

Assessment of ecotoxicological impact of pesticides on soil microbial functioning in agroecosystems: how to define suitable indicators and for what purposes?



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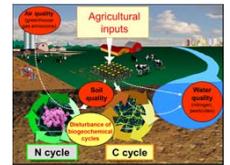
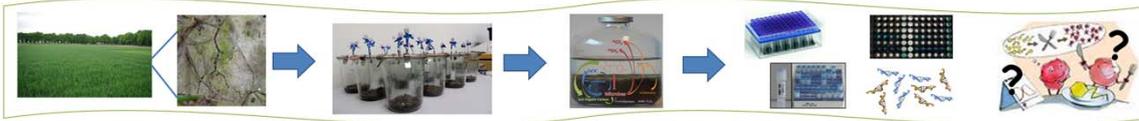


Background

- **Microbial communities are key actors for soil processes and services** (Costanza et al. 1997, Kibblewhite et al., 2008; MEA, 2005) MILLENNIUM ECOSYSTEM ASSESSMENT
Total bacterial / fungal communities & functional groups => => nutrient recycling, water filtration, pathogen regulation, biomass production
- **European directive in Pesticide risk assessment: Develop specific protection goals in relation to biodiversity and ecosystem services** (EFSA, 2016).
- **Yet, only two standardised tests for pesticide homologation** (91/414/CEE): *C and N mineralization test [OCDE 216 & 217]* & *Pesticide biodegradation [OCDE 301B]*
→ Lack of understanding of pesticide impacts on diversity – function relationships !

OBJECTIVES:

- ⇒ **Testing the relevance of original microbial descriptors** (biochemical and molecular tools) **in comparison with these regulatory tests**
- ⇒ **Target microbial function ensuring regulation services** : Nutrient recycling, Pesticide degradation



Microcosm Experiments and microbial descriptors

- **Selection of three soil types** (under similar cropping systems with low chemical inputs)
=> sandy (DBZ), loamy (La Cage) and clay (Chambère) soils

→ Pesticide selection

- Agricultural chemical input survey : database on actual exposure on the field
- Selection of some model compounds by using TyPol method (Servien et al., 2014)
=> isoproturon, tebuconazole, chlorpyrifos

→ a priori assessment by microcosm approach (Tier II)

- Compound tested alone and in mixture
- Initial contamination at the same dose (5 µg / g_{sol-sec}); Incubation at 25°C and 80% WHC
- Sampling days : 4 (initial effect) and 28 (residual effect)

→ Microbial descriptors

	Standardized descriptors	Original / innovative descriptors (standardization in progress)	
Nutrient recycling	Carbon mineralization (OECD test 216, with acetate)	Extracellular enzyme (AEE) Catabolic diversité	qPCR Quantification of microbial phyla
Nitrogen transformation	Potential N (NH4+) mineralization	Potential Nitrification Potential Denitrification	qPCR quantification of functional genes - N-cycling - pesticide degraders
Pesticide degradation	Modified Test Sturm (kinetic mineralization)		

AEE involving in biogeochemical cycles: β-Glucosidase, phosphatase, arylsulfatase, arylamidase, urease
qPCR on bacterial phyla (16S rDNA): α-, β-, γ-proteobacteria, planctomycètes, actinobacteria, firmicutes, acidobacteria, bacteroidetes, verrucomicrobia, gemmatimonadetes

Results on pesticide impact on communities structure and function

- **Identification of the most sensitive descriptors (short-term: T+4j ; and middle term: T+28j)** (2 way ANOVA, (soil * treatment) at each date

→ Acetate, 2,4-D and Glyphosate Mineralization (V max and cumulated mineralization (%)):

	T +4j						T +28j					
	Acé v _{max}	ACE A65	24D v _{max}	24D A65	Gly V _{max}	GlyA65	Acé v _{max}	ACE A65	24D v _{max}	24D A65	Gly V _{max}	GlyA65
Sol	< 0.0001	< 0.0001	0.313	< 0.01	< 0.0001	< 0.0001	0.087	< 0.0001	0.0008	< 0.0001	< 0.0001	< 0.0001
traitement	< 0.0001	< 0.0001	< 0.01	< 0.001	0.595	0.084	< 0.0001	0.001	< 0.0001	0.736	0.433	0.497
Sol * traitement	0.025	0.215	0.003	< 0.01	0.531	0.042	0.268	0.335	0.626	0.122	0.377	0.510

❖ Mainly Short-term effect => transitory impacts

❖ Strong Soil type effects

❖ Higher sensitivity of « Functional » descriptors (microbial activities, e.g. nitrification, minéralisation des pesticides) compared to molecular descriptors (qPCR on bacterial phylum)

❖ The most sensitive descriptors (impacted in all soils at days 4 and 28):

- => V max of 2,4-D mineralization (V_{max} 2,4-D)
- => Potential Nitrification (PNA)

→ Enzym activities (AEE) & potential nitrification (PNA):

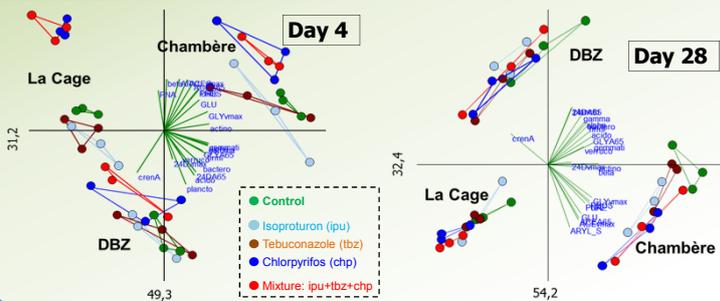
	T +4j				
	Phos	Glu	Aryl-S	Urease	PNA
Sol	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
traitement	< 0.0001	0.01	0.007	0.0004	< 0.0001
Sol * traitement	0.0007	0.012	0.002	0.170	< 0.0001

	T +28j				
	Phos	Glu	Aryl-S	Urease	PNA
Sol	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
traitement	0.0005	0.0036	0.813	0.650	< 0.0001
Sol * traitement	0.0067	< 0.0001	0.0015	0.0008	0.0055

→ qPCR abundances of bacterial phyla (% of total 16S rDNA):

- => Some significant differences in several soils at day 4... BUT no effect recorded at day 28.
- => β-proteobacteria increased while Actinobacteria decreased in all soils, 4 days after Chlorpyrifos and Mixture treatments.

- **Overall responses of soil microbial communities to pesticides: short-term (4 d) and residual (28 d) impact** (ACP, correlation matrices)



❖ Day 4: impact of chlorpyrifos et mixture >> impact isoproturon ≈ tebuconazole

❖ Microbial communities from soils with higher pH (Chambère, clay soil, pH 7.8, and DBZ, sandy soil, pH 8.2) were more resistant than those of the soil from La Cage (loamy soil, pH 6.9)

❖ Resilience of microbial communities from La Cage soil at day 28

❖ Further analyses for sensitivity: Hierarchization / ranking of indicators ?
robustness: confounding environmental factors ?

Conclusions & Perspectives

- ❖ Suitability of innovative descriptors at community level were identified, but referential data (Normal Operating Range) are required to interpret pesticide impacts.
- ❖ Currently, the regulation considers that 25 % of change in microbial processes is acceptable ... **BUT is not too high in a global change context ?**
→ **Need for a greater consideration of community resilience to pesticide effects.** (-> define tolerance thresholds).
- ❖ Develop methodologies to assess soil functions and services, on the basis of ecosystem properties (e.g. physico-chemical, biodiversity).
=> **It will improve multicriteria assessment of cropping system effects on soil services.**



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