</sub>	Biomass-C mg <sub>C</sub> .kg <sup>-1</sup> <sub>ds</sub>
	Sand	Silt	Clay						
<b>Control</b>	16,3 ± 0,7	73,5 ± 1,4	10,2 ± 0,4	1,34 ± 0,03	15,2 ± 5,1	1,1 ± 0,2	6,3 ± 0,03	118 ± 4	238
<b>Straw</b>	23,6 ± 0,3	68,6 ± 0,5	7,8 ± 0,1	1,38 ± 0,13	20,4 ± 6,7	1,5 ± 0,3	5,9 ± 0,03	91 ± 4	465
<b>Conifer Compost</b>	20,2 ± 0,4	70,6 ± 0,7	9,2 ± 0,1	1,33 ± 0,08	30,3 ± 3,9	1,6 ± 0,1	6,3 ± 0,03	102 ± 5	492
<b>Fescue</b>	22,9 ± 0,6	68,7 ± 1,1	8,3 ± 0,1	1,35 ± 0,09	17,3 ± 4,2	1,3 ± 0,2	6,1 ± 0,02	89 ± 9	430
<b>Clover</b>	30,1 ± 0,6	64,3 ± 0,6	5,7 ± 0	1,33 ± 0,07	23,6 ± 3,6	1,9 ± 0,2	5,6 ± 0,1	106 ± 6	432

➔ The soil treatments induced mainly increases  
of Organic Carbon content and microbial biomass

➔ All other parameters remained almost constant

# Particle size distributions (PSD) of the five soils obtained by laser granulometry with and without ultrasonication

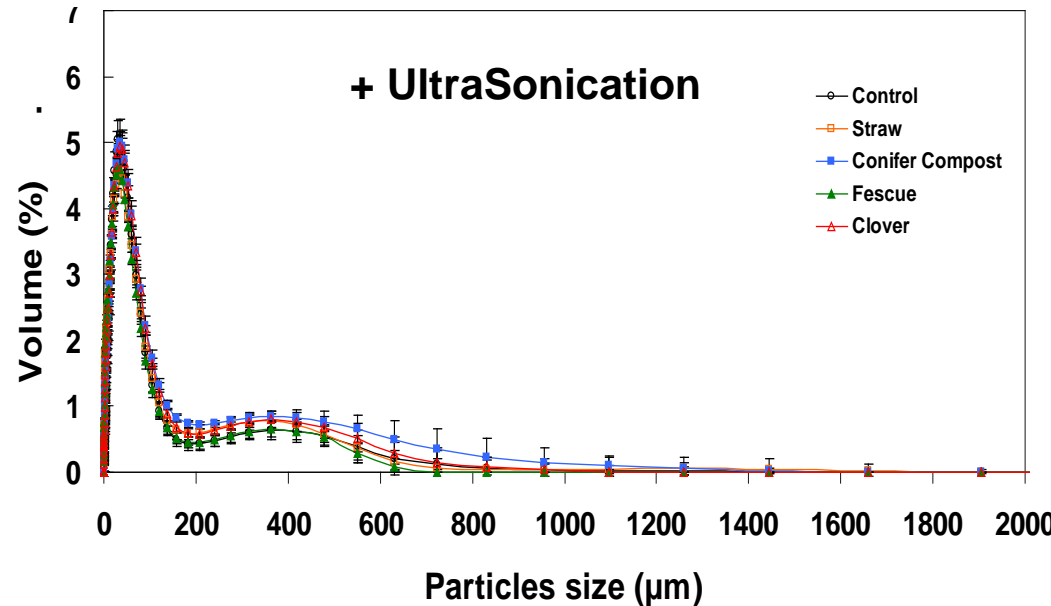


→ Strong modification of the soil PSD by the 20 years of organic amendment and vegetation

→ Increased aggregation of the smallest particles (<50μm) into larger aggregates of sizes ranging from 0.1 up to 2 mm with the **Conifer compost and clover**

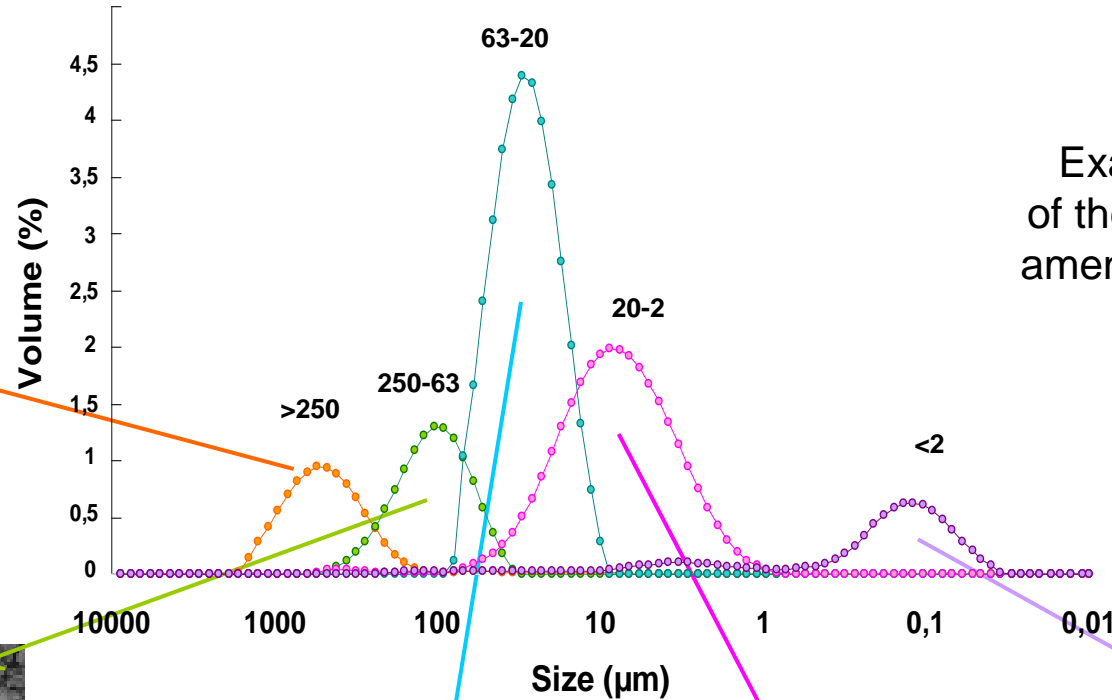
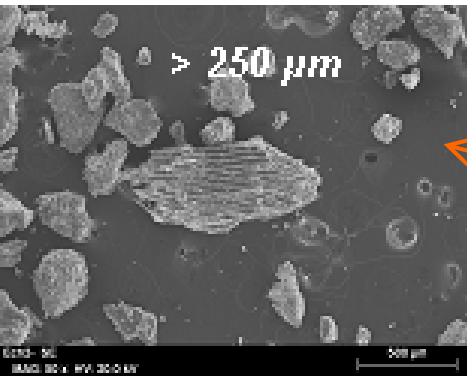
**Ultrasonication destroys soils aggregates**

→ **Same texture of the 5 soils**

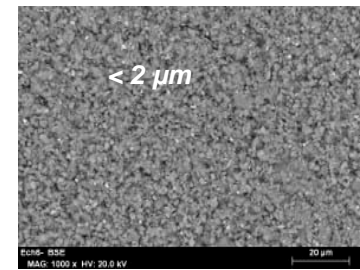
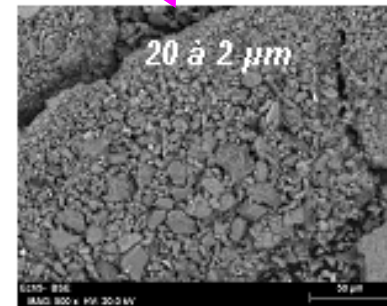
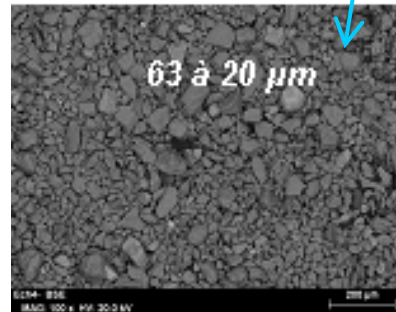


# Particle size distributions (PSD) of the size-fractions of the 5 soils

Laser granulometry coupled with SEM

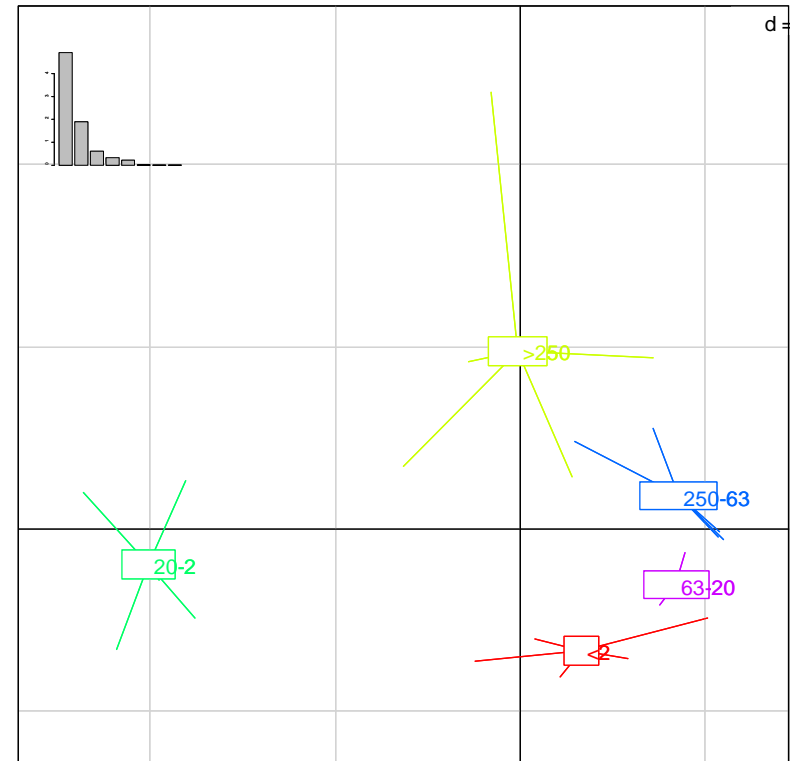
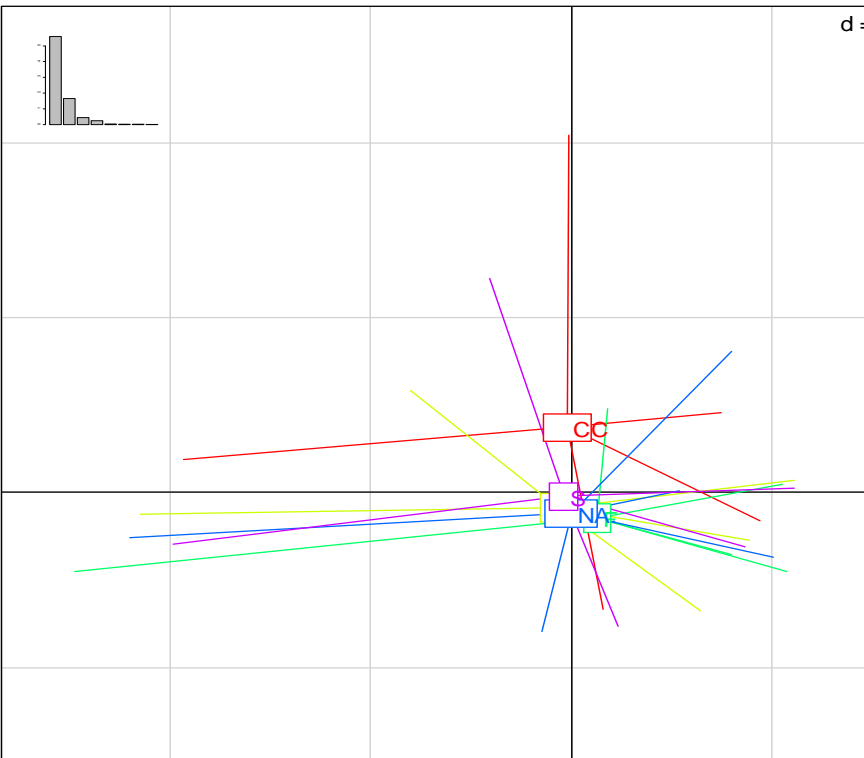


Example of the Straw amended soil



➔ Good separation of the soil compartments and preservation of their aggregated structure by the physical fractionation procedure

# PCA of the major elements contents in the five soils and in their size-fractions

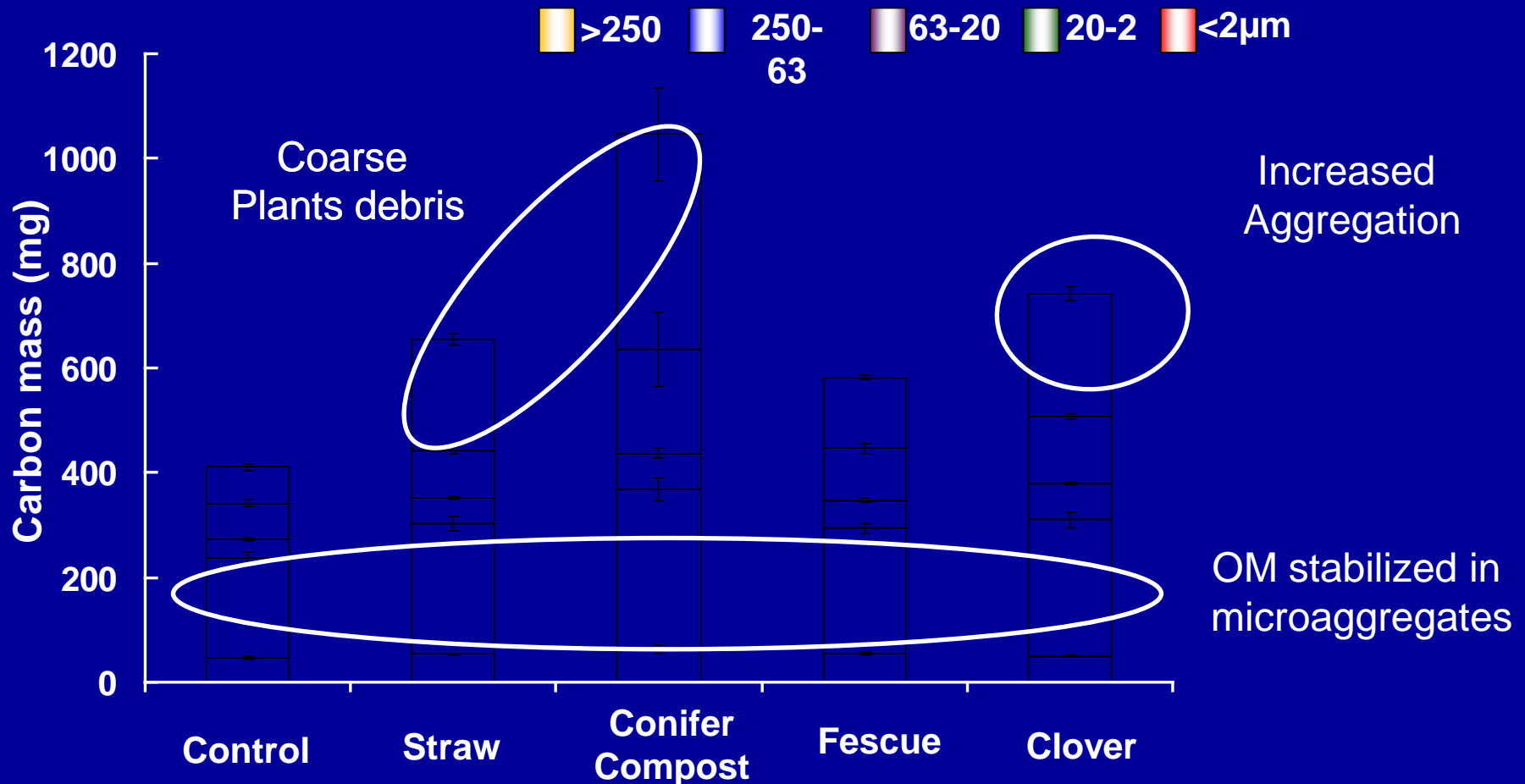


No significant difference between soils  
→ soil treatments did not modify soil's elementary composition.

The 5 subfractions of the soils appeared strongly different in terms of chemical composition confirming that

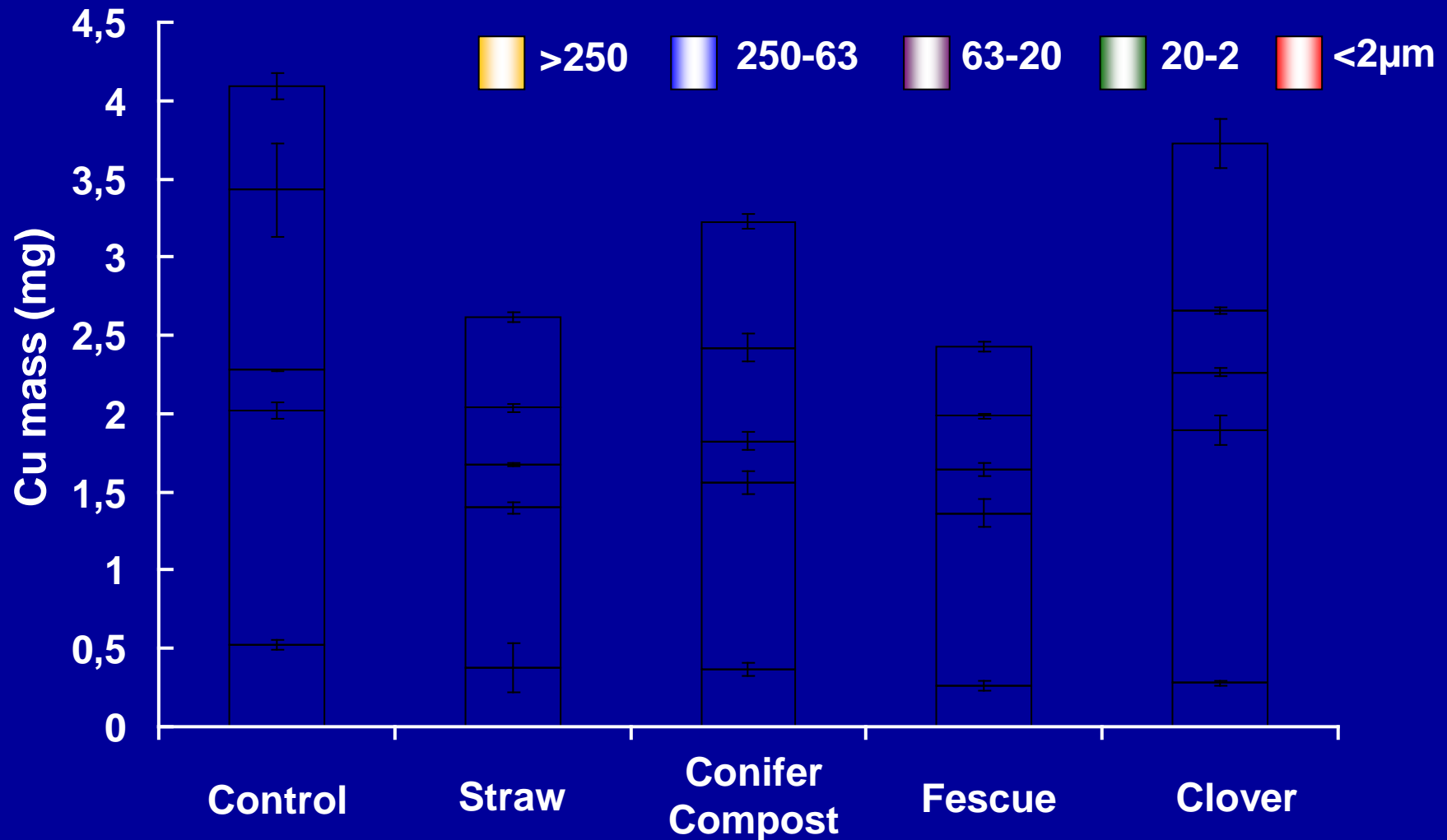
→ each soil size fraction constitutes an independent biogeochemical fabric.

# Distribution of the mass of total organic carbon in the five size-fractions of the Clessé soil



**<2µm and 63-20µm fractions are insensitive to soil treatments**

# Distribution of the mass of copper retained in the five size-fractions of the Clessé soil



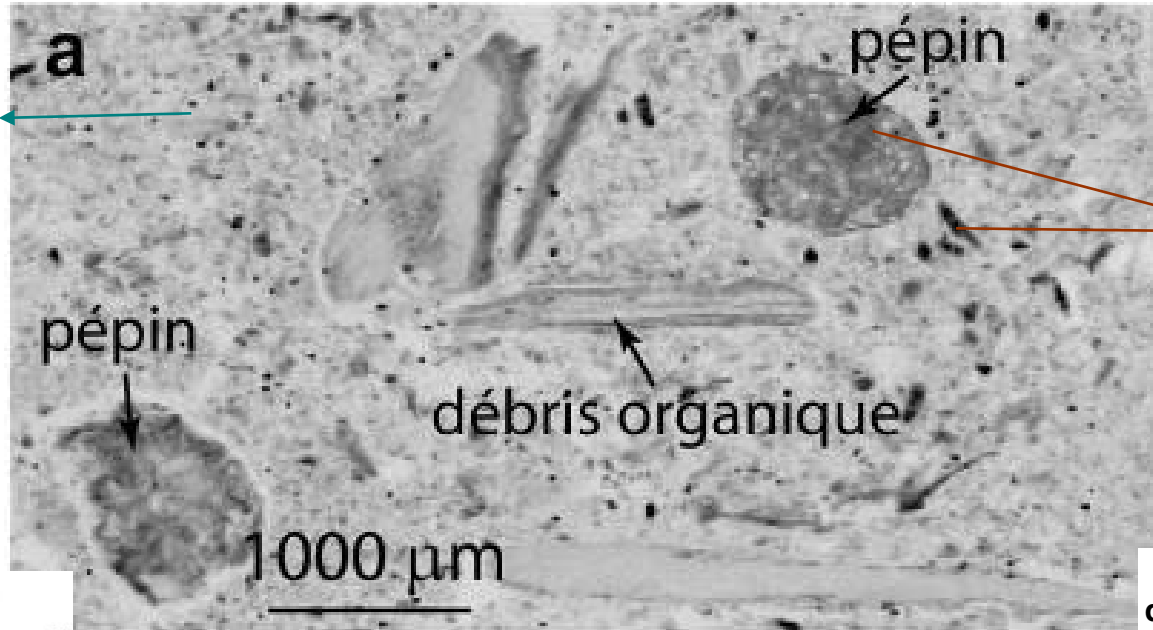
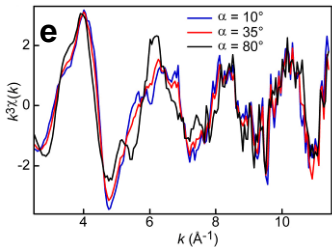
Copper accumulates preferentially in the finest (>20µm) and coarser (>250µm) fractions → up to 80% of Cu

# Solid Copper speciation in the bulk soils

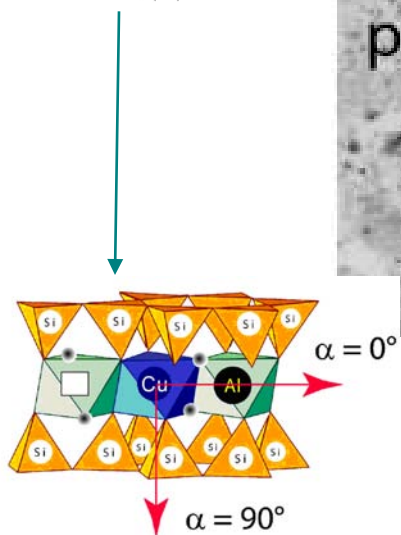
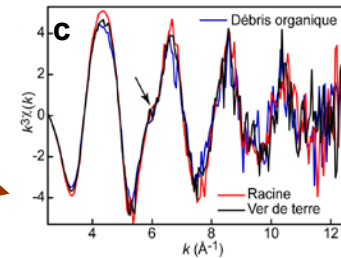
(Data from Alain Manceau)

## Map of Cu distribution at the Cu K-Edge

### Cu-Clay associations



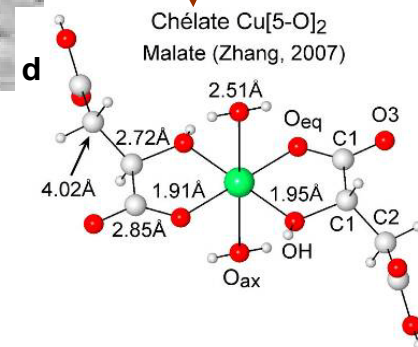
### Organic elements



Courtesy of Alain Manceau

[Cu] = grey scale

### Similar spectra



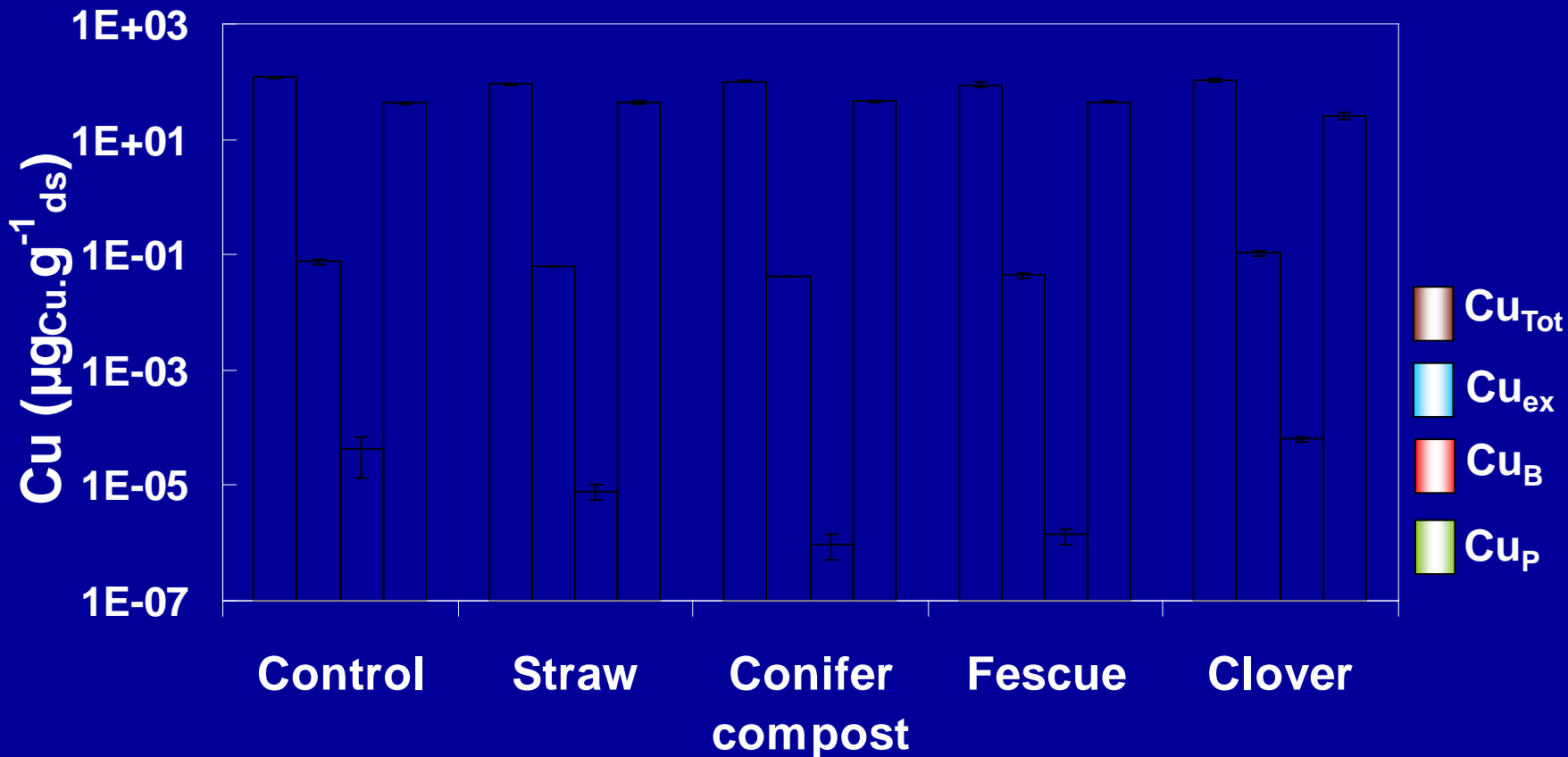
Copper penetrated the whole soil matrix : **organic** and **inorganic** compartments.

→ Diffuse association of Cu with the crystalline network of clays (light grey)

→ Specific Cu accumulation in organic components (dark grey or black).



# Effect of soil treatment on copper retention, speciation and bioavailability



$\text{Cu}_{\text{Tot}}$  similar in the five soils

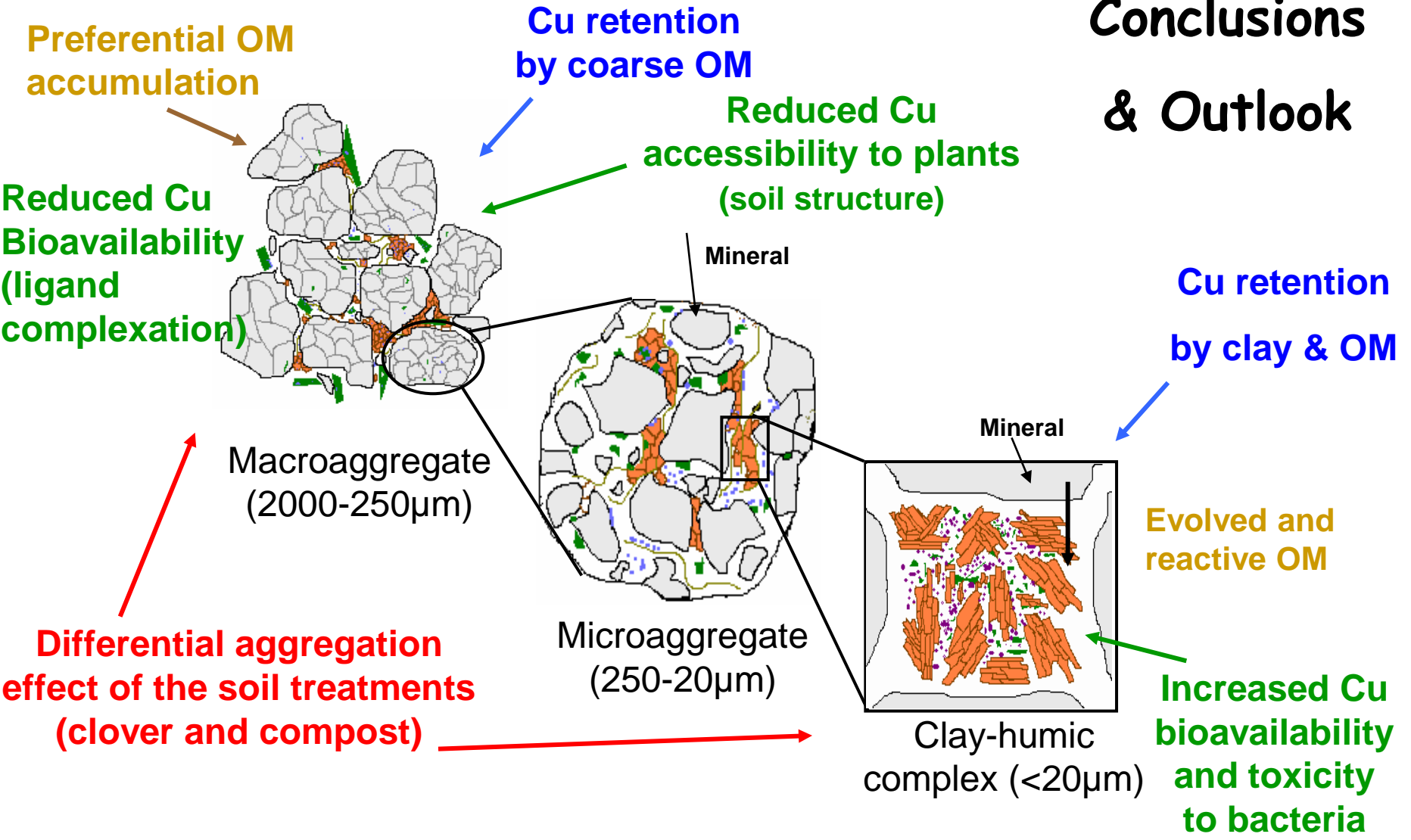
Cu exchangeable with Ca is lower in the amended soils (decreased mobility)

Cu bioavailable to bacteria is lower in S, CC and F soils (decreased toxicity)

Cu accumulated in the ryegrass *Lolium perenne* is lower in the clover soil (decreased Cu accessibility)

# Soils are constituted of a mosaic of functional compartments (habitats)

## Conclusions & Outlook



LTHE



**MARTINS**  
Jean



**SPADINI**  
Lorenzo

LGIT



**SPADINI**  
Lorenzo



**MANCEAU**  
Alain

G2R



**FAURE**  
Pierre

INRA  
Pessac



**LAMY**  
Isabelle

INRA  
Dijon



**RANJARD**  
Lionel



**NAVEL**  
Aline



**VINCE**  
Erwann



**MARON**  
Pierre-Alain

Thank you for your attention

Acknowledgements

Vine owner : **LACROZE Daniel**

Chambre d'agriculture de Saône et Loire : **CROZIER Philippe**

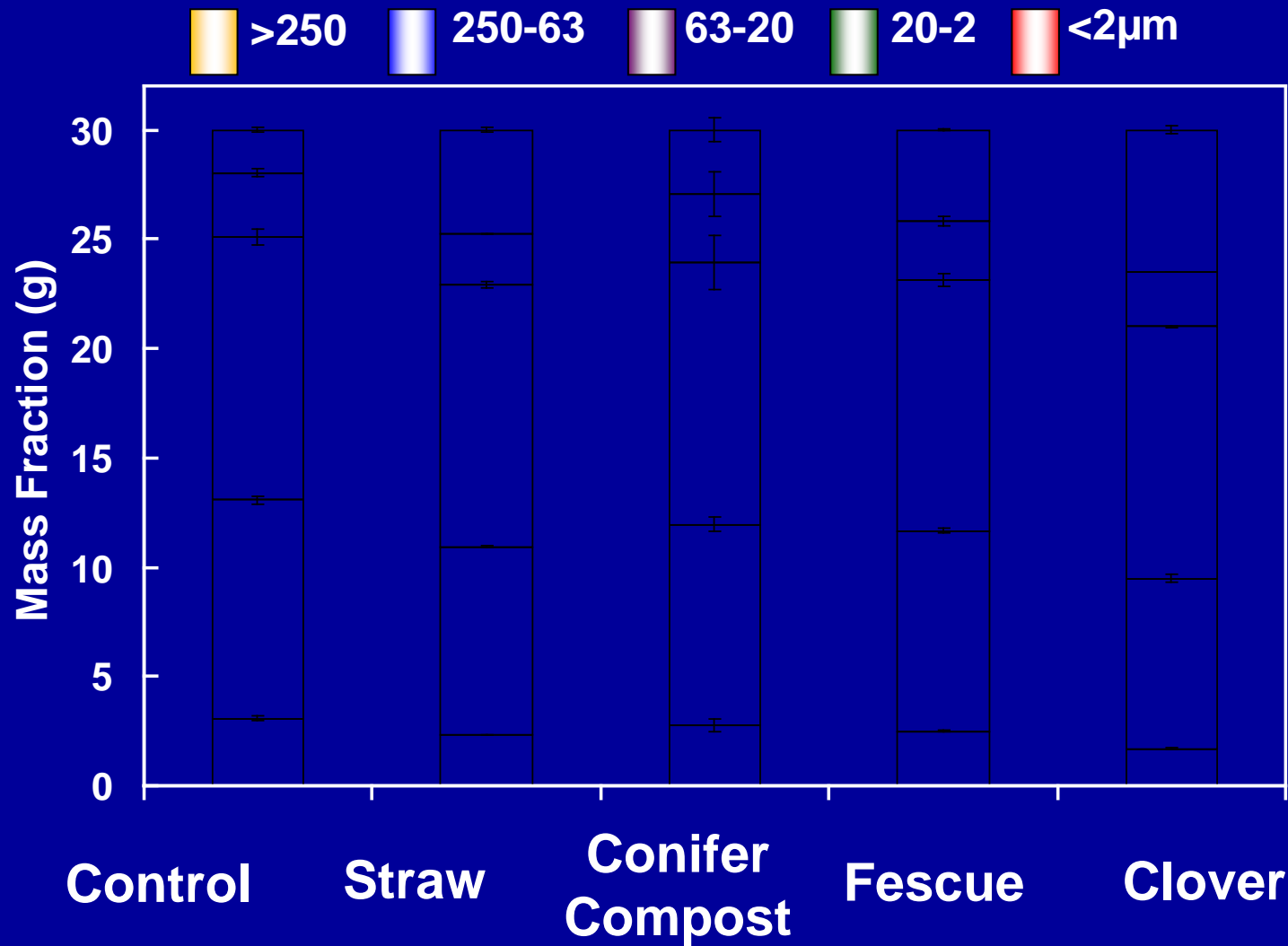
**EC2CO**



Bourse ministérielle



# Distribution of the solid mass of the five soil size-fractions of the Clessé soil



# Conclusions and outlook

**Both amendment and vegetation treatments induced an increase of the soil TOC content, which distributed dominantly in the two coarser size-fractions of the soil.**

**Compost and clover treatments of the soil induced a strong aggregation of the finest soil constituents, thus modifying its spatial structure and copper accessibility to plants.**

**The size fractions of the five soils were shown to present very different trace elements composition, thus constituting differentiated habitats of very different copper retention capacities.**

**The most reactive fractions of the five soils were the finest (<20 $\mu$ m) and the coarser (>250 $\mu$ m) fractions, which accumulated about 80% of the total Cu content.**

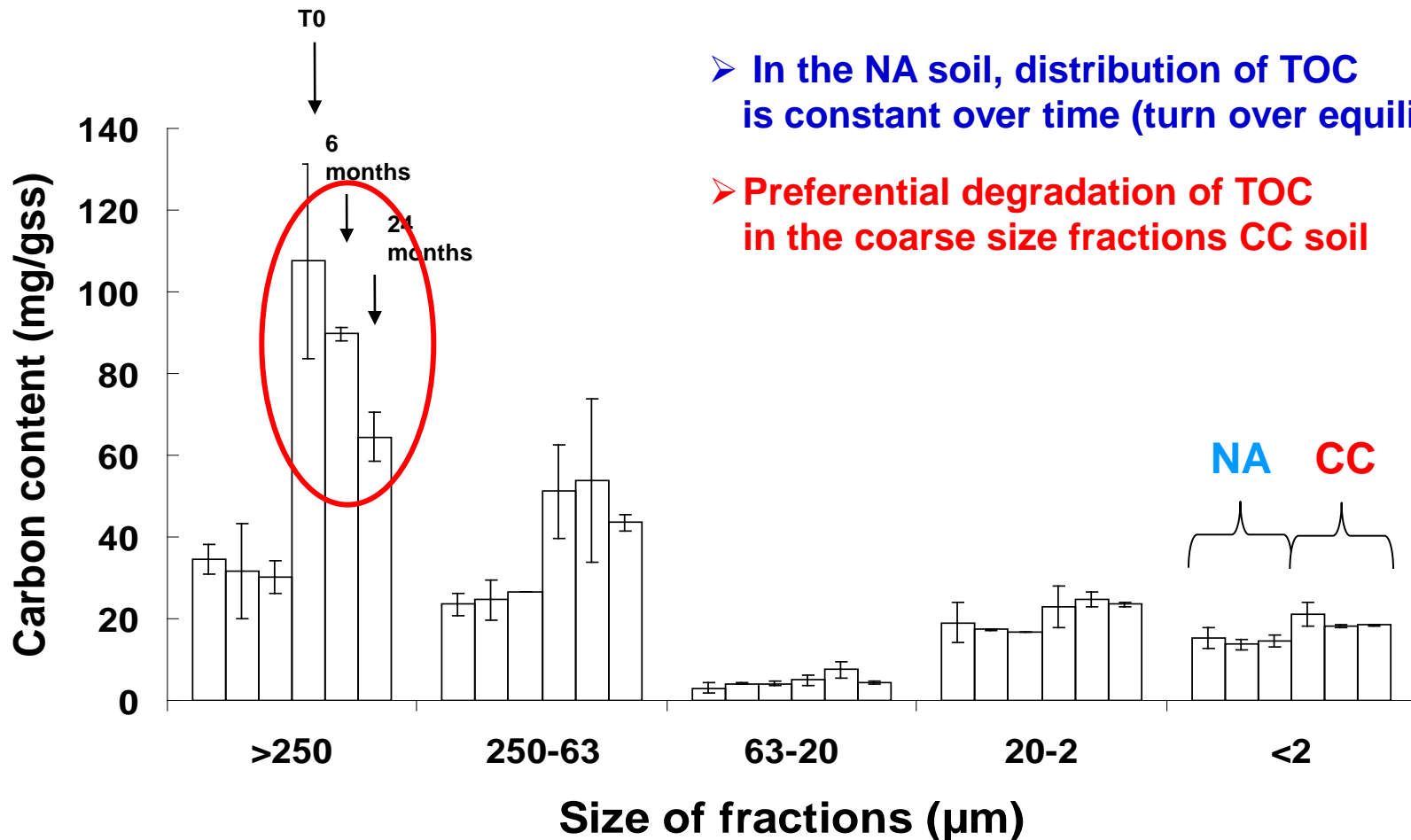
**Copper speciation and bioavailability to bacteria and plants varied significantly between soil treatments:**

- organic amendments reduced strongly the concentrations of Ca-exchangeable and bioavailable copper (toxic) by Cu-complexation with organic ligands**
- clover vegetation modified the soil pH, which increased copper solubility and bioavailability to bacteria**

**The field scale organic management of vineyard soils controls copper distribution and bioavailability at the micro-aggregate scale through differential effects**

# Temporal evolution of the TOC contents in size-fractions of the soils

- the coarser fractions of the 2 soils present the higher OM contents



- In the NA soil, distribution of TOC is constant over time (turn over equilibrium)

- Preferential degradation of TOC in the coarse size fractions CC soil

# Conclusions

Le projet MOBIPO n'est pas un échec !

=> **Meilleure compréhension du fonctionnement des sols (boite grise?) :**

**Les amendements organiques des sols modifient**

1/ la **distribution du cuivre** à microéchelle

2/ la structure des communautés microbiennes à macro et à microéchelle

=> **Impact différencié du cuivre** sur **les communautés microbiennes** selon les compartiments structuraux (fonctionnels?) des sols et l'amendement en MO.

The compost amendment modified Cu speciation, distribution and ageing in the soil and reduced its impact on the microbial compartment

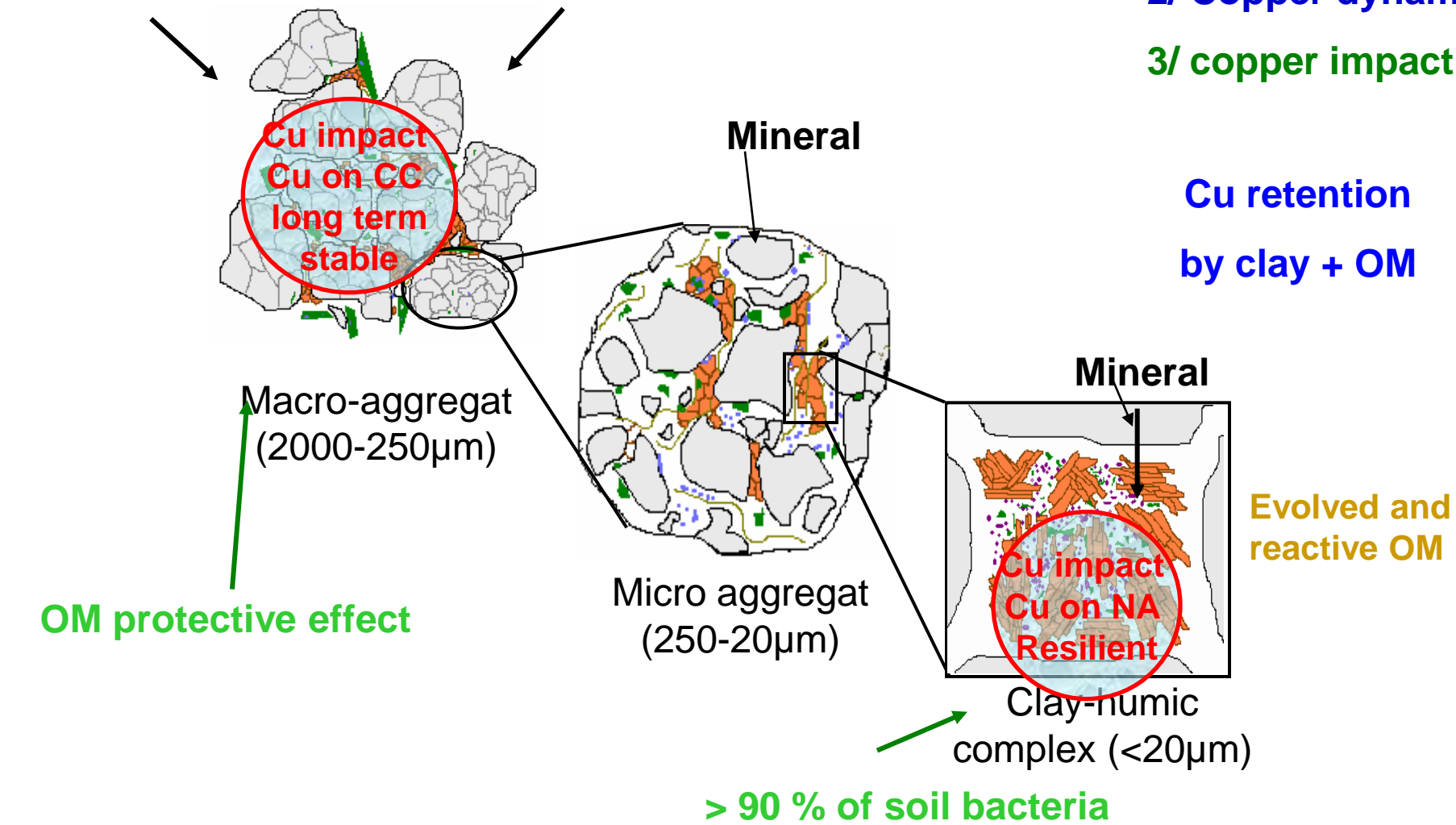
# Conclusions

- 1/ Carbon dynamics
- 2/ Copper dynamics
- 3/ copper impact

Cu retention  
by clay + OM

Cu retention  
by coarse OM

Preferential OM  
accumulation and  
degradation  
(protective effect)



OM protective effect

Macro-aggregat  
(2000-250µm)

Micro aggregat  
(250-20µm)

Clay-humic  
complex (<20µm)

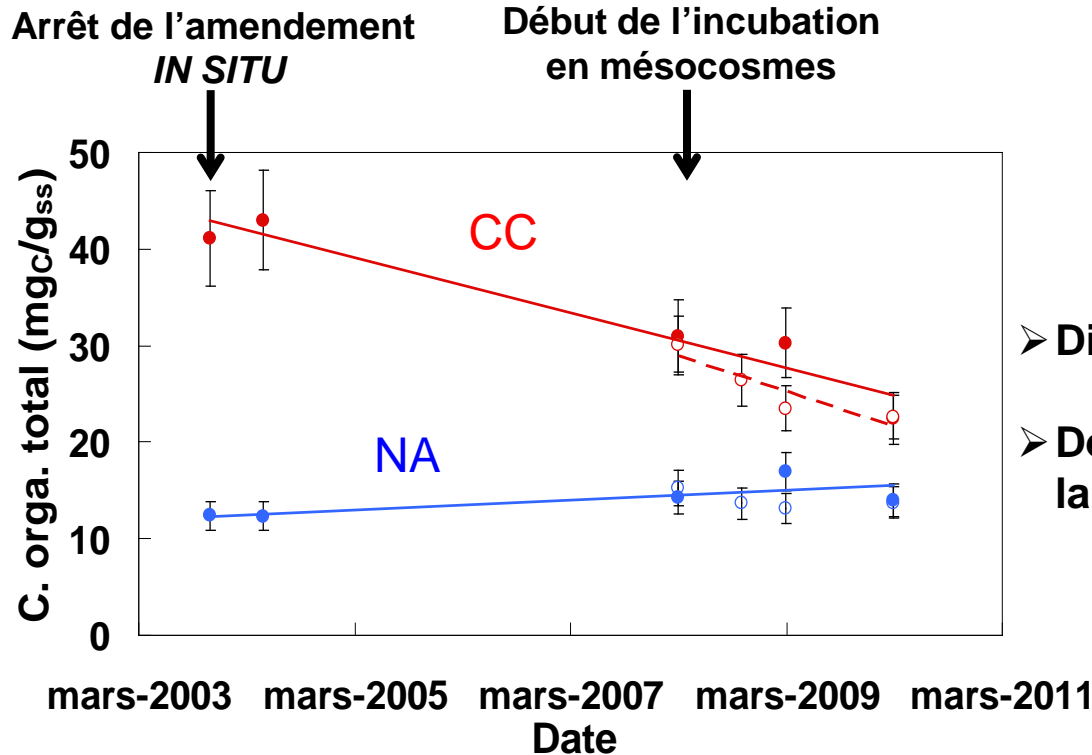
Evolved and  
reactive OM

> 90 % of soil bacteria

The compost amendment modified Cu speciation, distribution and ageing in the soil and reduced its impact on the microbial compartment



# Evolution temporelle du COT dans les sols



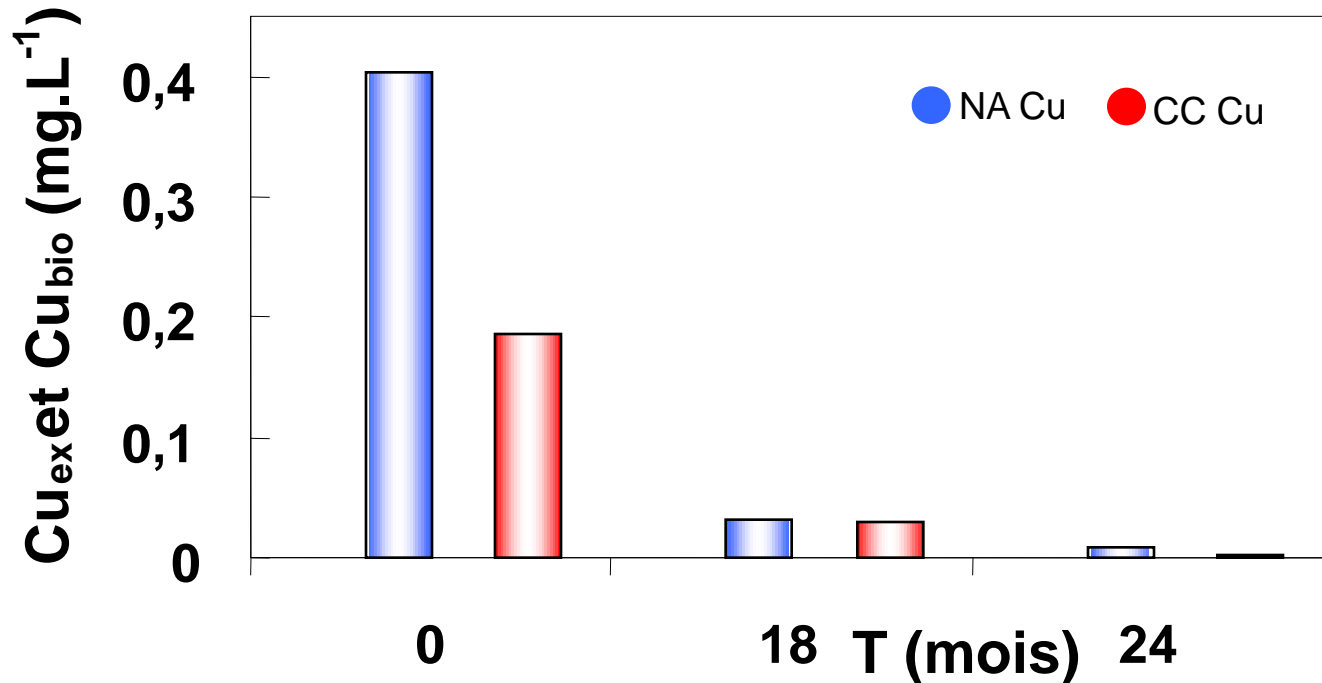
- Diminution du  $C_{org}$  dans le sol CC in situ
- Dégradation légèrement plus rapide de la MO en conditions contrôlées.

Rôle des MO dans la relation spéciation/biodisponibilité/impact?

Quel(s) est le compartiment fonctionnel clé?

# Dynamics of bioavailable copper in soil solution

Cu-biosensor (*P. fluorescens* DF57)

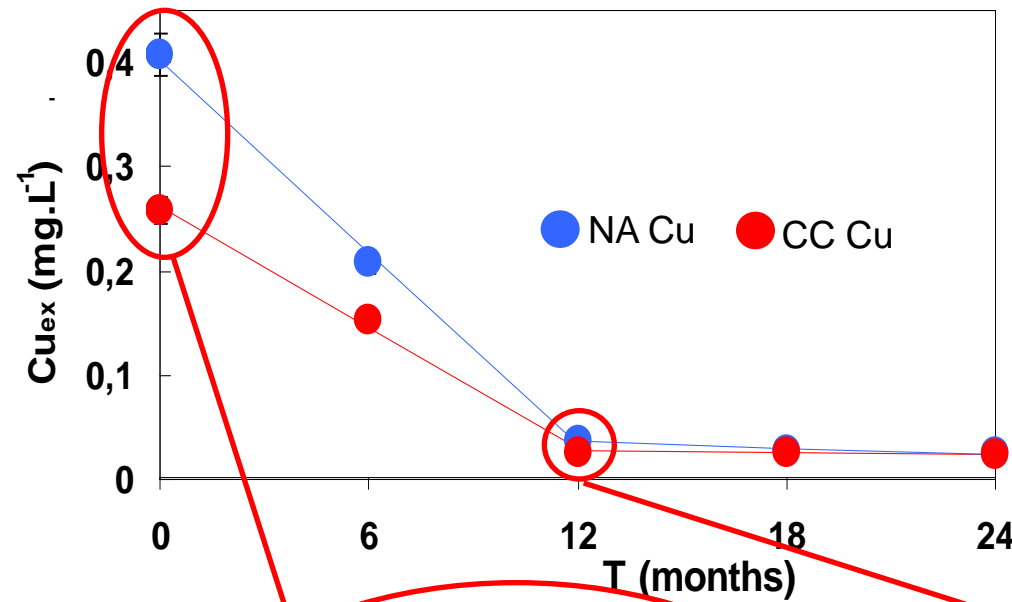


Copper is more bioavailable in the **NA** than in the **CC soil**.

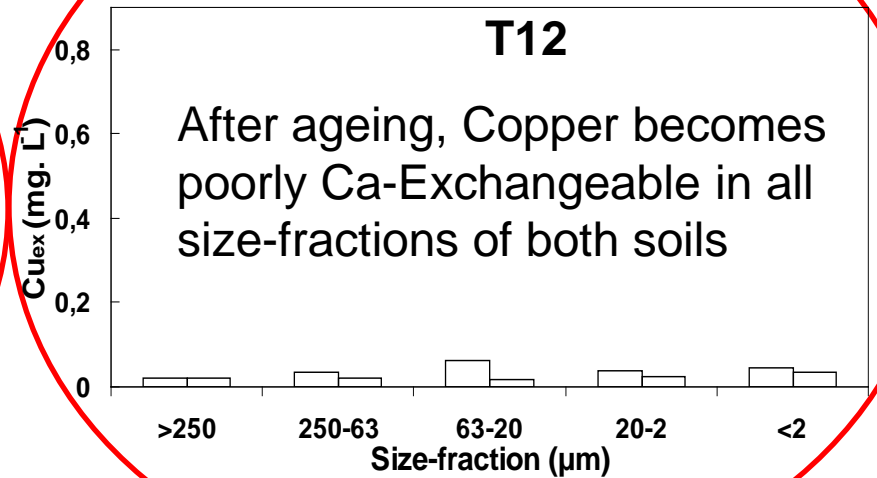
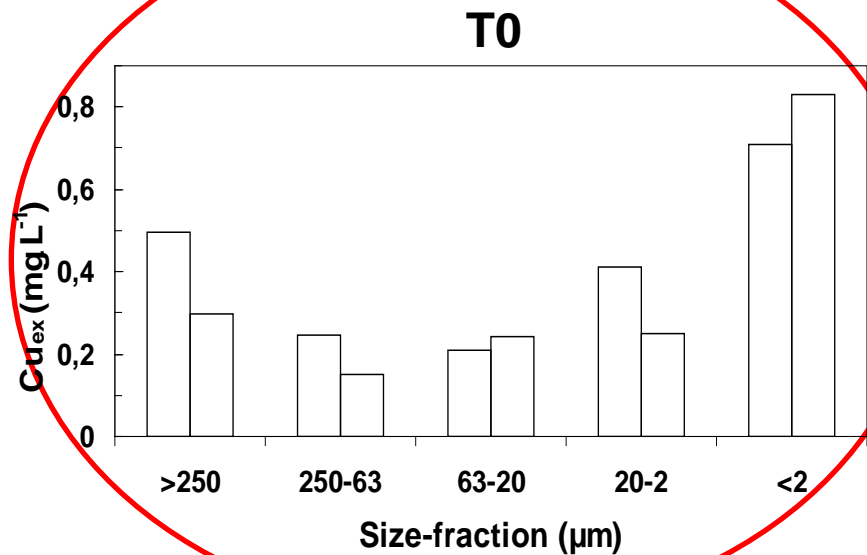
→ Confirms the protective effect of added OM

**These results indicate that Cu will probably be more toxic in the non amended soil**

# Speciation and dynamics of Ca-exchangeable copper in soil solution



- Only 0.1% of Cu exchanges with Ca!
- **NA** exchanges more Cu than **CC**
- **protection effect of the OM**
- Fast ageing of the Cu contamination



# Results

**1- Effect of amendments and vegetation**

**on the particle size distribution and aggregation of soils**

**2- Effect of amendments and vegetation on Cu speciation  
and bioavailability**

**3- Effect of amendments and vegetation on the mass  
distribution of total carbon, copper and soils size-fractions**

# Results

**1- Effect of amendments and vegetation on the particle size distribution and aggregation of soils**

**2- Effect of amendments and vegetation on Cu speciation and bioavailability**

**3- Effect of amendments and vegetation on the mass distribution of total carbon, copper and soils size-fractions**

# Results

**1- Effect of amendments and vegetation**

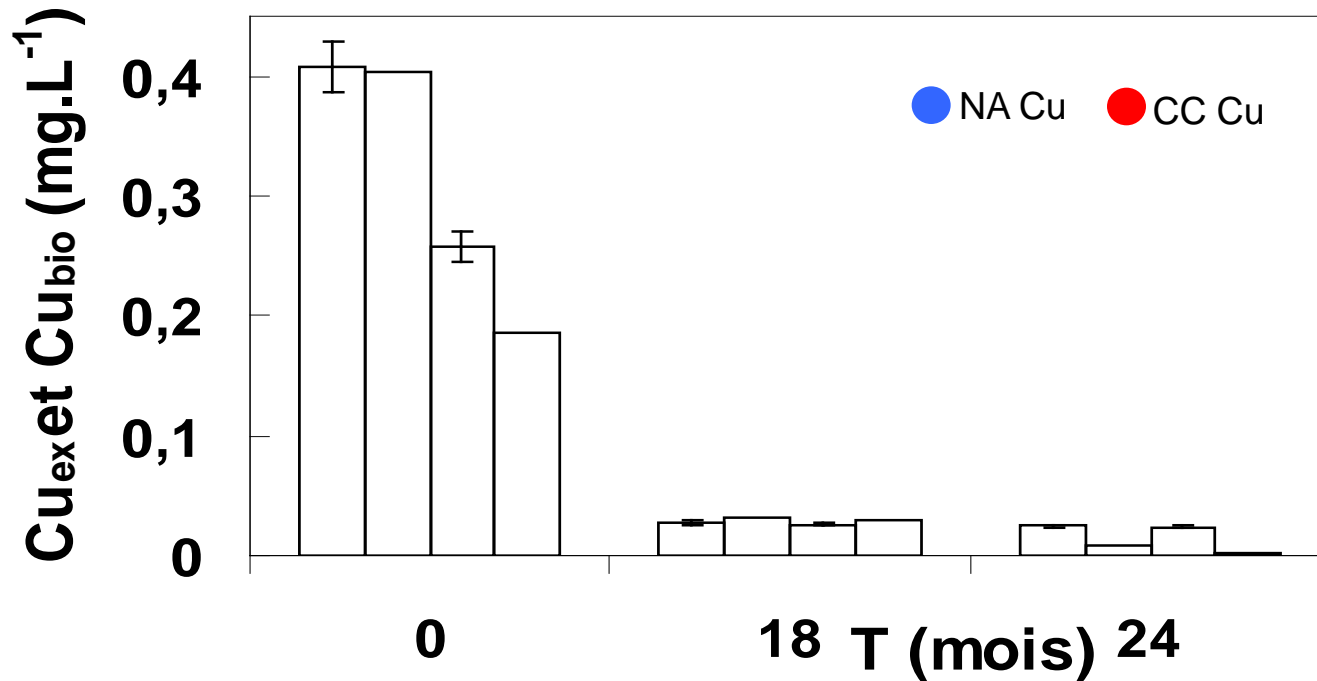
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# Dynamics of bioavailable copper in soil solution

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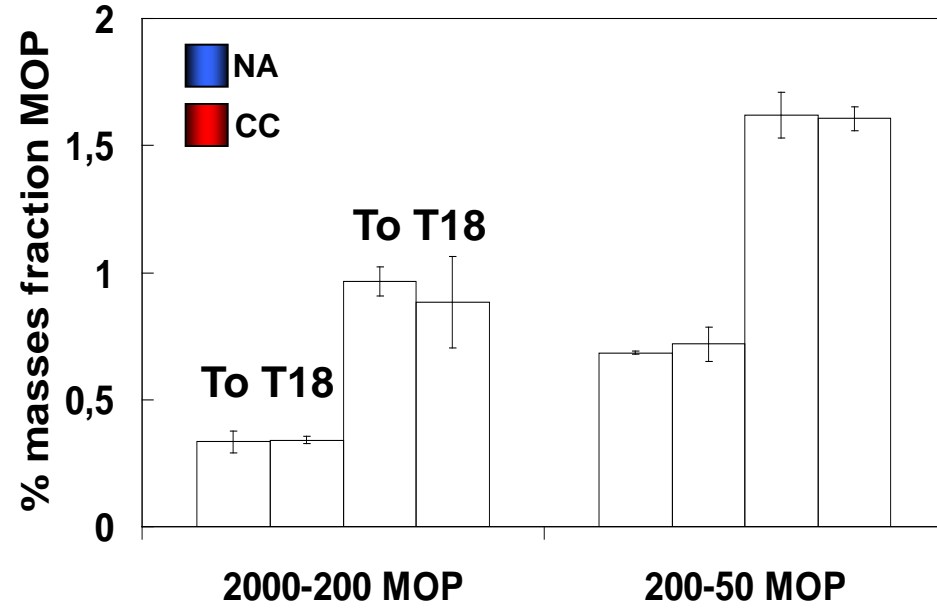
Copper is more bioavailable in the **NA** than in the **CC soil**.

→ Confirms the protective effect of added OM

These results indicate that Cu will probably be more toxic in the non amended soil

# Evolution sur 2 ans des Matières Organiques Particulaires (MOP)

- Cu

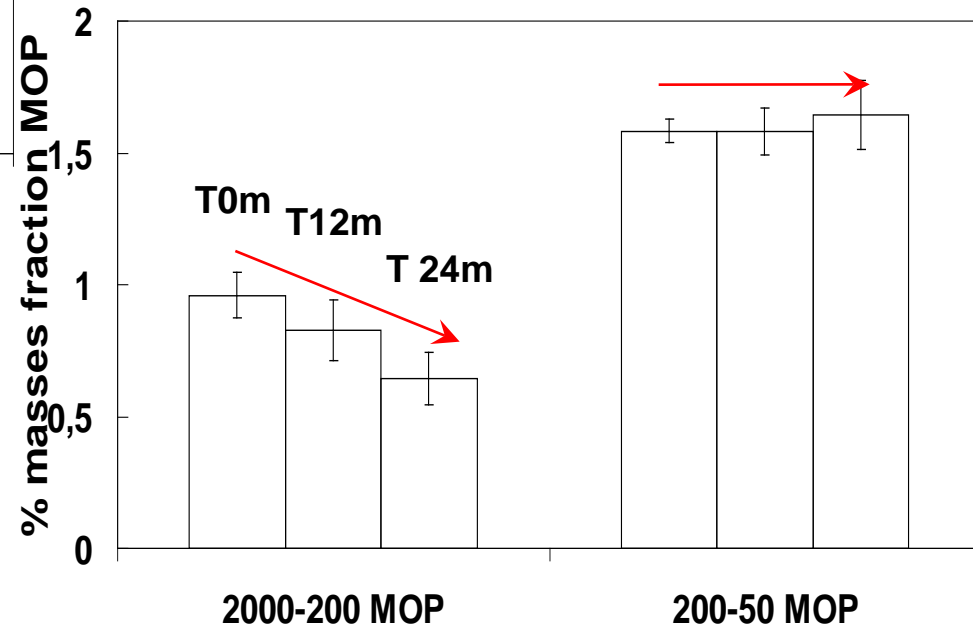


Pas d'évolution significative dans NA !

Le cuivre stimule la dégradation  
des MOP !!

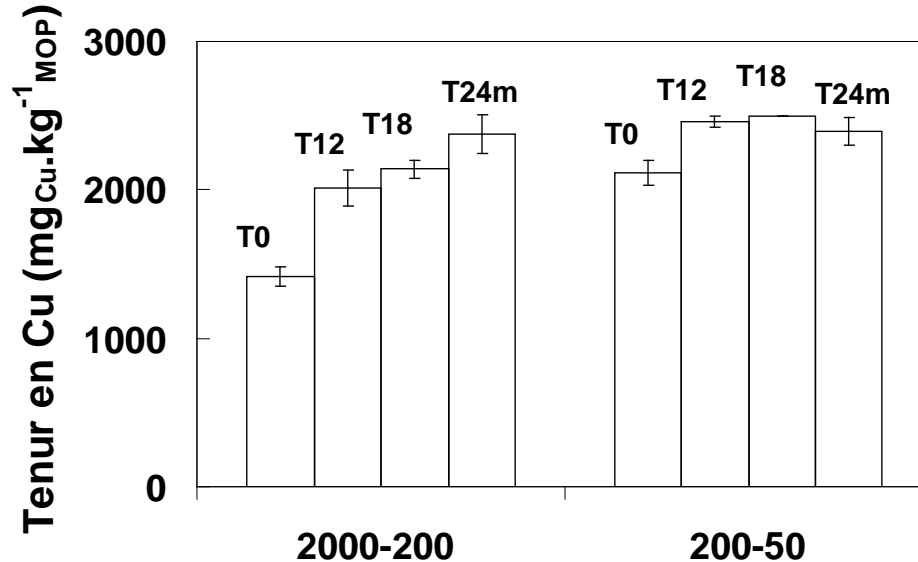
Plus de MOP dans le sol CC  
Dominance de la fraction 200-50  $\mu\text{m}$ .

+ Cu





# Evolution des teneurs en Cu dans les MOP sol amendé en compost de conifère



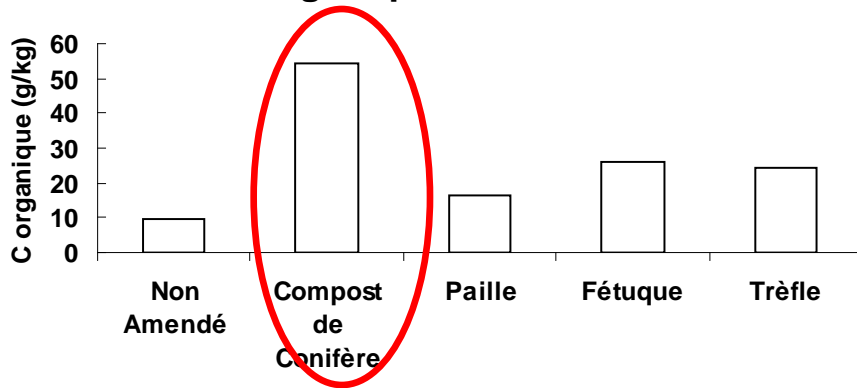
**Soil CC**  
**contaminé au Cu**  
**(+/- 300 ppm)**

- $[Cu]_{MOP}$  très élevées (7 x la moyenne).
- Enrichissement des MOP > 200 $\mu$ m en Cu au cours du temps (dégradation des MOP)
- Dynamique de puits de cuivre différente selon la taille des MOP

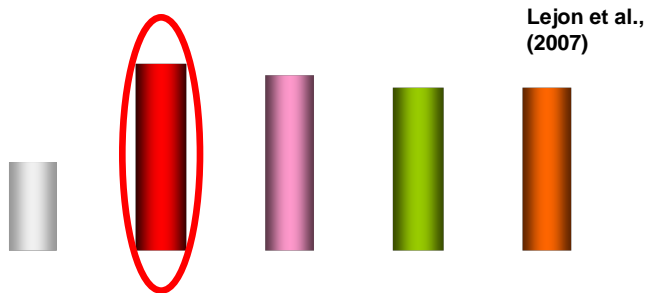
# Choix de 2 sols parmi les 5 statuts organiques

## Caractérisations **Bio-géo-chimiques**

### Teneur en C organique

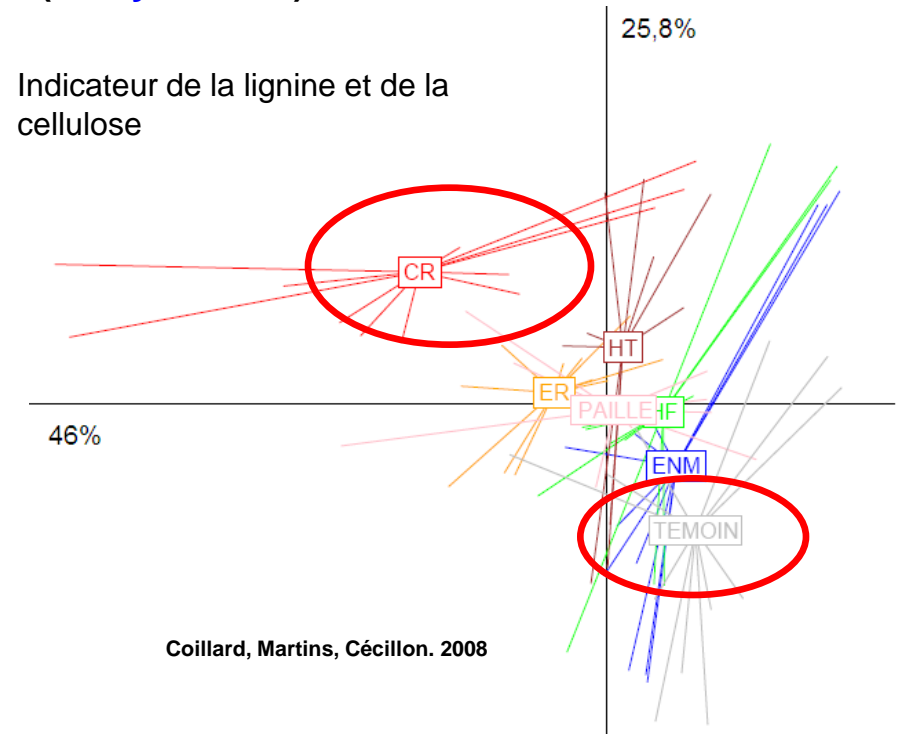


### Teneur en biomasse microbienne



NA ≠ CC, P, F et T

### Effet de la nature de la Matière organique (Analyse NIRS)



➔ Choix du traitement de MO :

1 contrôle : **NA**

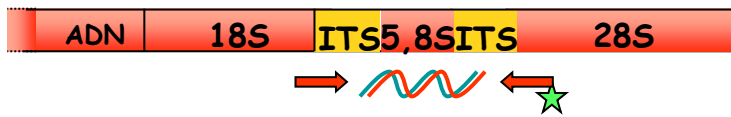
1 amendement : **CC**

# ARISA Analysis of bacterial community structures (INRA Dijon)

Extraction *in situ* of DNA of microbial communities  
(Ranjard, Lejon *et al.*, 2003)

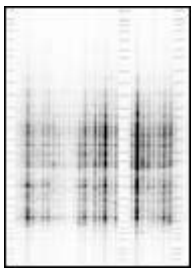
**ARISA**  
(Automated Ribosomal  
Intergenic  
Spacer Analysis)  
(Fisher et Triplett, 1999; Ranjard  
*et al.*, 2001)

IGS (intergenic spacer) or ITS controlled PCR amplification

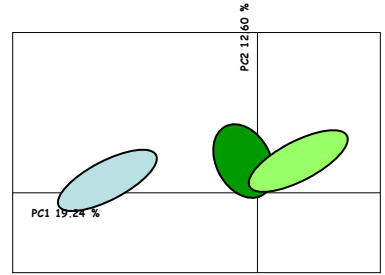


Electrophoretic (LiCOR-type gel)

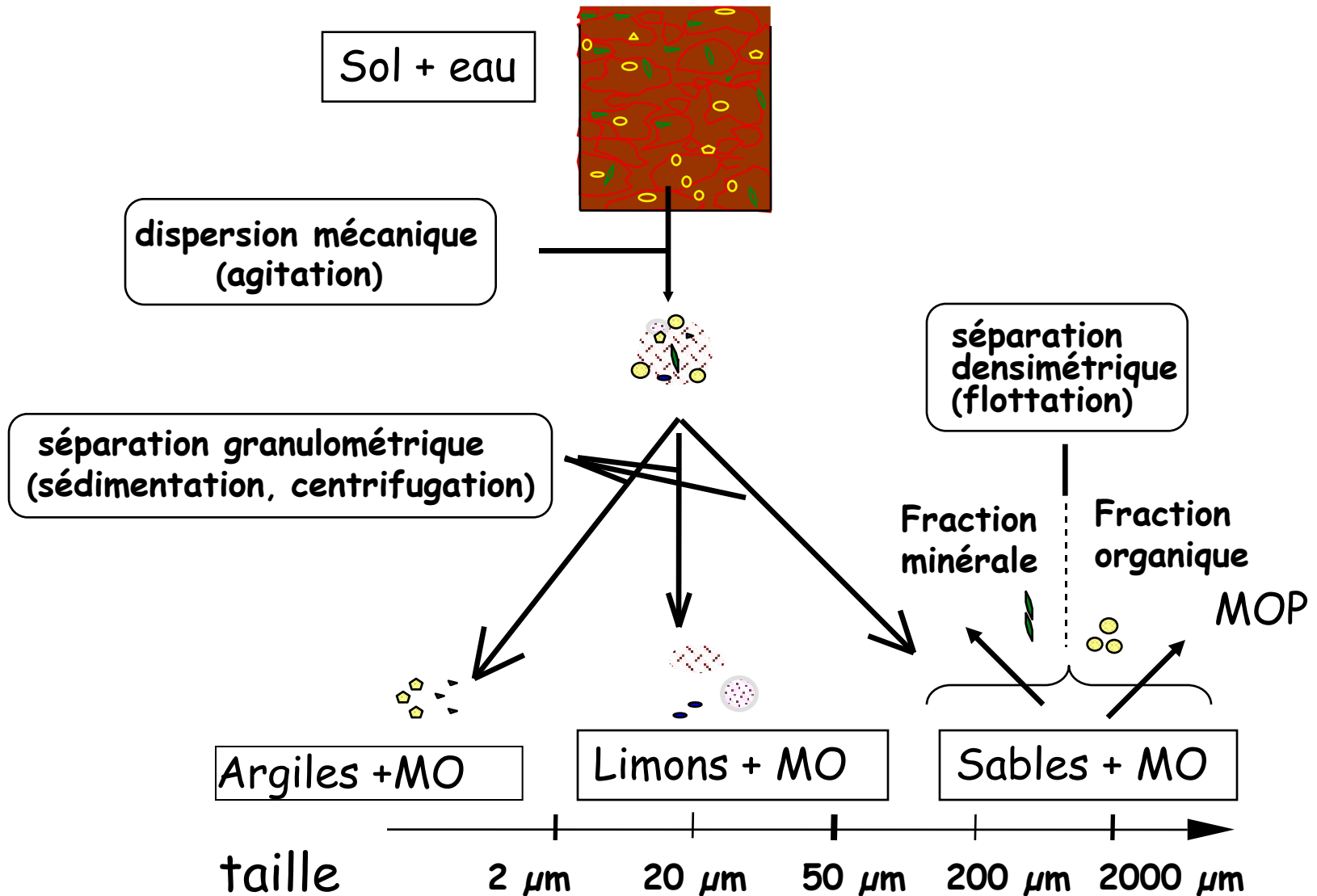
DNA ("*fingerprint*") of bacterial communities



Numerical analysis of profiles -  
Principal Component Analysis (PCA)



# Fractionnement granulo-densimétrique



# Impact du Cuivre sur la structure des communautés microbiennes dans les compartiments structuraux.

Fractions fines

10<sup>-2</sup>

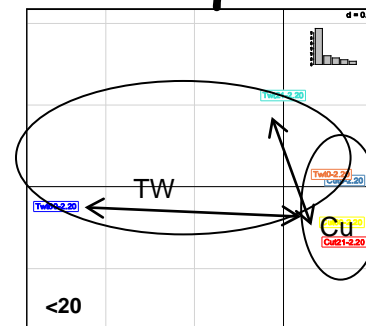
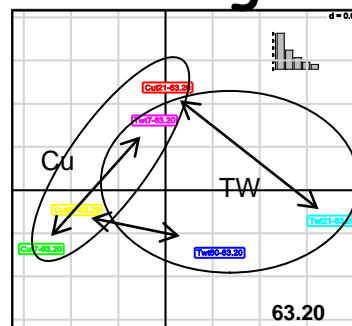
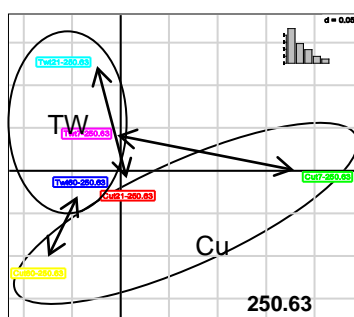
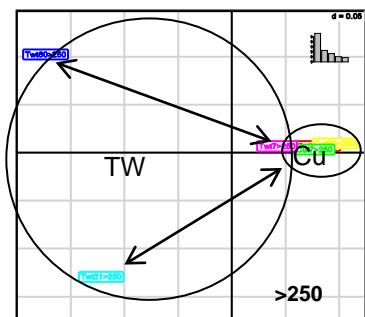
Différence de structure initiale de communautés dans les fractions et le sol total.

→ impact différent du Cuivre ?

## Partie Lionel Ranjard

### Biodiversité microbienne dans les fractions granulométriques

Impact Cu NA



Impact Cu CC

