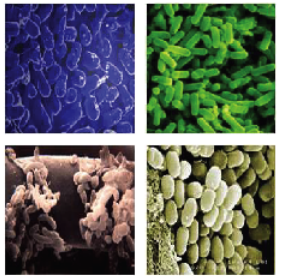
How pesticides are affecting soil microorganisms?

F Martin-Laurent

UMR 1347 Agroécologie, INRA Dijon, France

Soil: a unique reservoir of microbial diversity exposed to multiple stresses

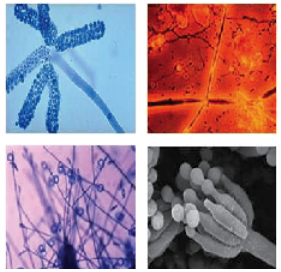
The soil :
-reservoir of microbial diversity
- natural patrimony



1 billion of bacteria
1 million of species

1.500 t/ha

1 g of soil



1 million of fungi
10 000 to 100 000 species

3.500 t/ha

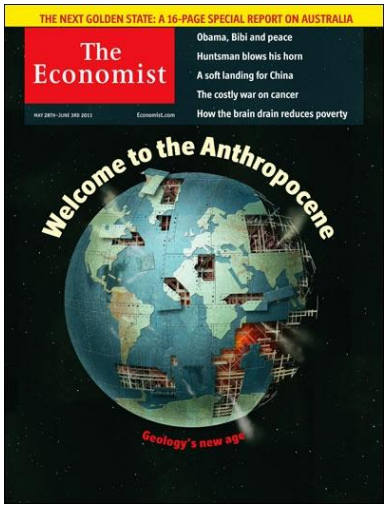
MILLENNIUM ECOSYSTEM ASSESSMENT

Microbiota = key driver of soil ecosystemic services

Quality of the environment
(human health and primary production)

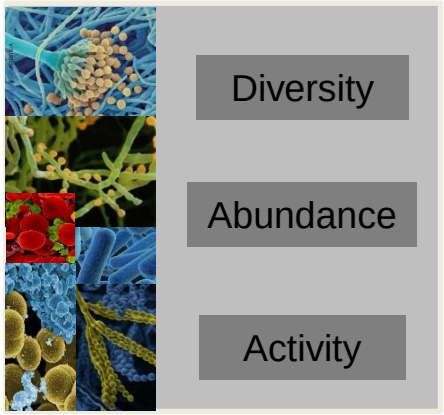
Soil ecosystemic services under ‘anthropic pressures’

Soil usage



Global change:
-climate change,
-anthropocene era,…

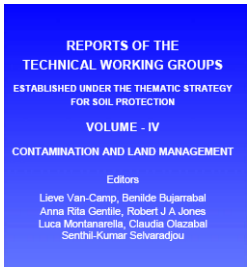
Agricultural practices



Soil microorganisms

Soil ecosystemic services

Soil microorganisms 'the poor parent from the regulatory point of view'



EUROPEAN COMMISSION DIRECTORATE-GENERAL Joint Research Centre ies

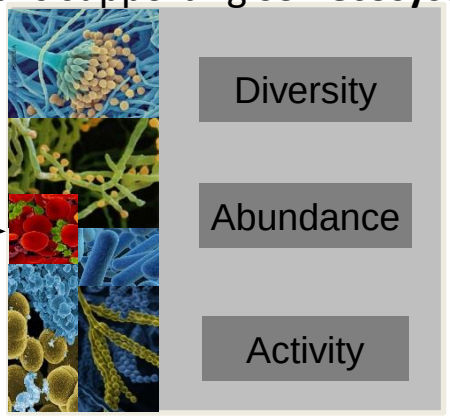
No regulatory requirements for *post-registration* assessment of pesticides effect on soil microorganisms in the absence of soil protection directive (proposed in 2006 to European Parliament)

- EU-regulation 1107/2009 for the authorization of pesticides: *pre-registration* evaluation of environmental risk of pesticides comprising soil ecotoxicity measurement:
- Impact on soil microorganisms: mineralization of nitrogen [OECD 216] and carbon [OECD 217]
- *(Biodegradability of pesticides: modified test of Stürm [OCDE 301])*

UNION EUROPÉENNE

- Global tests not sensitive enough to estimate the ecotoxicological impact of pesticides on microbial communities and functions supporting **soil ecosystemic services**

Pesticides



Soil microorganisms

Soil ecosystemic services

Martin-Laurent et al. (2013) Environ. Sci. Poll. Res. 20: 1203-1205

Towards a revision of the EU-regulation 1107/2009 to better protect soils

2010

SCIENTIFIC OPINION

Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002)[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

2016

Recovery in environmental risk assessments at EFSA

EFSA Scientific Committee

2017

1 DRAFT SCIENTIFIC OPINION

2 **Scientific Opinion addressing the state of the science on risk assessment**
3 **of plant protection products for in-soil organisms**[1]

4 EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]

5 European Food Safety Authority (EFSA), Parma, Italy

▷ **EFSA proposed:**
- **'soil ecosystemic services'** as specific protection goals for ERA of pesticides,
- '**microorganisms**' as key factors to be protected (at the 'functional group' scale)

▷ **EFSA proposed:**
- the '**ecological recovery**' concept for ERA of pesticides,
- '**normal operating range**' for each key factor to be protected (including soil microorganisms)

▷ **EFSA proposed:**
- A series of stantardized methods for pesticide risk assessment on in soil living non-target organisms (**for microbes**: cycle of nitrogen and endomycorhizal fungi)

Storck et al. (2017) Sci. Tot. Environ. 575: 1027-1033

Open challenges for pesticide risk assessment on soil microorganisms and supported ecosystemic services

-Which **microbial function(s)** and corresponding proxies ?

-For a given function (nutrient cycling or water purification) which attribute(s) to monitor (**function, diversity, abundance**) by targeting **functional communities** (in the C and N cycles and biodegradation of pesticides)?

-Need to have basic knowledge (genes and enzymes involved)

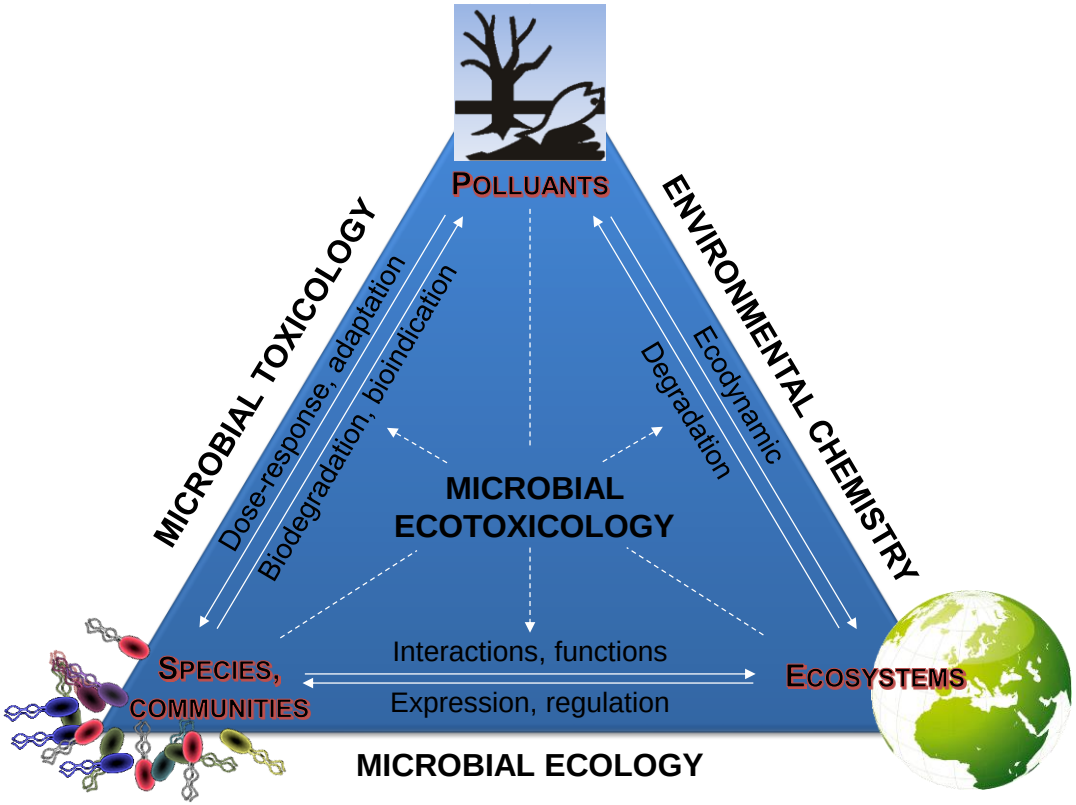
-Define the **normal operating range (NOR)** of the attributes measured in a given context? Influence of global change on NOR?

-Choose the experimental conditions to test the impact of a given stressor (i) *a priori* ERA: compound by compound and (ii) *a posteriori :* ERA multi-contamination (i.e. anthropocene)?

-Direct and indirect effect on non-target organisms ?

-…. (not exhaustive list of challenges)

Microbial ecotoxicology an emerging science for a better pesticide risk assessment on soil microorganisms



(i) Impacts of pesticides on microorganisms and microbial functions

(ii) Role of microorganisms and of microbial functions on the fate of pesticides

Environ Sci Pollut Res (2016) 23:3981–3983
DOI 10.1007/s11356-015-5763-1

EDITORIAL

Microbial ecotoxicology: an emerging discipline facing contemporary environmental threats

Jean-François Ghiglione[1] · Fabrice Martin-Laurent[2] · Stéphane Pesce[3]

RTP EcotoxicoMic

http://www.ecotoxicomic.fr/fr_FR/

https://fr.wikipedia.org/wiki/%C3%89cotoxicologie_microbienne

Example of advanced researches on RA of pesticides on soil microorganisms

pesticide application to agricultural soil

?

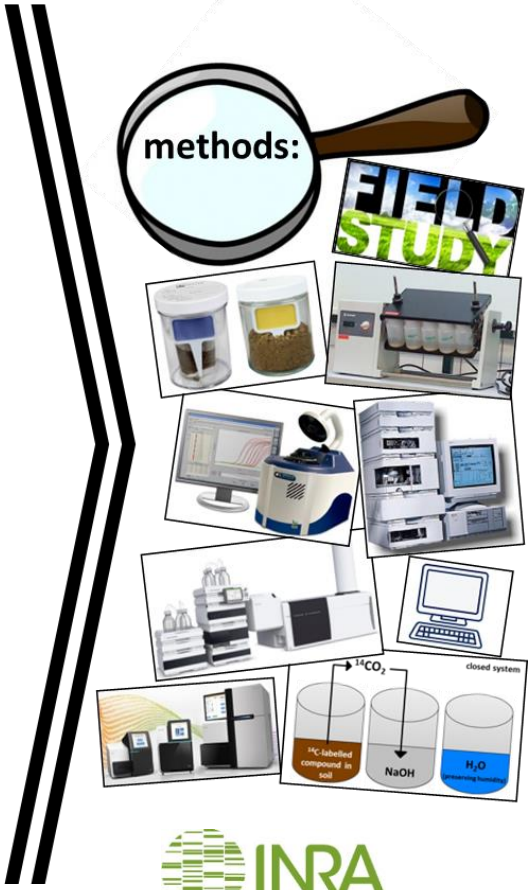
'LOVE'

- adaptation
- degradation

'HATE'

- toxicity

soil microbial communities



ECOFUN-MICROBIODIV: an FP7 European project for developing and evaluating innovative tools for assessing the impact of pesticides on soil functional microbial diversity—towards new pesticide registration regulation?

Fabrice Martin-Laurent · Ellen Kandeler · Ines Petric · Simonida Djuric · Dimitrios G. Karpouzas

INRA SCIENCE & IMPACT

University of Thessaly

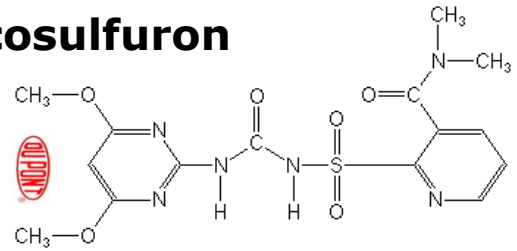
UNIVERSITÄT HOHENHEIM

Lab-to-Field set-up: *field experiment (Tier II)*

- Novi Sad, Serbia, June to October 2011.
- full randomized block pattern (6m x 5m)
- 5 repeats per treatment
- 3 treatments : x1, x2, x5 of the recommended agronomic dose (80 g a.i. ha^{-1})
- control plots
- **Tier II for toxicity assessment:** representing realistic exposure scenario

Zea mays variety NS640

Test compound – sulfonylurea herbicide Nicosulfuron



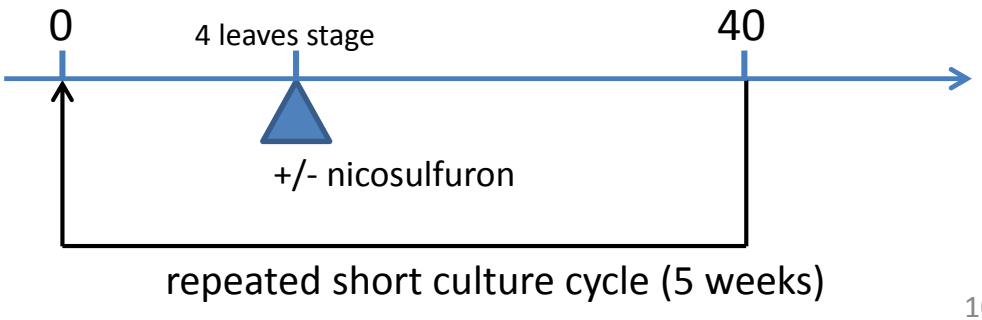
50-100 g of ai /ha

- ✓ used for the post-emergence control of annual grass and broad-leaf weeds in maize
- ✓ application rate 10-1000 times lower than conventional herbicides
- ✓ inhibition of the *actohydroxyacid synthase* (AHAS) → biosynthesis of valine, leucine and isoleucine (branched chain amino acids)

Lab-to-Field set-up: *lab experiment (Tier I)*

- Dijon, France, under controlled conditions

- 5 repeats per treatment
- 3 treatments: x10, x100, x1000 of the recommended agronomic dose (80 g a.i. ha^{-1})
- control pots
- **Tier I for toxicity assessment** – under extreme long-term exposure scheme
- 5 culture cycles



Pesticide toxicity for soil microorganisms - methodology

Testing pertinence of **existing ISO standards** for estimating pesticide impact on soil microbial communities

Methods to study the abundance (ISO14240:2), diversity (ISO/TS29843-1) and activity (ISO/TS 22939) of soil microorganisms

Methods targeting the microorganisms (based on direct soil DNA extraction (ISO 11063) and further PCR analyses)

- **qPCR analysis** assesing abundance of the main microbial groups supporting ecosystem functions (*ISO 17601*)
- **NGS analysis** (16S rRNA and AHAS amplicons)

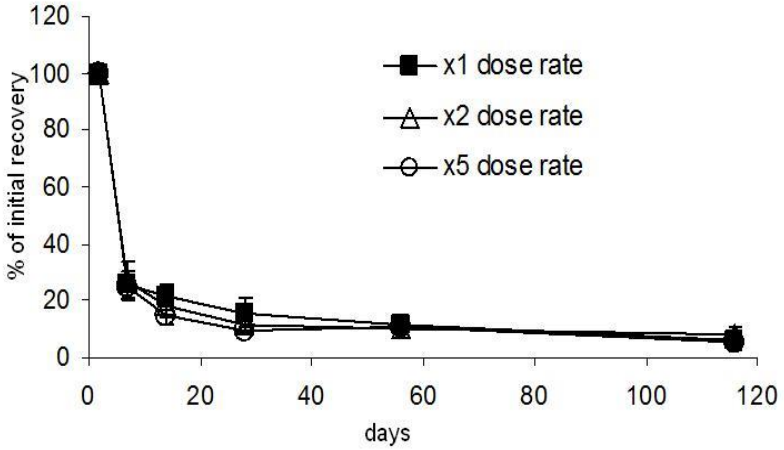
Develop, test and **propose new methods** for estimating pesticide impact of soil microbial communities for standardization

Methods targeting **specific microbial groups**

- **Arbuscular mycorrhizal fungi :** root colonization and composition of AM fungal community
- **Nitrogen cycle**: abundance and diversity of nitrifyers
- **Nicosulfuron-tolerant bacterial strains**: abundance, diversity and activity (culturable)

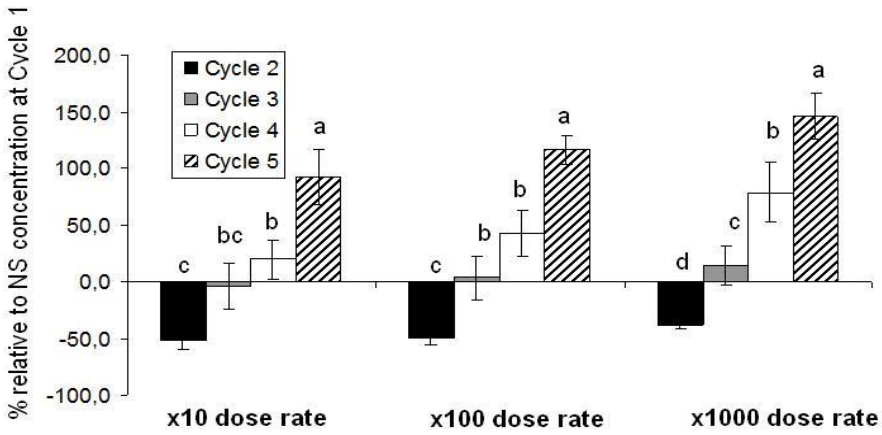
Contrasted scenarii of exposure of soil microorganisms to nicosulfuron

Tier II (*field experiment*): dissipation



Model	Parameters	Nicosulfuron dose rates		
		x1	x2	x5
First order kinetics	$t_{1/2}$ (days)	4.7	4.0	4.5
	r^2	1.000	0.956	0.944
	χ^2 (%)	25.1	19.8	21
Hockey stick	$t_{1/2}$ (days)	3.6	3.5	3.7
	r^2	0.999	0.998	0.998
	χ^2 (%)	29.9	26.4	29.1

Tier I (*lab experiment*): accumulation



➡ **Rapid dissipation of nicosulfuron under *TierII* scenario**: nicosulfuron half-life comprised between 4.0 and 4.7 days

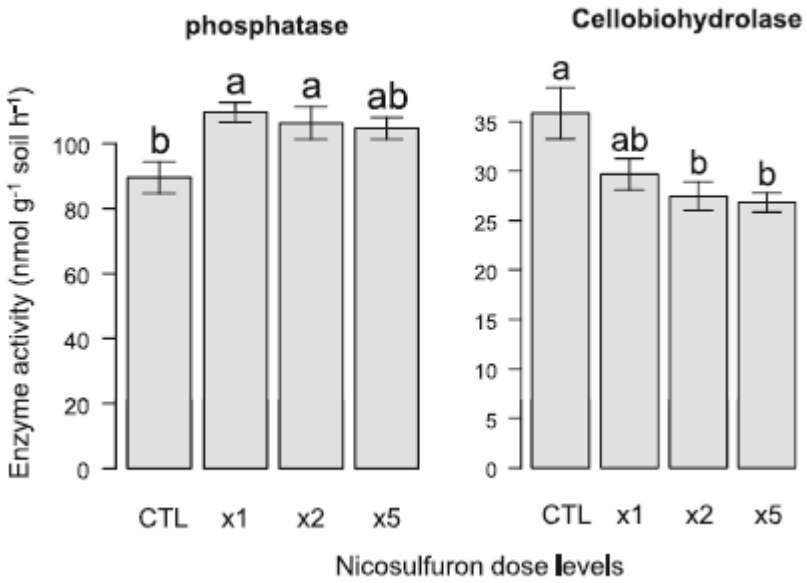
➡ **Accumulation of nicosulfuron in *TierI* scenario**

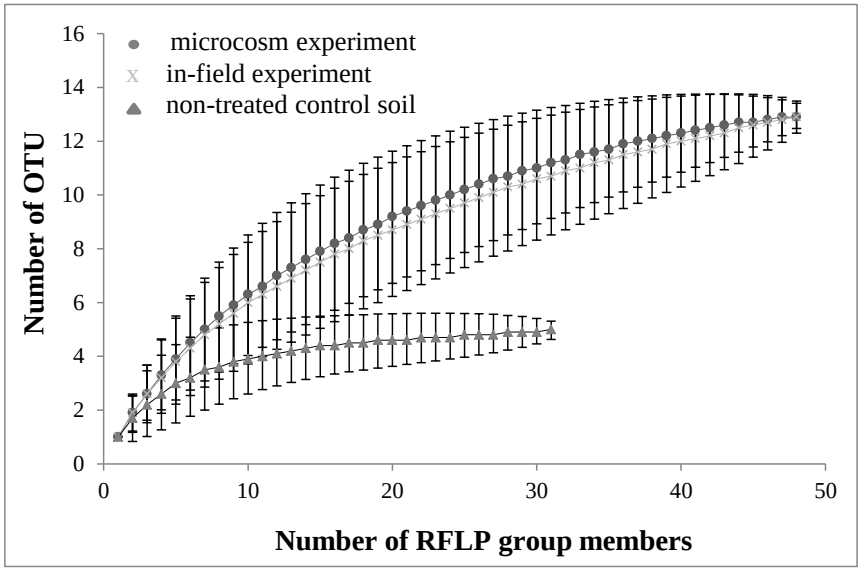
Effects of nicosulfuron on the abundance and diversity of arbuscular mycorrhizal fungi used as indicators of pesticide soil microbial toxicity

D.G. Karpouzas[a,*], E. Papadopoulou[a,b], I. Ipsilantis[c], I. Friedel[d], I. Petric[e], N. Udikovic-Kolic[e], S. Djuric[f], E. Kandeler[g], U. Menkissoglu-Spiroudi[b], F. Martin-Laurent[d]

Ecological Indicators 39 (2014) 44–53

TierII: estimation of the ecotoxicological impact of nicosulfuron





Following nicosulfuron exposure:
- significant increase in phosphatase activity,
-significant decrease in cellobiohydrolase.

-Transitory effect (resilience after 50 days)

Following nicosulfuron exposure :
- significant increase in the abundance and diversity of bacteria resistant to nicosulfuron (i.e. harboring AHAS gene non-sensitive to nicosulfuron)

A tiered assessment approach based on standardized methods to estimate the impact of nicosulfuron on the abundance and function of the soil microbial community Soil Biology & Biochemistry 75 (2014) 282–291

D.G. Karpouzas [a], E. Kandeler [b], D. Bru [c], I. Friedel [c], Y. Auer [b], S. Kramer [b], S. Vasileiadis [d], I. Petric [e], N. Udikovic-Kolic [e], S. Djuric [f], F. Martin-Laurent [c,*]

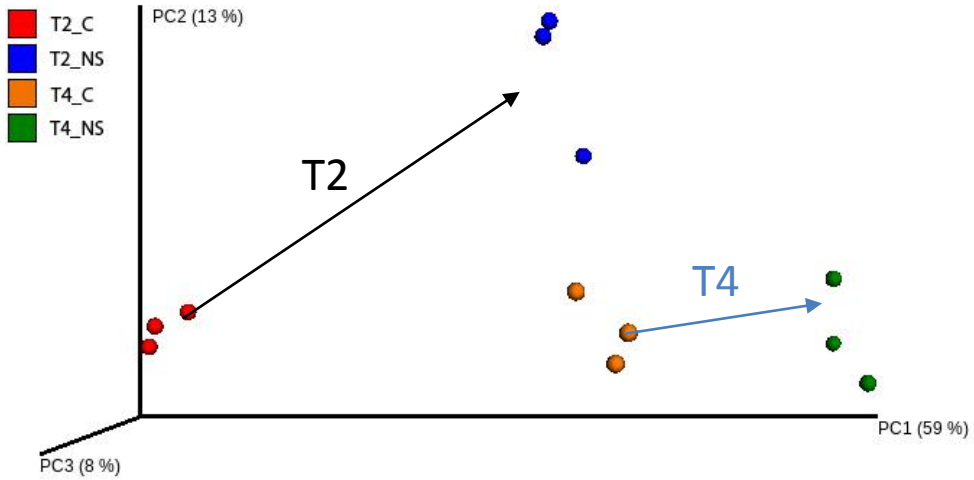
Nicosulfuron application in agricultural soils drives the selection towards NS-tolerant microorganisms harboring various levels of sensitivity to nicosulfuron

Environmental Science and Pollution Research, 23:4320–4333

Petric, Ines; Karpouzas, Dimitrios G.; Bru, David; Udikovic-Kolic, Nikolina; Kandeler, Ellen; Djuric, Simonida; Martin-Laurent, Fabrice

TierI: estimation of the ecotoxicological impact of nicosulfuron

Diversity analysis of soil microbial community: metagenomic analysis by 454 pyrosequencing of AHAS amplicons



Exposure to nicosulfuron led to:

⇨The evolution of the AHAS bacterial diversity

at T2 (T2_C ≠ T2_NS)

and, to a lesser extent, at T4 (T4_C ≠ T4_NS)

⇨Decrease in AHAS abundance was recorded in response to nicosulfuron exposure (data not shown)

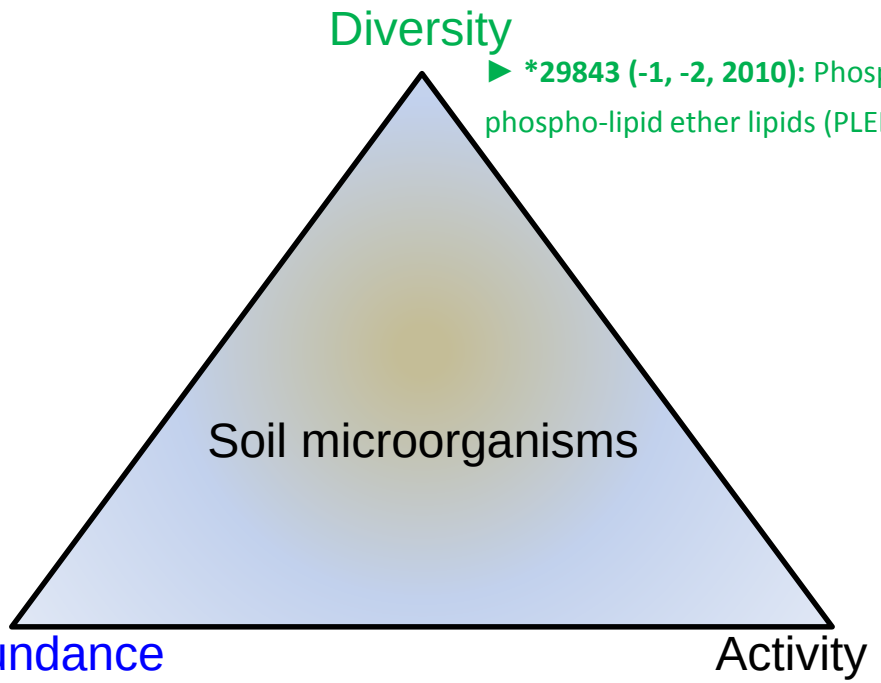
Summary of ecotoxicological impact of nicosulfuron on soil microorganisms

	Methods	Attribute	TierI *Greenhouse experiment*	TierII *Field experiment*
	PLFA	Composition/abundance	+	-
	Soil enzyme activity	Activity	+	+/- (2/6)
	qPCR taxa specific	Composition	+	-
	16S rRNA NGS	Diversity	+	nd
*	AMF_root colonization	Activity	+	-
*	AMF_18S rRNA	Composition	+	+ (at x5)
**	NS tolerant strain	Activity	+	+
**	NS tolerant strain_16S	Diversity	+	+
**	AHAS_qPCR	Abundance	+	+
**	AHAS_NGS	Diversity	+	nd

* Direct or indirect effect on AM fungi (obligate symbiont) ?

** microorganisms 'non-target organisms' harboring the target of the herbicide : indicator of exposure ?

State of the art of ISO standards for soil microorganisms



► ***29843 (-1, -2, 2010):** Phospholipid fatty acid analysis (PLFA) and phospho-lipid ether lipids (PLEL) analysis

► **14240 (-1, -2, 1997):** Soil microbial biomass (SIR, fumigation)

► ***16072 (2002):** Microbial soil respiration

► **17155 (2002) :** Abundance and activity of microbes (respiration curves)

► ***11063 (2011):** Direct soil DNA extraction from soil samples

► ***17601 (2016):** Abundance of microbial gene sequences

► **14238 (1997):** Nitrogen mineralization and nitrification in soils

►* **14239 (1997, 2017):** Mineralization of organic chemicals

► **15685 (2004):** Potential nitrification and inhibition of nitrification

► **23753 (-1, -2; 2005):** Dehydrogenase activity in soils (TTC and INT)

► **22939 (2010):** Enzyme activity patterns in soil (fluorimetry)

► **18187 (2017):** Deshydrogenase activity *A. globiformis*

► ***20130 (2017):** Enzyme activity patterns in soil (colorimetry)

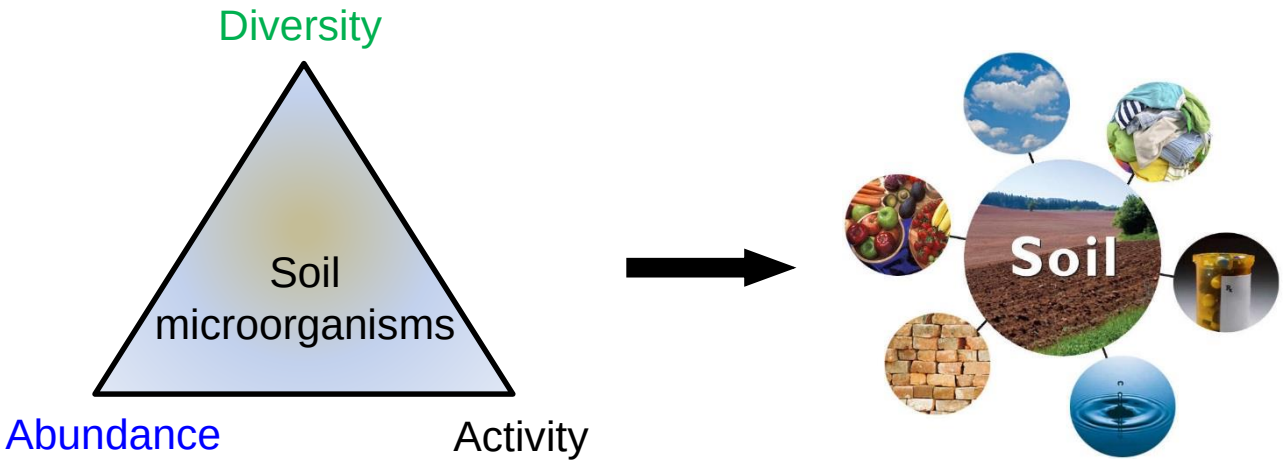
► ***20131 (-1, -2, 2017):** Soil denitrification, a process source of N_2O

***** AFNOR scientific leadership

New standards to assess proxies of soil ecosystemic services responsive to various stressors

ISO/TC 190/SC 4/WG 4 N 451 List of criteria for the selection of indicators for microbial functional indicators

ISO/TC 190/SC 4/WG 4 N 4 Identification of the most suitable functional indicators (and the methods to measure them) in soil microbiology



⇨ <u>Perspectives :</u> Package of standards to measure of the abundance, diversity and activity of functional guilds supporting soil ecosystemic services (N cycle, filtration,…) in response to various stressors (including pesticides)

Environ Sci Pollut Res
DOI 10.1007/s11356-014-3390-x

CONFERENCE REPORT

The coming of age of microbial ecotoxicology: report on the first two meetings in France

Jean-François Ghiglione · Fabrice Martin-Laurent · Sabine Stachowski-Haberkorn · Stéphane Pesce · Stéphane Vuilleumier

MANY THANKS FOR YOUR ATTENTION

Environ Sci Pollut Res
DOI 10.1007/s11356-015-5763-1

EDITORIAL

Microbial ecotoxicology: an emerging discipline facing contemporary environmental threats

Jean-François Ghiglione[1] · Fabrice Martin-Laurent[2] · Stéphane Pesce[3]

https://www6.inra.fr/ecotox

https://www.ecotoxicomic.fr/en_GB/

6th Sud Symposium IPM

European Parliament, 31st January 2018