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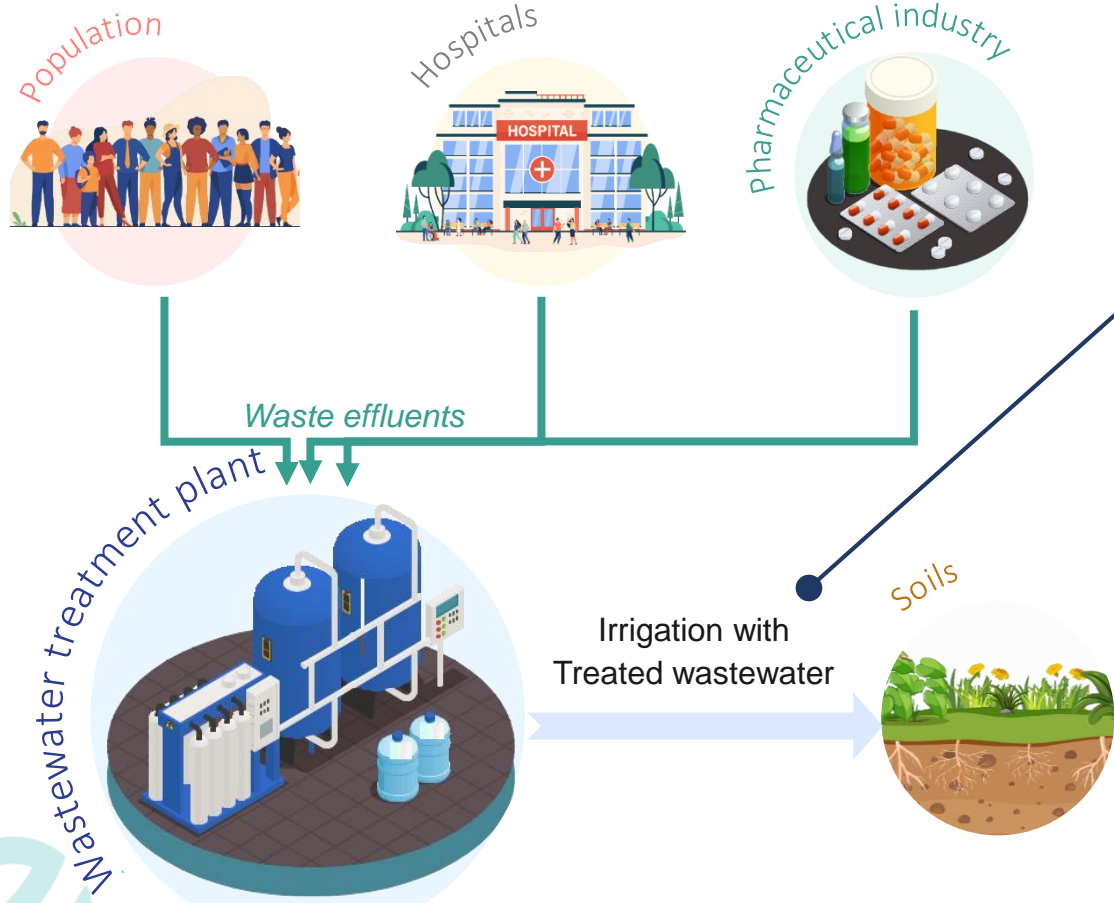
➤ Which are the antibiotic resistance strategies that soil bacteria adopt when irrigated with treated wastewater?

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➤ Context & objectives

▶ Water scarcity = major issue of XXIth century:

➡ Reuse of treated wastewater for irrigation



TWW contains
→ high N, P, S, OM contents
→ **pharmaceutical residues**
→ heavy metals
→ antibiotic resistance genes
→ mobile genetic elements
→ resistant bacteria & pathogens

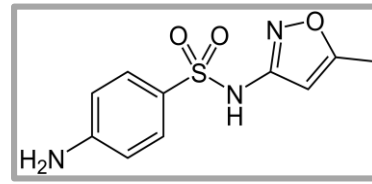
Few $\mu\text{g}/\text{kg}$ dry soil to mg/kg dry soil



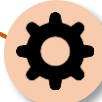
Investigate the effect of **antibiotic nature & concentration**, on:
→ the evolution of soil endogenous microbial communities
→ the spreading of resistant bacteria,
→ ARGs, MGEs in soils
→ the fate of antibiotics in soils



➤ Experimental design



Operating conditions



- 1 set with **historical soil** irrigated for 2 years with TWW
- 1 set **autoclaved** to reduce bacterial abundance & diversity
=> *Endogenous bacteria control*
- 1 set **autoclaved & irrigated with emQ**
=> *Exogenous bacteria control*
- Triplicate, sacrificial mode
- Maintained at 80% moisture content
- 1.5 month incubation at 35°C

Impact on bacterial communities



- 16S rRNA sequencing

Physico-chemical analysis

- Monitoring of **N, sulfate, phosphate**

Effect of antibiotic concentration



Irrigated with TWW (+ control with emQ)

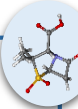
Spiked with sulfamethoxazole at different concentrations

- 0
 - 10 µg/kg_{dry soil}
 - 100 µg/kg_{dry soil}
 -
 - 500 µg/kg_{dry soil}
 - 1000 µg/kg_{dry soil}
- } [Environmental]*
- } > [Environmental]



Soil microcosms (from Murviel-lès-Montpellier platform, France)

Fate of antibiotics



- LC-MS analysis using a Q-Exactive Orbitrap
=> quantification & **monitoring of SMX**
=> detection of SMX **TP**

Impact on degradation/resistance gene markers

- nb of copy of key **ARG & MGE**
- nb of copy of **efflux pump**
- nb of copy of **degradation genes**



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*M. Cycoń, et al., *Front. Microbiol.* **2019**, 10

➤ Sulfamethoxazole biodegradation

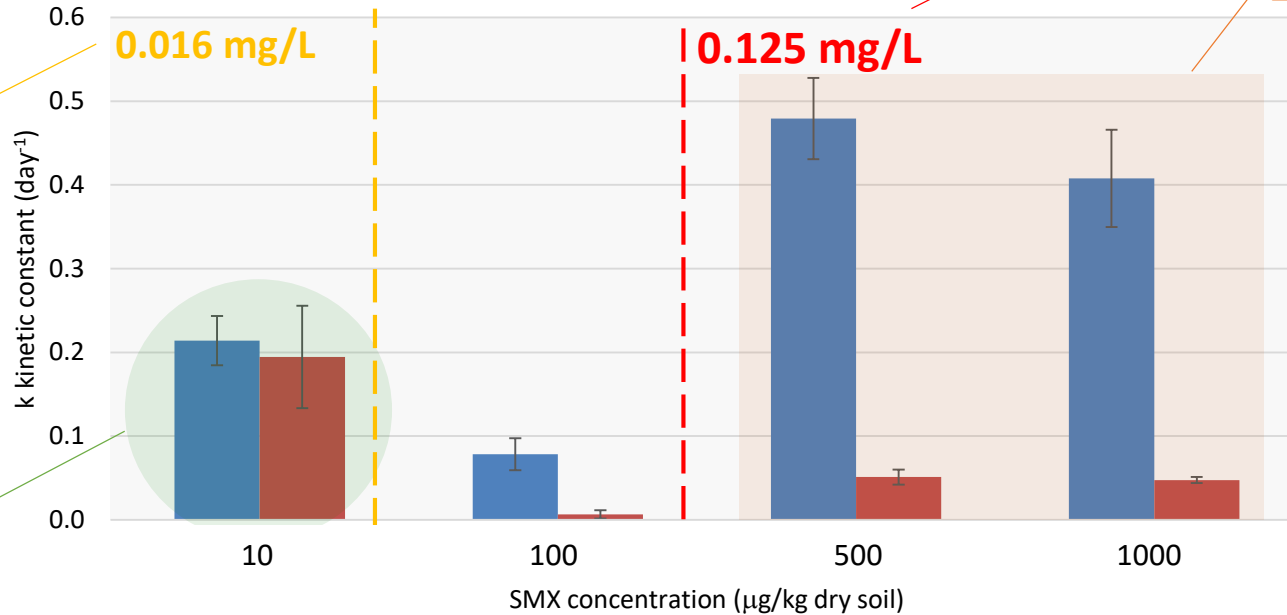
Kinetics

MIC minimum inhibitory concentration (for bacterial growth)*

Higher degradation rate

PNEC predicted no effect concentrations (for resistance selection)*

Fast degradation probably due to co-metabolism



Soil & irrigation conditions

- Soil_reuse + TWW
- Soil_autoclaved + TWW
- Soil_autoclaved + emQ

Hyp: 1st order kinetic: $v = \frac{d[SMX]}{dt} = -k \times [SMX] \Rightarrow \ln[SMX] = \ln[SMX]_0 - k \times t \quad R^2 [0.8-0.96]$

- ➡ Complete degradation for soil_reuse in each case
- ➡ No degradation in soil_autoclaved + emQ (⇒ no photodegradation)
 - ⇒ degradation in soil_autoclaved + TWW due to TWW exogenous bacteria or soil-resistant ones stimulated by TWW irrigation)



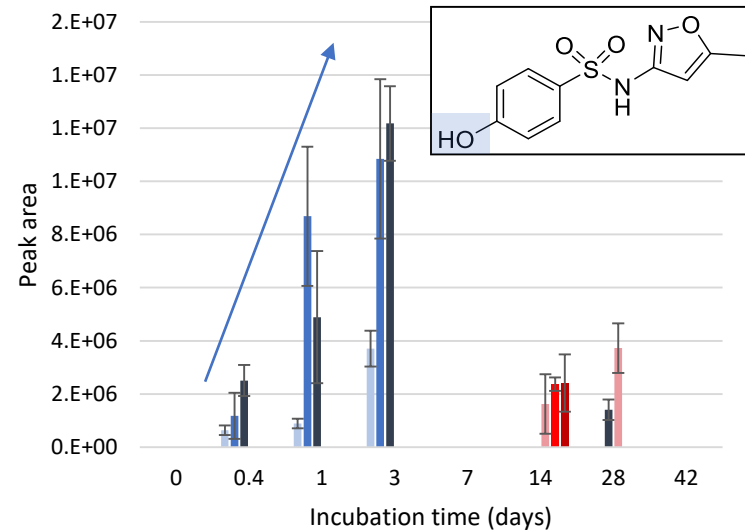
➤ Sulfamethoxazole biodegradation

Suspected identification of sulfamethoxazole transformation products (LC-HRMS)

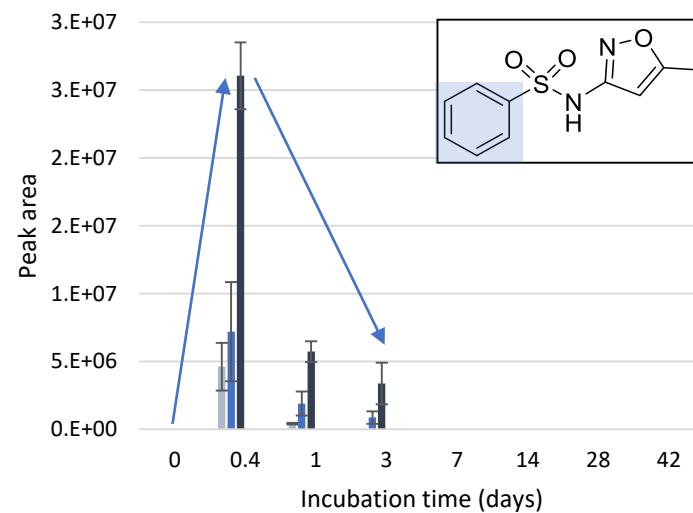
Soil reuse

- SMX = 100 µg/kg dry soil (in TWW)
 - SMX = 500 µg/kg dry soil (in TWW)
 - SMX = 1000 µg/kg dry soil (in TWW)
- Soil autoclaved + TWW
- SMX = 100 µg/kg dry soil (in TWW)
 - SMX = 500 µg/kg dry soil (in TWW)
 - SMX = 1000 µg/kg dry soil (in TWW)

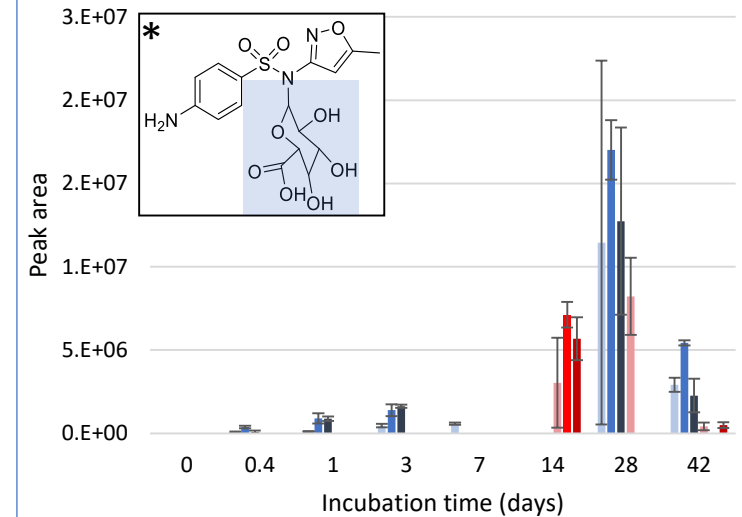
Oxidation



Desamination



Glucuronidation



* Structure to be confirmed with commercial reference

Possible co-metabolism by **autotroph nitrifying bacteria**¹

-> Transformation generally performed by **ammonia oxidizing bacteria** during **nitrifying/denitrifying periods**²
 -> Correlated with **nitrate & nitrite evolution**

-> Human metabolite
 -> **Glucuronidation to transport and release SMX across the cell membrane (efflux pump)**³



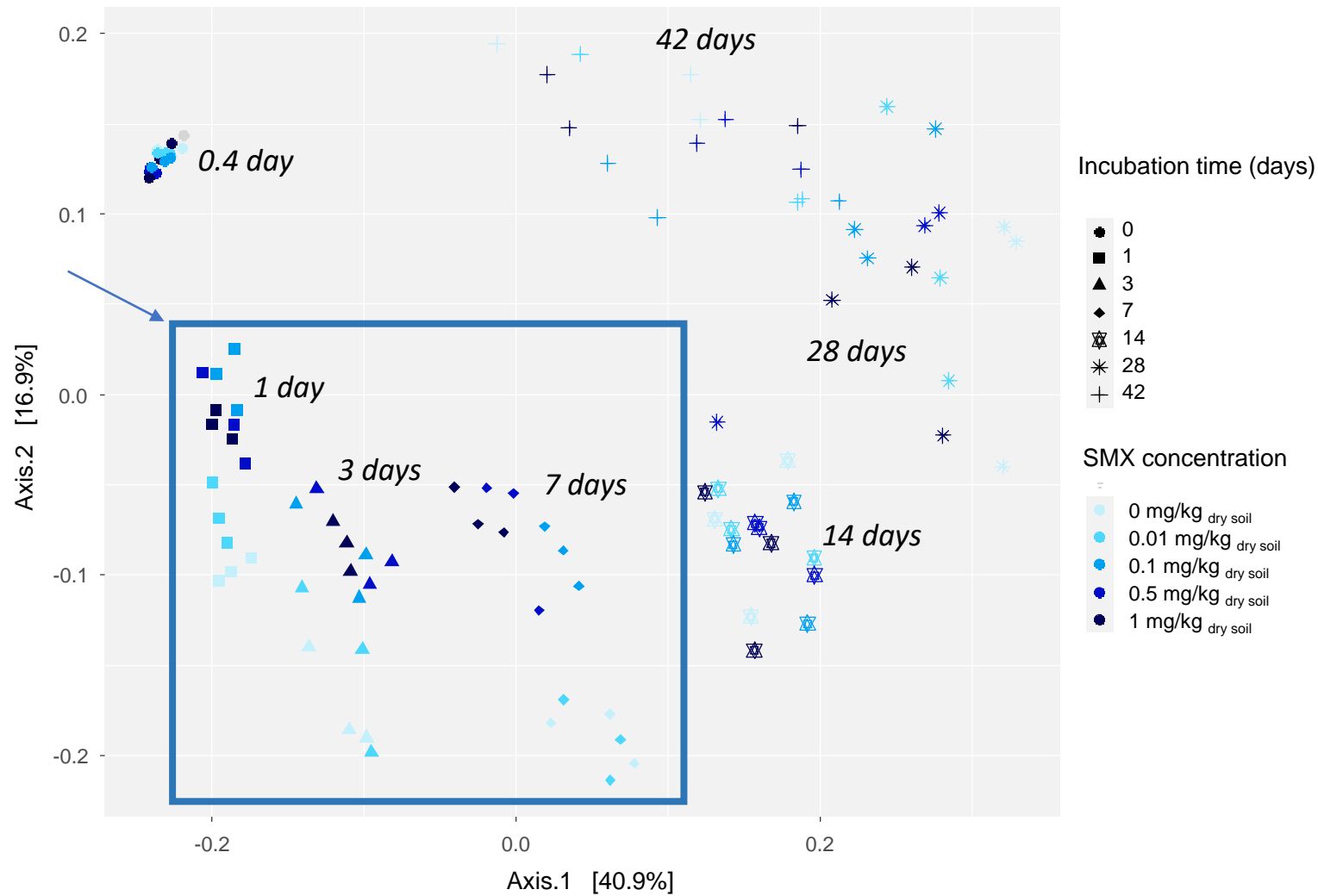
¹E. Müller et al., *Chemosphere*. **2013**, 92(8), 969-978

²E. Kassotaki, et al., *Water Research*. **2016**, 94, 111-119

³M. Radke, et al., *Environmental Science & Technology* **2009** 43 (9), 3135-3141

➤ Biomarkers of sulfamethoxazole concentrations

PCoA from 16S rDNA sequencing analysis



Differences in microbial communities between 1 & 7 days



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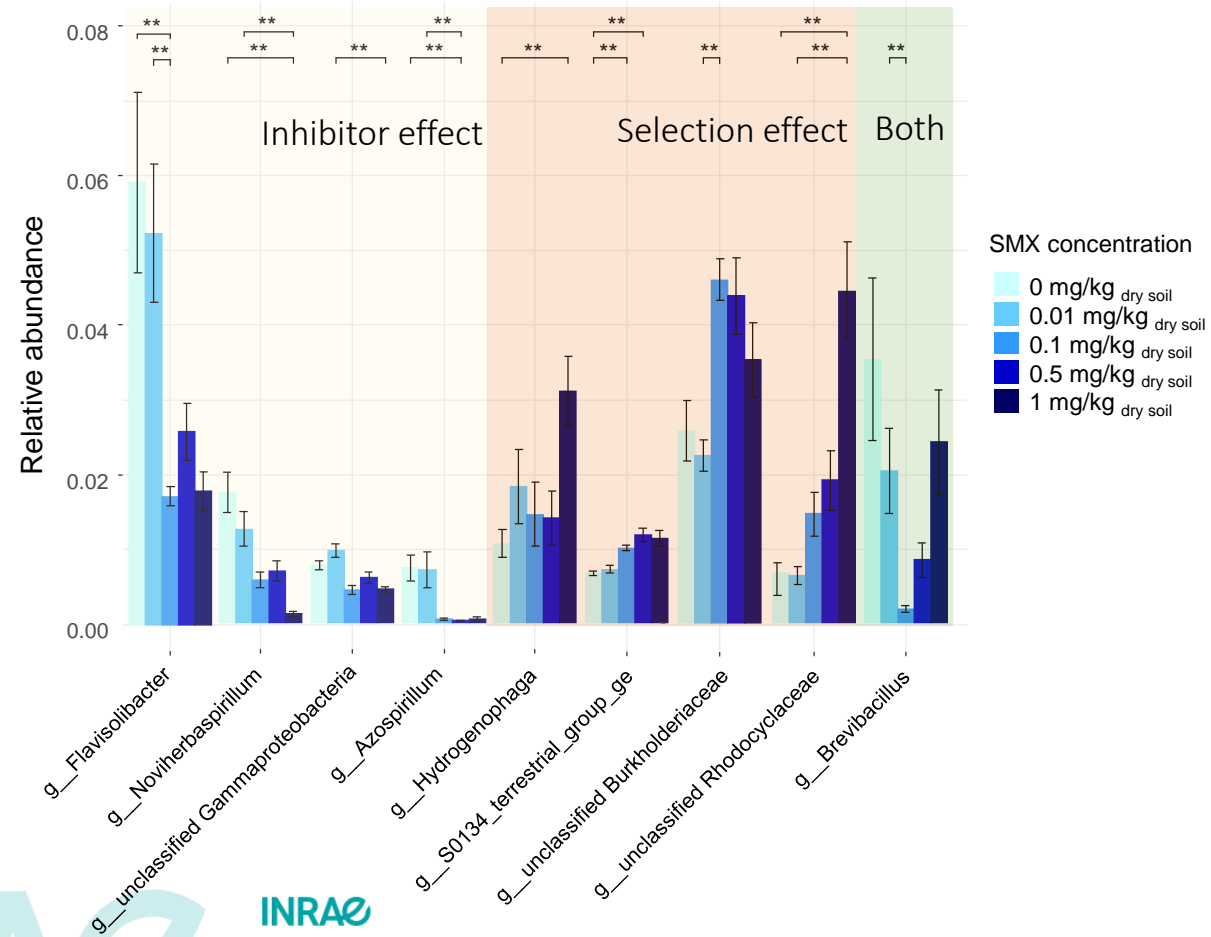
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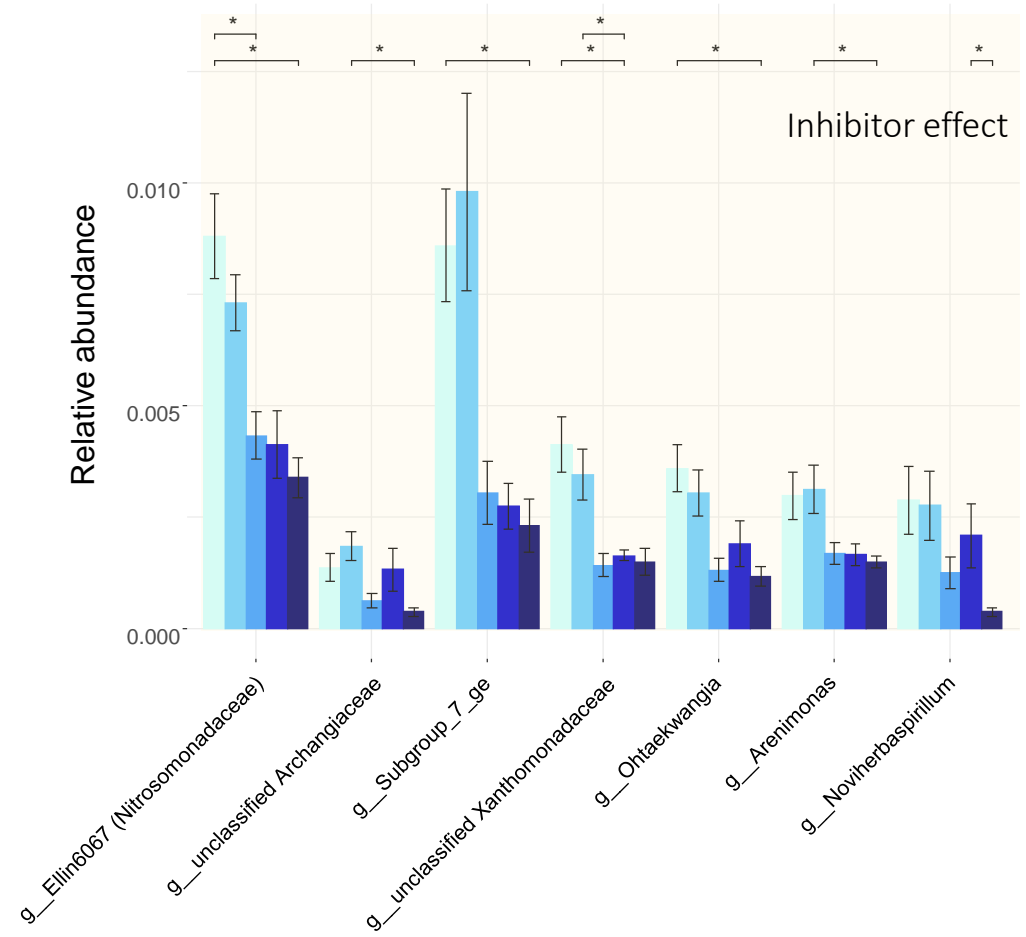
➤ Biomarkers of sulfamethoxazole concentrations

LEfSe

LEfSE on times 1,3,7 days



LEfSE on times 14,28,42 days



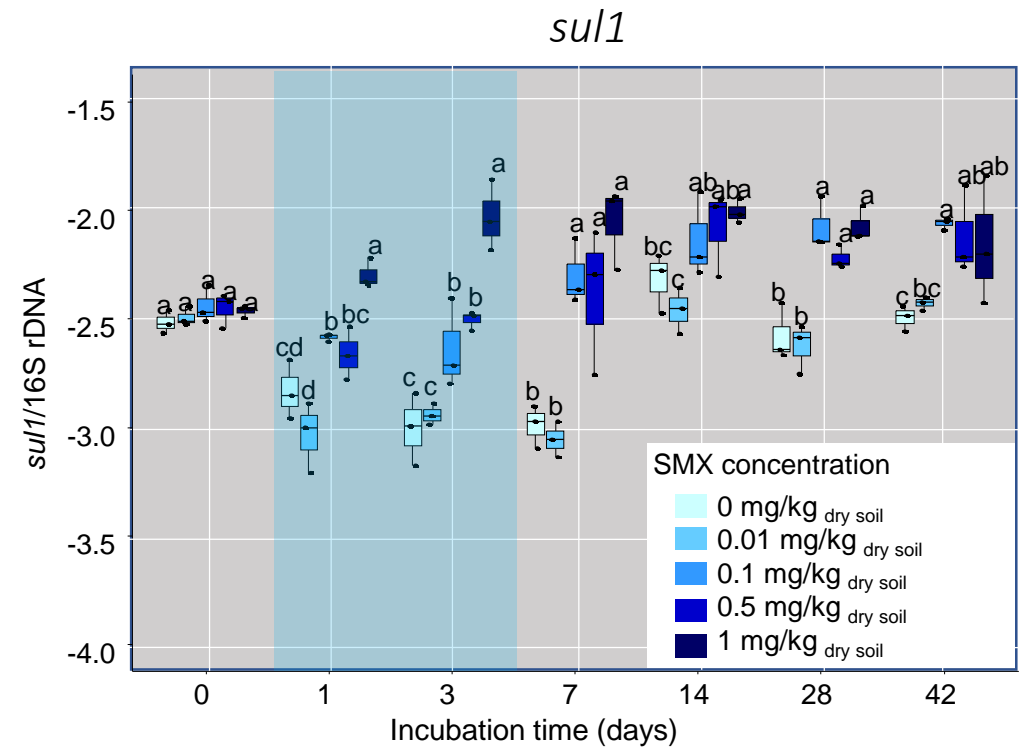
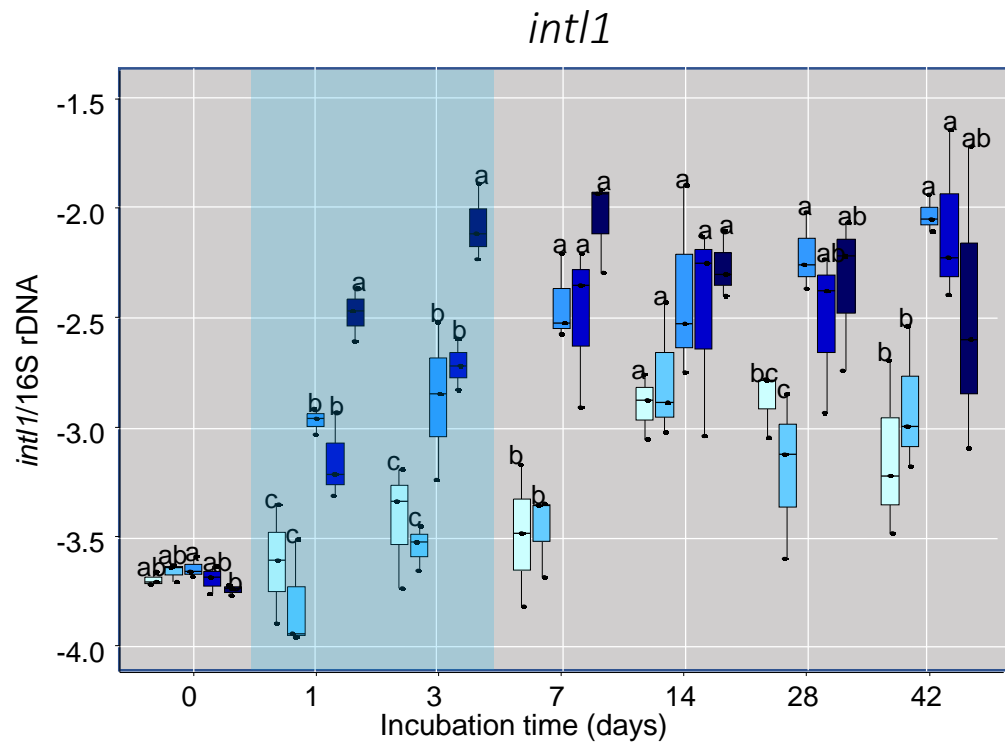
- ➡ Less marked effect
- ➡ Maintains a fingerprint of SMX despite its transformation

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➤ Evolution of *sul1* and *int1* in *soil_reuse*

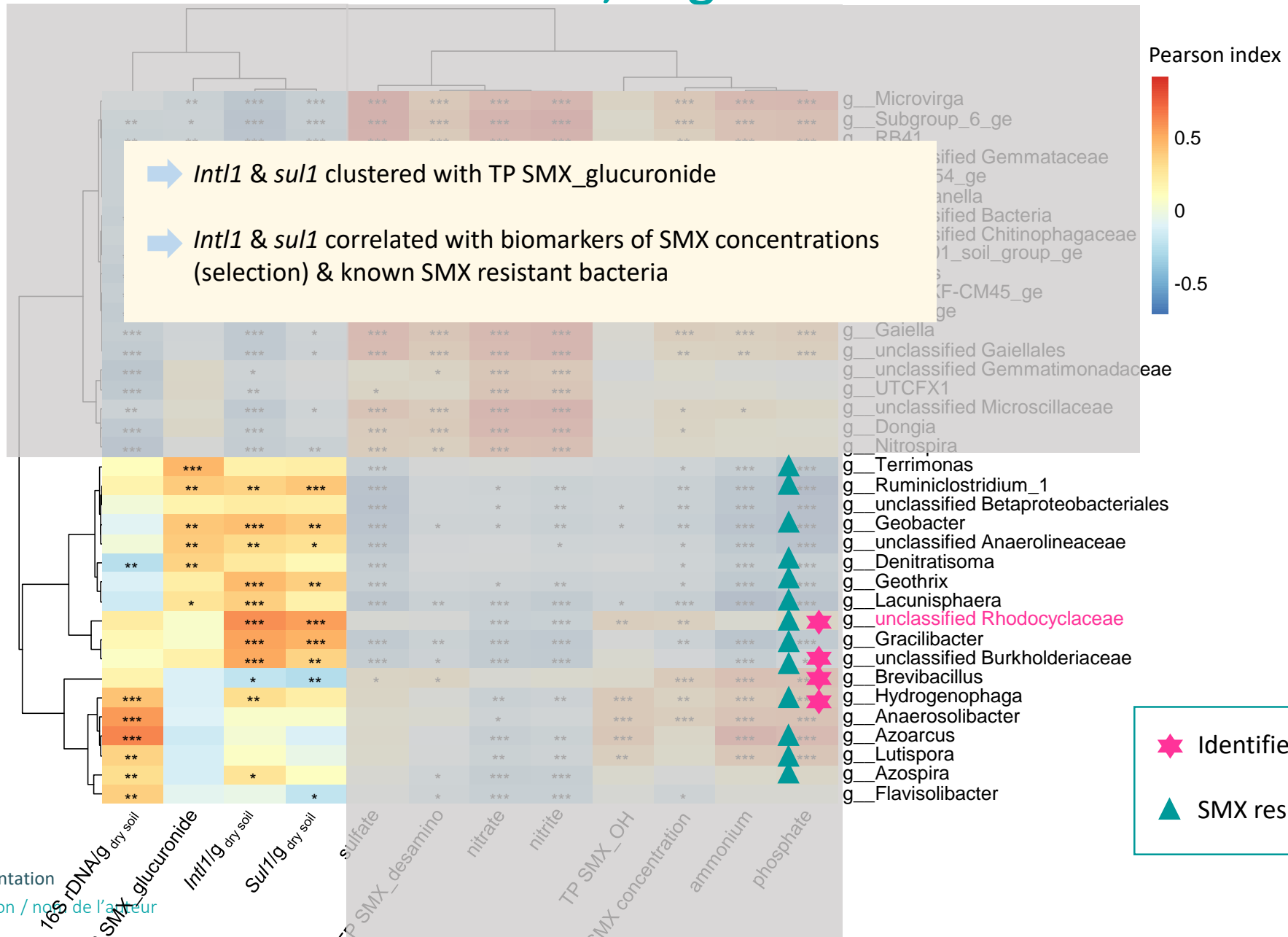


- ➔ **Decorrelated from 16S rDNA** evolution
Enrichment of the copy number of *int1* gene in microbial community after TWW irrigation (day 42)
- ➔ Concentration effect: **3 levels** between 1 & 3 days (0, 0.01 // 0.1, 0.5 // 1 mg/kg_{dry soil}), then **2 levels** (> 0.01 mg/kg_{dry soil})

- ➔ Similar trends with *sul1* but less marked
- ➔ **At day 42** enrichment of the copy number of *sul1* gene for **[SMX] > 0.01 mg/kg_{dry soil}** suggesting resistance transfer

From **[SMX] > 0.01 mg/kg_{dry soil}** fingerprint of SMX use for *sul1* and *int1* gene even after 42 days of incubation

➤ Correlations between resistance, degradation & biomarkers



> Conclusions & Perspectives

- ➔ For $[SMX] > 0.1 \text{ mg/kg}_{\text{dry soil}}$ (\sim MIC), \nearrow degradation rate
 - identification of TPs associated with \neq bacterial groups

➔ What about the other antibiotics?

- ➔ Bacterial inhibition & selection observed for $[SMX] > 0.01 \text{ mg/kg}_{\text{dry soil}}$ (\sim PNEC)
 - main effect observed between 0 & 7 days
 - fingerprint of SMX use still observed after 1.5 months

➔ Link to residual SMX TPs?

- ➔ \nearrow *sul1* & *int1* gene abundance for $[SMX] > 0.01 \text{ mg/kg}_{\text{dry soil}}$
 - increase remains after 1.5 months of incubation suggesting resistance transfer

➔ What about the effect of repeated and long term irrigation?

> Acknowledgments



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