



Assessing the toxicity of biopesticides on soil microbiota using a modification of the OECD 216 test

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Introduction

Synthetic pesticides are widely used in agricultural systems to protect crops against diseases and pests. Despite their undeniable importance for crop production, studies indicate that the indiscriminate use of synthetic pesticides threatens the environment and human health. As an alternative, biopesticides (chemicals of biological origin), including many natural products of plants and microbes, are gaining the attention of the general public and the pesticide market. In general, biopesticides are considered low-risk compounds and environmentally safe even though hard evidence to support this idea are still lacking.

The aim of the present work was to assess if bio-pesticides are non-toxic, or less toxic, than synthetic pesticides to soil microorganisms. In this frame we used nitrogen biotic transformations and specifically nitrification as a proxy to assess the soil microbial toxicity of biopesticides on soil microorganisms.

Material and Methods

A modified OECD 216 test was followed:

- Two soils from **France** (silt clay loam) and **Germany** (sandy loam).
- Six biopesticides plus **a synthetic pesticide**:

- **Azadirachtin** • **Spinosad** • **Aliphatic Phenol** • **3.5 DCA***
- **Pyrethrins** • **Isoflavone** • **Dihydrochalcone**

- **Dose rates:** Recommended dose (1x) and 10x the recommended dose.
- Sampling at 0, 7, 14, and 28 days after the beginning of incubation.

Nitrogen transformation analysis

The effects of biopesticides on the soil microbiota and soil nitrogen transformation were assessed by measuring **potential nitrification (PNT), ammonium (NH₄⁺) and nitrate (NO₃⁻) levels.**

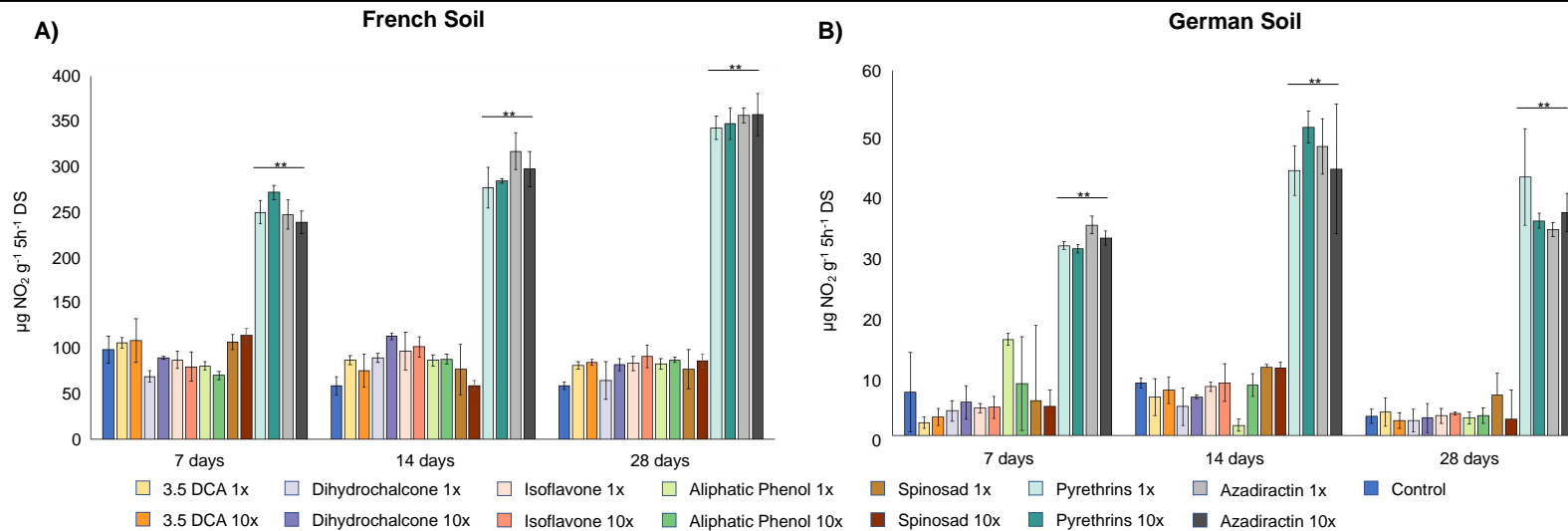


Figure 1. The effect of biopesticides on **potential nitrification rates** in the French (A) and the German (B) soils. Error bars represent the standard deviation of three replicates. ** refer to significant differences between treatments and the control at each sampling date based on ANOVA test followed by Tukey post-hoc test ($P \leq 0.05$).

Results

The impact of biopesticides on potential nitrification (PNT)

- Regardless of the soil and dose rates, the use of Dihydrochalcone, Aliphatic Phenol, Isoflavone, Spinosad, and the synthetic compound 3.5 DCA did not show significant effects on PNT rates.
- **Azadirachtin** and **Pyrethrins** at both dose rates and in both soils showed a strong stimulatory effect on PNT.



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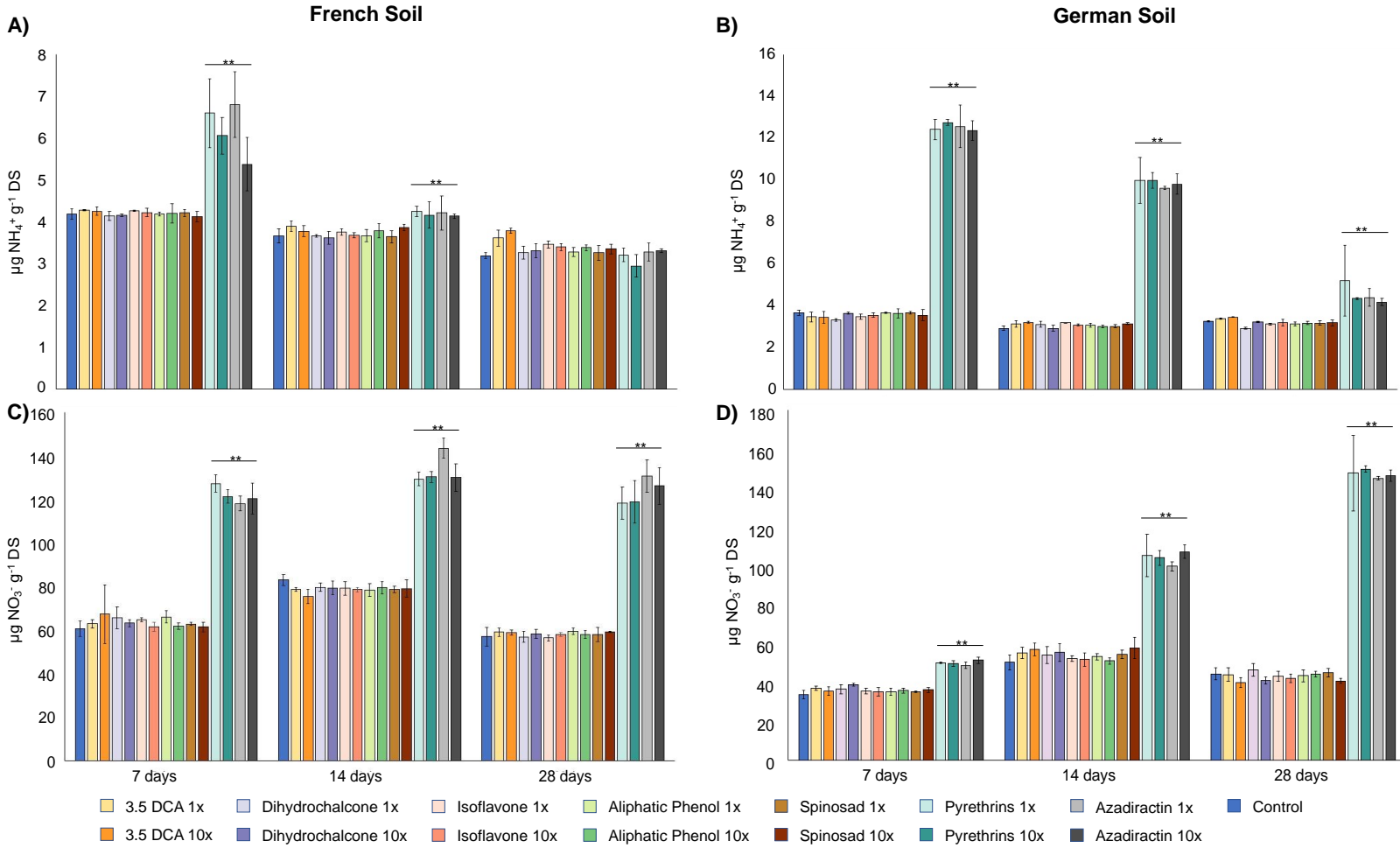
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The impact of biopesticides on the dynamics of inorganic N-pools

- Regardless of the dose, **Azadiractin** and **Pyrethrins** induced significant increases in NH_4^+ levels at 7 and 14 days in both soils. However, a recovery in NH_4^+ levels was evident in French soil at 28 days.
- Regardless of the dose, **Azadiractin** and **Pyrethrins** induced significant increases in NO_3^- levels. This effect persisted for at least 28 days.
- Dihydrochalcone, Isoflavone, Aliphatic Phenol, Spinosad, and the synthetic compound 3.5 DCA showed no significant effects on inorganic N pools during the 28 days.

On-going work

- Follow up experiments to identify the mechanism behind the stimulatory effect of **Azadiractin** and **Pyrethrins** on N transformation in soil.
- Determine the dissipation of biopesticides in both soils via HPLC-UV.
- Assess the effects of biopesticides on the soil microbial diversity via amplicon sequencing.

Figure 2. The effect of biopesticides on ammonium (A and B) and nitrate (C and D) rates in the French and the German soils. Error bars represent the standard deviation of three replicates. ** refer to significant differences between treatments based on ANOVA test followed by Tukey post-hoc test ($P \leq 0.05$).

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