# Significance of soil algal and cyanobacterial communities, as a model for the pesticide risk assessment.

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INRA SCIENCE & IMPACT

**Pesticide Behaviour in Soils, Water and Air – York 2017**

# INTRODUCTION

## Pesticides in the environment

➲ **Environmental pesticide contamination and ecological awareness**

- **Very high and diverse compounds** (fungicides, herbicides, insecticides, etc . . .)
- **Remanence and transfer** => local and diffuse environmental contamination
- **Side-effects on biodiversity => ecosystem functioning**

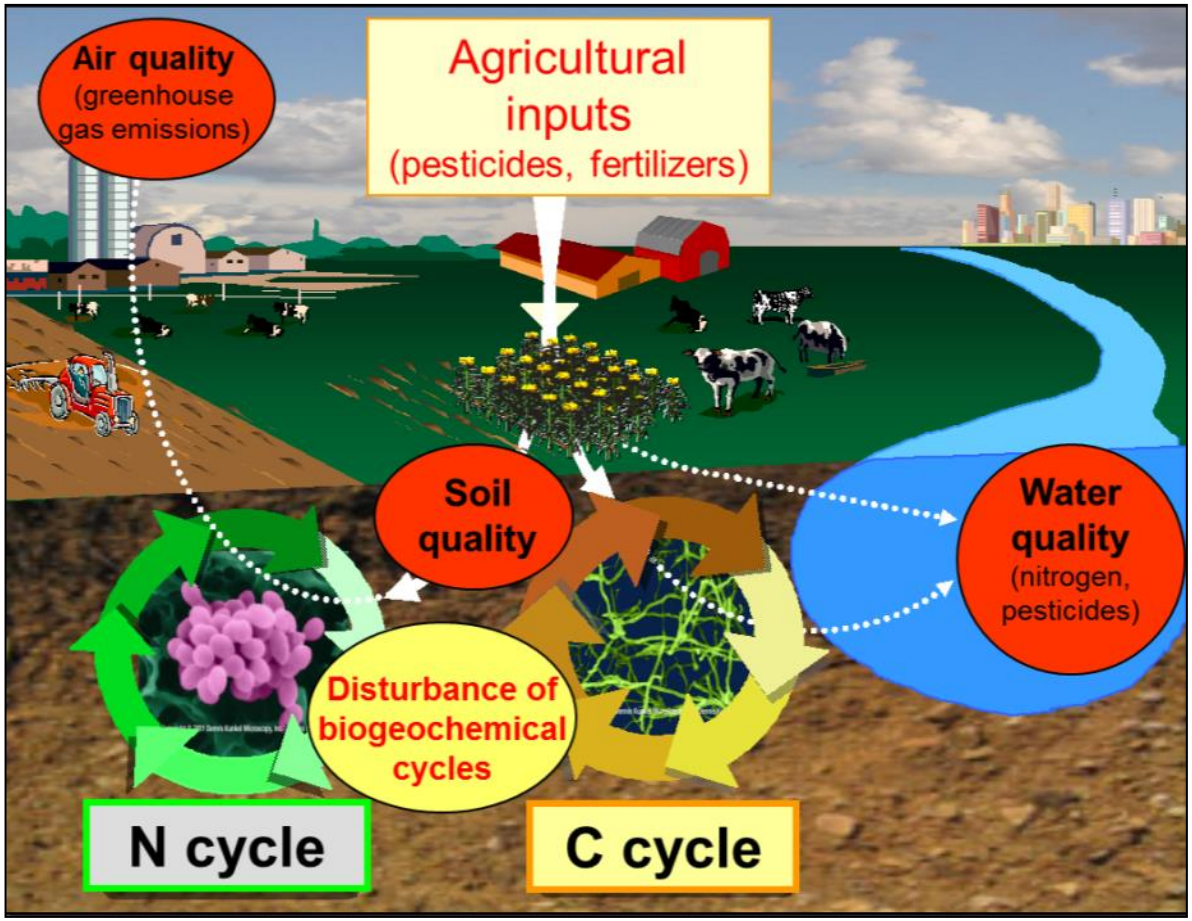
➲ **European and national frameworks to decrease the environmental risk**

- *PAN Europe => Strategy on the sustainable use of pesticides ; (EC) No 1107/2009*
- *Ecophyto French project (2010 – 2018)*

- Develop innovative farming practices & alternative pest management strategies
- Secure and prevent the risks (renewal of approval of many products)
- **Improve Risk Assessment processes** (Regulatory & legislative policies)
  - Relevant exposure routes (EFSA, 2016)
  - Effects on biodiversity & functions (EFSA, 2016),
  - Recovery Time (EFSA, 2017)

# INTRODUCTION

## Significance of microbial processes for environmental quality




**Microbial communities**

**FONCTIONS / PROCESSES**

**SERVICES**

- Nutrient recycling
- Soil buffering
- C storage
- …

# SCIENTIFIC BACKGROUND

## Photosynthetic microorganisms in agricultural soils

➲ **What can we expect and learn from soil algae and cyanobacetria, as indicators of herbicide impacts on soil functioning ?**

# SCIENTIFIC BACKGROUND

## Photosynthetic microorganisms in agricultural soils

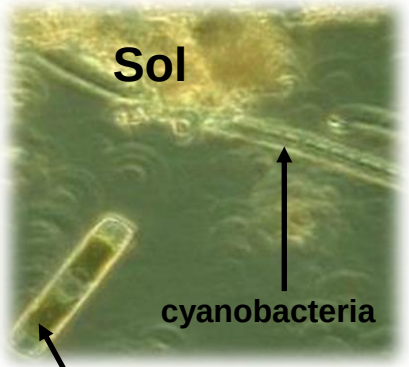
➲ **Why soil photosynthetic microorganisms ?**

- Various metabolic pathways could be disrupt by herbicides
- First soil microbial interface receiving pesticides
- Many knowledges from aquatic systems on direct and indirect impacts

Expected as very sensitives

➔ ***Impacted at low herbicide concentrations***

(Crouzet et al., 2013, in revision
Bérard et al. 2004; Joly et al., 2015)




**Algal & cyanobacterial biofilms**

# SCIENTIFIC BACKGROUND

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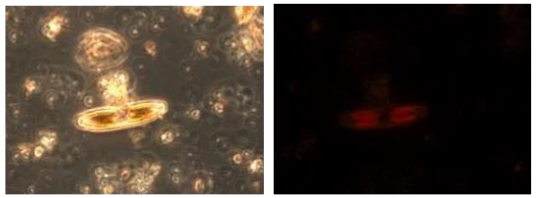
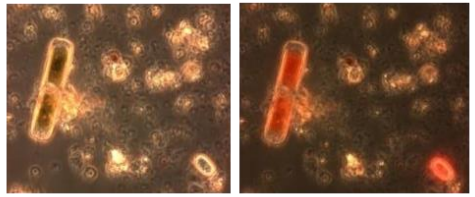
➲ **An unrecognized abundance and diversity of soil algae and cyanobacteria**

- present in all temperate agricultural soils
- numerous trophic strategies

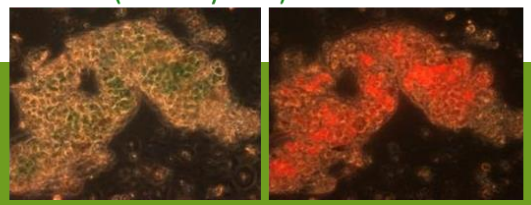
(Metting, 1981; Pipe & Schubert, 1984; Hoffman, 1989; Bérard et al., 2005; Zancan et al., 2006; Reisser, 2007 ; Davis et al., 2013)

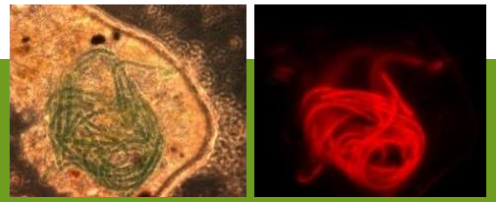
**Diatoms, chlorophyceae, xanthophyceae,** eustigmatophyceae, etc… (Eukaryotic algae)



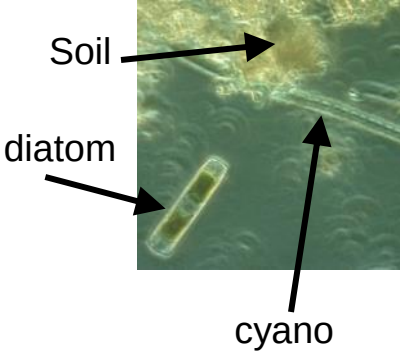


**Cyanobacteria** (Prokaryotes)





# SCIENTIFIC BACKGROUND

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➲ **Influence on soil processes**

- Soil surface aggregate stability (Bailey et al. 1973; De Caire et al. 1997; Crouzet et al., in revision)
- 5 – 10 % of microbial production and C storage (Shimmel and Darley 1985; Reisser 2007)
- $N_2$ fixation by cyanobacteria (Wegener et al. 1985; Pardo et al. 2009)

# Current issues & Objectives

- **Methods & descriptors to study soil microbial photosynthetic microorganisms.**

  Suitable bioassay for soil algae -> field sampling strategies

  Biochemical and genetic descriptors for structural endpoints

  Development of functional approaches *(e.g. photosynthetic activity)*

- **Effects of herbicides at soil algal and cyanobacterial communities ?**

  Identifying suitable indicators of exposure or impact of herbicide :

  - biochemical / molecular taxonomic signatures
  - herbicide community tolerance acquisition (PICT)

- **To what extent herbicide-related communities shifts could induce changes in their functions** (soil aggregation, C fluxes) **?**

# Field experiments (long-term and low dose effects)

➢ **Comparative approach of Long-term cropping systems** (loamy soil, Versailles)

***ORGANIC***

**Winter wheat,**
Alfalfa, pea

No amendment
mechanical weed
control,

≠ **chemical inputs** (**pesticides**, fertilizers), and some ≠ in rotation and soil tillage

***CONVENTIONAL***

**Winter wheat**,
rapeseed, pea

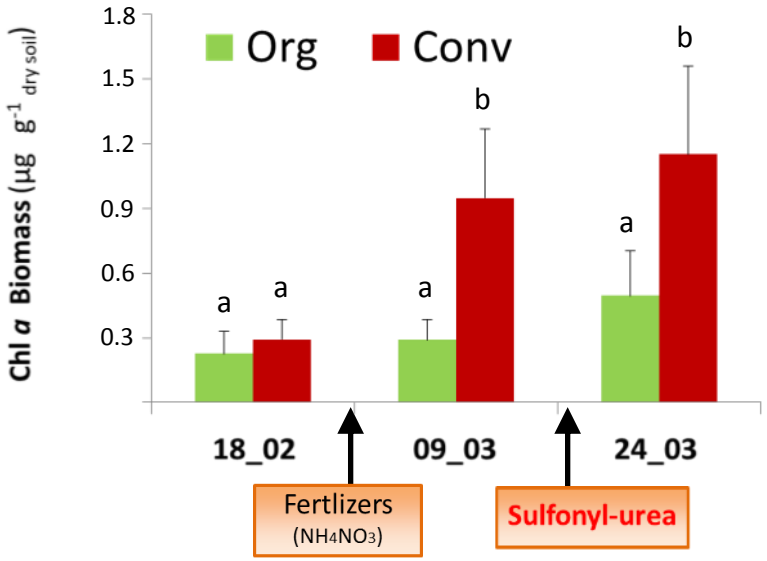
**Fertlizers (N, P, K),**

**Pesticides (herbicides, fungicides, insecticides)**

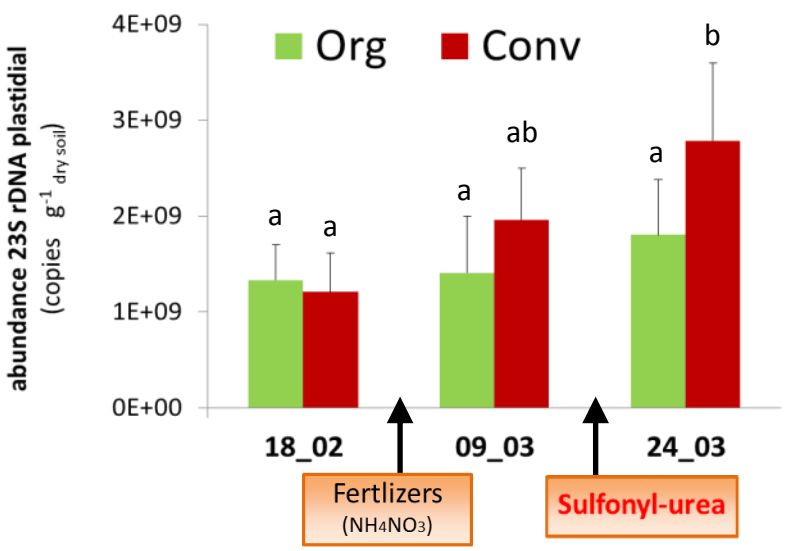
# Field experiments

➢ **Biomass & Abundances of algae & cyanobacetria : CONV *vs.* ORG**

**Chl-*a* Biomass** (spectrophotometry)




**Plastidial 23S rDNA abundance** (qPCR)




➔ **Higher microbial photosynthetic biomass in conventional cropping system**

- Fertilizers -> favour the growth of algae and cyanobacteria in **CONV soils**
- Higher frequency of soil tillage for weeding, limit their growth in **ORG soils**

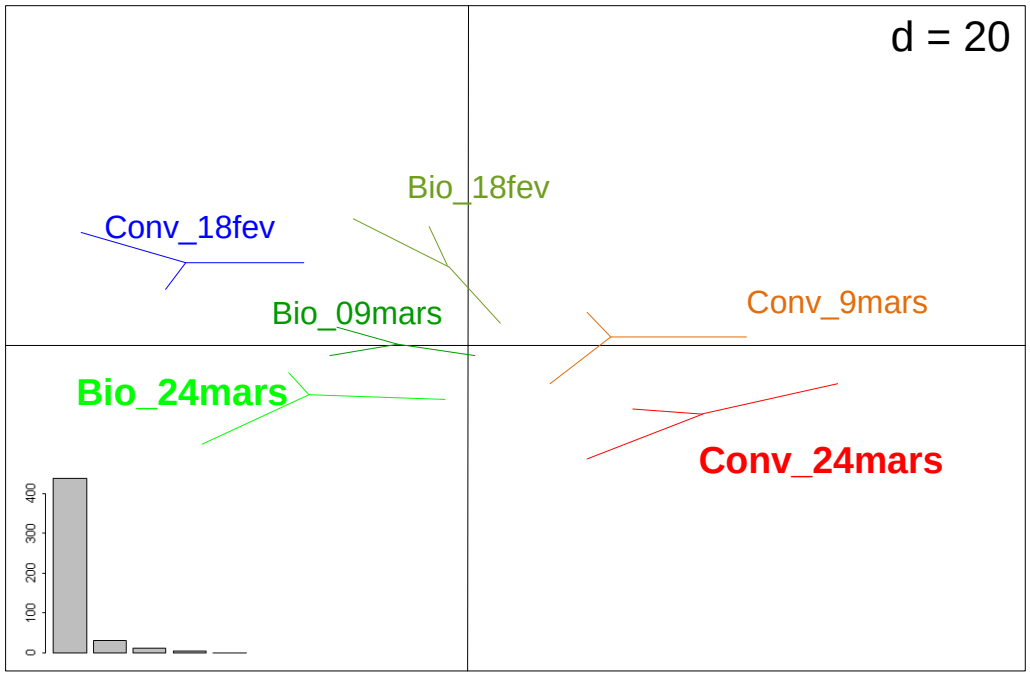
➔ **No evidence of sulfonyl-urea herbicide effect** (single event) **on these endpoints.**

# Field experiments

➢ **Community structure and Diversity : CONV** *vs.* **ORG**

➔ **All photosynthetic microorganisms -> 23S rDNA plastidial**

**Temporal shift of the genetic structure of soil photosynthetic microorganisms**



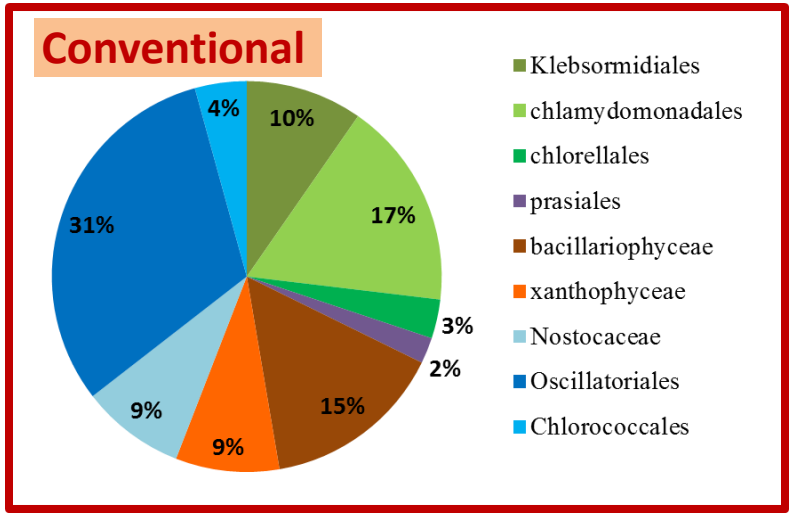
# Field experiments

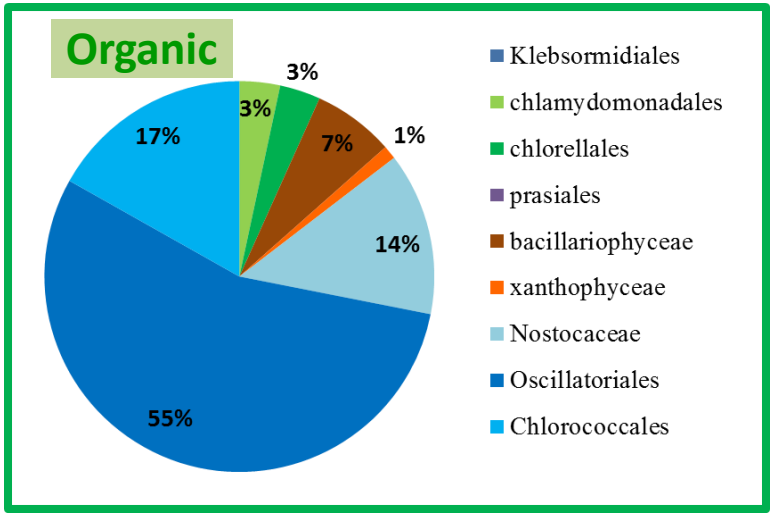
➢ **Community structure and Diversity : CONV** *vs.* **ORG**

➔ **All photosynthetic microorganisms -> 23S rDNA plastidial**

***H'*** **2,43**

***E*** **0,85**




***H'*** **1, 71**

***E*** **0,63**

⇒ **Strong differences at the middle spring** (after herbicide treatments) **:**

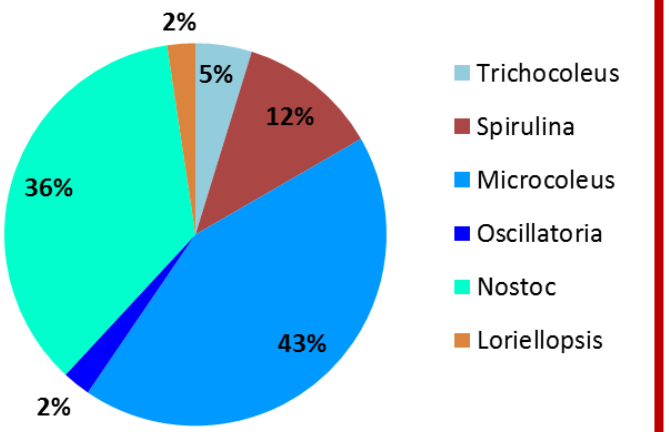
- Higher diversity and Eveness indices (H' and E) in soils from **Conventional system**
- Cyanobacteria dominated in soils from **Organic system**

# Field experiments

➢ **Community structure and Diversity : CONV** *vs.* **ORG**

➔ **Focus on cyanobacterial community -> 16S rDNA**

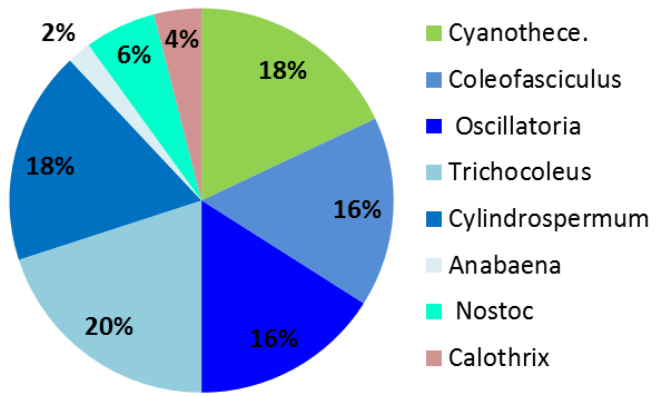
**Conventional**




***H'*** **1,51**

***E*** **0,71**

**Organic**




***H'*** **2,12**

***E*** **0,89**

⇒ **What is different in cyanobacetrial communities ?**

Higher diversity index (H') and Eveness in soils from **Organic system**

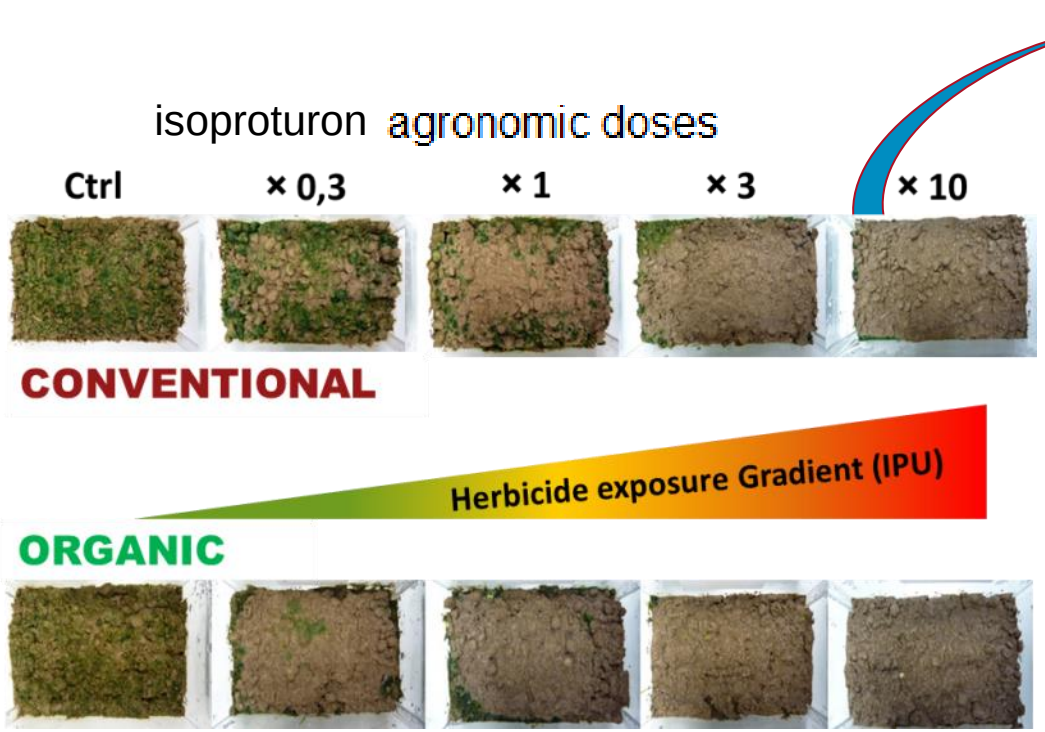
*Microcoleus* and *Nostoc* dominated soils from **Conventional system**

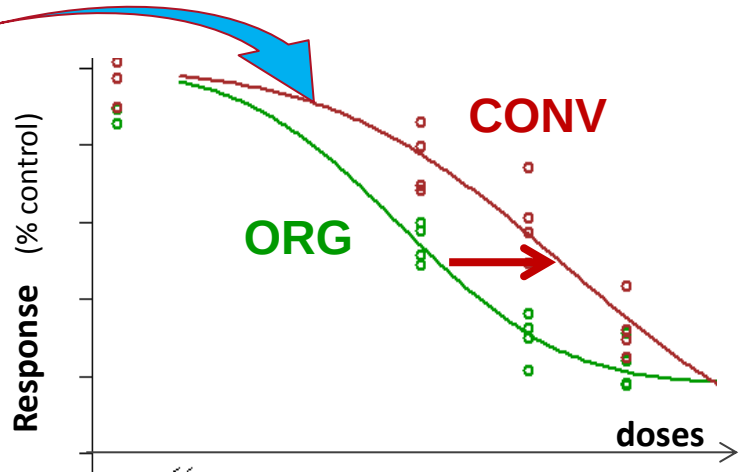
# Field experiments

➢ **Pollution Community Tolerance acquisition** (PICT assay).

✓ ***Specificity towards a contaminant*** *(or a mode of action)*

✓ ***Robustness to confounding factors***




**Higher $CE_{50}$ for soil communities from Conv system**

⇒ ***Tolerance to Isoproturon (Matin EL ®)***

⇒ *No tolerance sulfonyl-urea herbicide*

# Lab microcosm experiments

➢ **Functional significance for aggregate stability in cropped soils:**

✓ Indigenous soil algae and cyanobacteria influence soil aggregate stability

➲ **Does herbicide disturb functional roles of soil algae and cyanobacteria ?**

**Field soil aggregates**
**Ø : [3 – 5mm]**

**Photoperiod 16:8**

**Photoperiod 16:8**
***+ herbicide IPU***

**Dark**

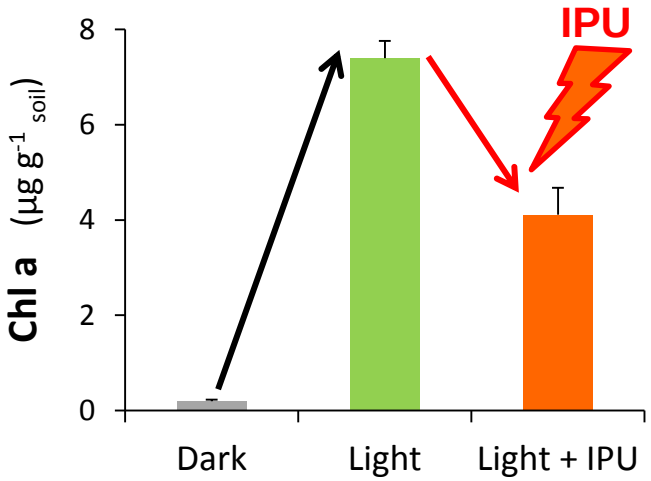
**20°C, 80% WHC**
**50 days**

⇒***Analysis:*** *- Chl a biomass, pigments*
*- Total microbial biomass*
*- Aggregate stability test*

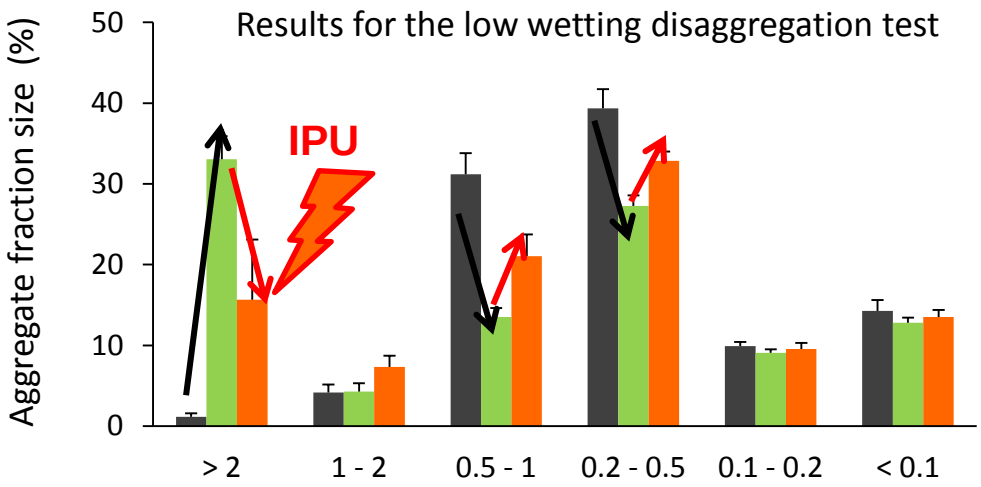
# Lab microcosm experiments

➢ **Functional significance for aggregate stability in cropped soils:**

**Chl-*a* Biomass**

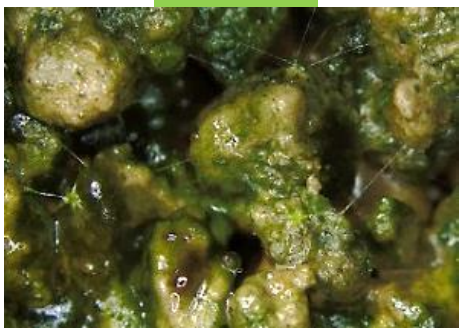



**Structural stability of soil aggregates**



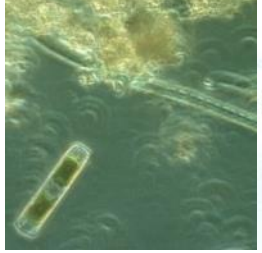

**Dark**


**Light**



**Light + IPU**

# Conclusion & Perspectives



## Indicators of herbicide impact

- **Soil algae and cyanobacteria appear as more sensitive to herbicides than other microbial communities, in agricultural soils** *(Crouzet et al., 2010 ; 2013 ; Joly et al., 2015)*
  - ⇒ NOEC and LOEC detected at herbicide doses lower than agricultural application rates
- **Proof of concept of an "in-soil" herbicide – PICT assay** *(Crouzet et al, in prep)*
- **Advances in genetic diversity analysis of algae and cyanobacteria in agricultural soils** *(Bérard et al., 2005; Davies et al., 2013; Crouzet et al, in prep)*

## Further insights into the community ecotoxicology

- **Greater investigations on responses of algal and cyanobacterial diversity to herbicides:**
  - Acquiring dataset across different field experiments, in order to hierarchize environmental and agricultural driving factors to highlight taxonomic signatures at the community level in relation with herbicide gradients.
- **Functional trait approach on algae and cyanobacteria**

Thanks to technical staff and students:

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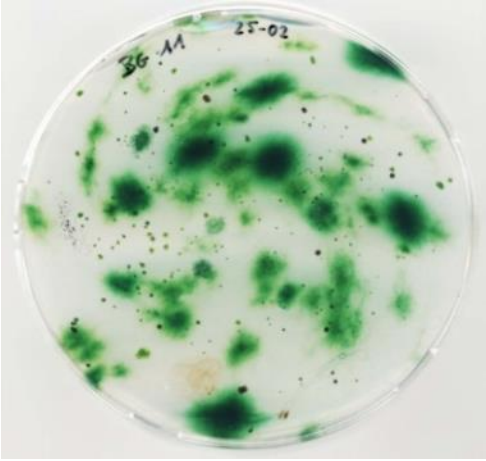
And INRA Experimental Farm Unit




# Thanks for your Attention

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