In vitro assessment of toxicity of pesticides on activity of non-target ammonia-oxidizing microorganisms

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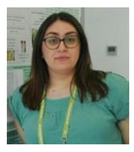

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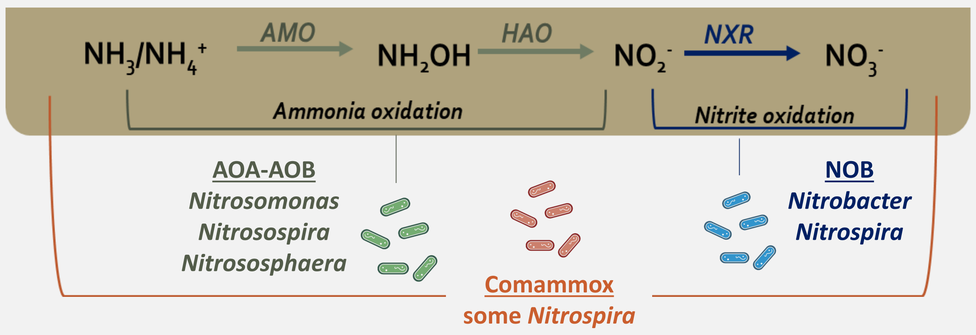
Introduction

- **Pesticides** are major **environmental pollutants**, regulated by the EU framework based on established toxicity assays for aquatic and terrestrial macro-organisms.
- Assessment of soil microbial toxicity of pesticides does not provide a view of potential effects on microbial diversity and key microbe-mediated functions in soil.
- **Ammonia-oxidizing microbes** (AOM), which perform the first and typically rate-limiting step of nitrification, are **ideal microbial indicators of agrochemicals toxicity** because of their key functional role, sensitivity to xenobiotic compounds, and availability of established tools to measure activity and abundance *in vitro* and *in situ*.



Objective

- Determine sensitivity of phylogenetically and ecophysiologically distinct soil AOM to establish and standardize *in vitro* protocols for the toxicity of pesticides.

Culture experiment

- Soil ammonia-oxidizing archaea (AOA) and bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were grown in culture.

AOA strains
- *Ca.* Nitrosotalea sinensis
- *Ca.* Nitrosocosmicus franklandianus
- *Nitrosphaera viennensis*

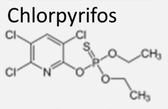
AOB stains
- *Nitrosomonas europaea*
- *Nitrosomonas communis*
- *Nitrospira multiformis*

NOB stains (Data not shown)
- *Nitrobacter sp. NHB1*
- *Nitrospira defluvii*
- *Nitrobacter winogradski*

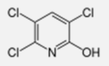
- Strains were exposed to a range of pesticides at different concentrations.

Insecticides

Chlorpyrifos

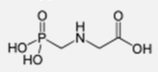


3,5,6-Trichloro-2-pyridnole (TCP)

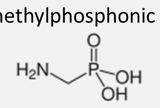


Herbicides

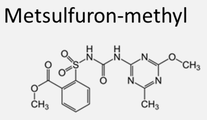
Glyphosate



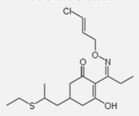
Aminomethylphosphonic acid (AMPA)



Metsulfuron-methyl

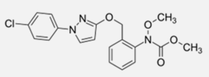


Clethodim

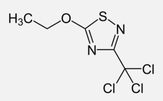


Fungicides

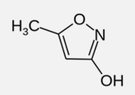
Pyraclostrobin



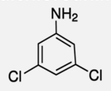
Etridiazole



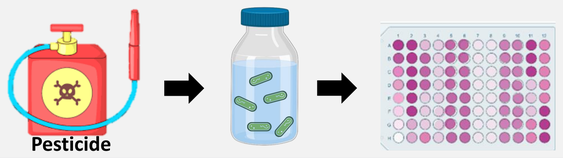
Hymexazol



3,5-dichloroaniline (3,5 DCA)

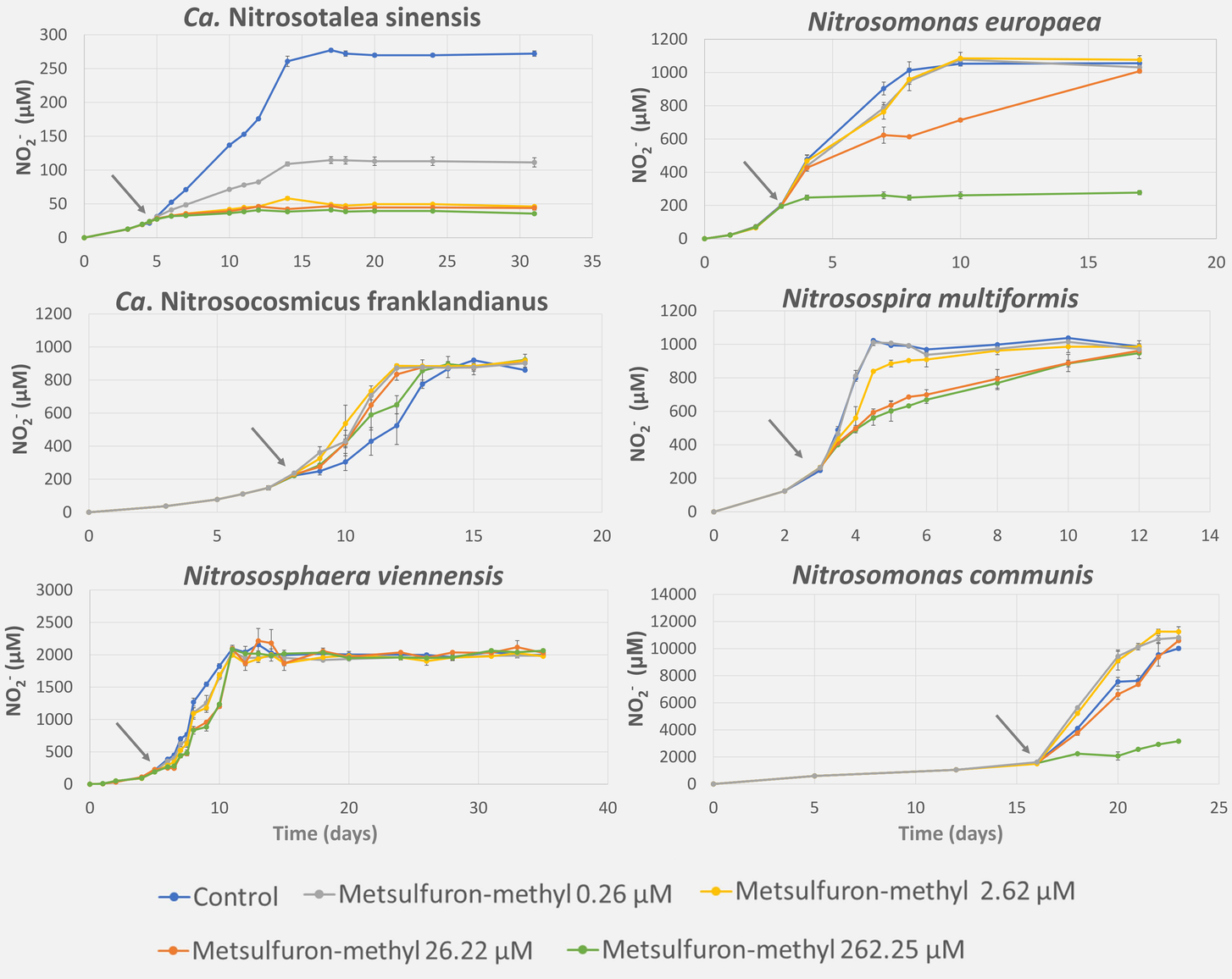


- Nitrite production was measured colormetrically.



Results

Impact of Metsulfuron-methyl on nitrite production of AOM strains



Arrows indicate the time at which pesticides were added.

Potency of pesticides to ammonia oxidation activity of AOM strains

		AOA		
Pesticide group	**Pesticide**	***Ca.* N. sinensis**	***Ca.* N. franklandianus**	***N. viennensis***
Insecticide	**Chlorpyrifos**	**++++**	**++++**	**++++**
	TCP	**++++**	**-**	**-**
Herbicide	**Glyphosate**	**+**	**-**	**-**
	AMPA	**+**	**-**	**-**
	Metsulfuron-methyl	**++++**	**-**	**-**
	Clethodim	**+++**	**-**	**-**
Fungicide	**Pyraclostrobin**	**++++**	**++++**	**++++**
	Etridiazole	**+++**	**++++**	**++++**
	Hymexazol	**++**	**-**	**++**
	3,5 DCA	**++++**	**+++**	**+++**

		AOB		
Pesticide group	**Pesticide**	***N. europaea***	***N. communis***	***N. multiformis***
Insecticide	**Chlorpyrifos**	**-**	**-**	**-**
	TCP	**-**	**-**	**-**
Herbicide	**Glyphosate**	**+**	**++**	**+**
	AMPA	**-**	**-**	**-**
	Metsulfuron-methyl	**+++**	**+++**	**++++**
	Clethodim	**+++**	**-**	**-**
Fungicide	**Pyraclostrobin**	**++++**	**++++**	**+++**
	Etridiazole	**++++**	**++++**	**++++**
	Hymexazol	**++**	**-**	**-**
	3,5 DCA	**+++**	**+++**	**++++**

++++ 0-10 µM +++ 10-100 µM ++ 100-1000 µM + 1000 µM
- >max tested concentration

Spectrum of pesticide toxicity based on calculated mean EC_{50} values. Symbols +/– show toxicity level of pesticides.

Conclusions

- ✓ Pesticides affected at least one non-target AOM, with AOA and AOB strains varying in sensitivity.
- ✓ Categorizing by pesticide target revealed contrasting patterns, with fungicides highly toxic to all AOM strains while insecticides and herbicides had greater effect on AOA and AOB strains, respectively.
- ✓ Among the AOA strains, acidophilic *Ca.* N. sinensis was the most sensitive. Neutrophilic AOAs showed the same toxicity pattern in all tested pesticides, with exception of hymexazol that was more toxic for *N. viennensis*.
- ✓ Sensitivity of AOBs was pesticide dependent.
- ✓ Findings will contribute to development of novel ecotoxicity tools and risk assessment scheme characterizing impact of pesticides on non-target soil microbes.

Acknowledgements

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