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

Oriane Della-Negra, Maria Vittoria Barbieri, Marilia Camotti Bastos, Valérie Bru-Adan, Gaëlle Santa-Catalina, Nassim Ait-Mouheb, Marc Heran, Serge Chiron, Dominique Patureau

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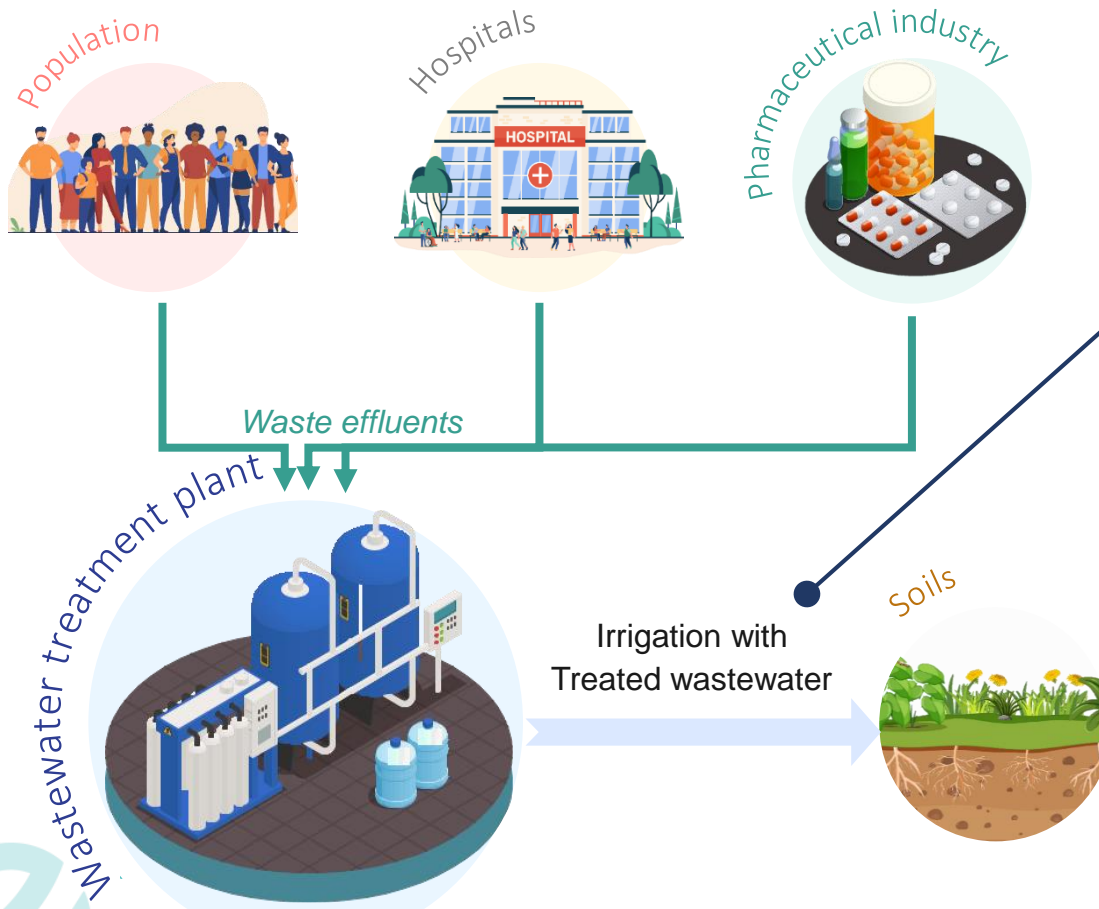
➤ Which are the antibiotic resistance strategies that soil bacteria adopt when irrigated with treated wastewater?

Della-Negra Oriane, Vittoria Barbieri Maria, Camotti Bastos Marilia, Bru-Adan Valérie, Santa-Catalina Gaëlle, Ait Mouheb Nassim, Heran Marc, Chiron Serge, Patureau Dominique

> 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



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➤ 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*

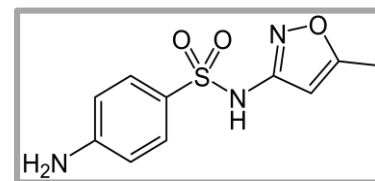
- Triplicate, sacrificial mode
- Maintained at 80% moisture content
- 1.5 month incubation at 35°C

Impact on bacterial communities

- 16S rRNA sequencing

Physico-chemical analysis

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



Sulfamethoxazole = SMX



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

Effect of antibiotic concentration

Irrigated with TWW (+ control with emQ)

Spiked with sulfamethoxazole at different concentrations

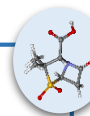
- 0
 - 10 µg/kg_{dry soil}
 - 100 µg/kg_{dry soil}
 - 500 µg/kg_{dry soil}
 - 1000 µg/kg_{dry soil}
- Right-side labels:
- 0, 10, 100 µg/kg → [Environmental]*
- 500, 1000 µg/kg → > [Environmental]

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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> Sulfamethoxazole biodegradation

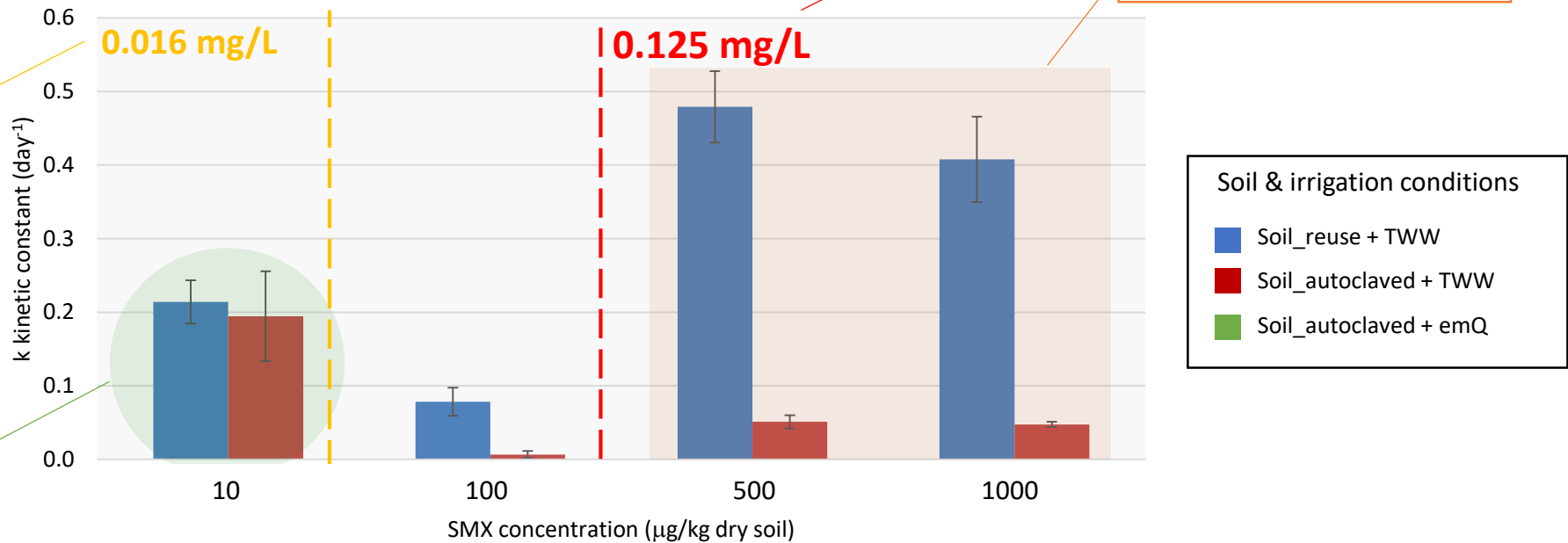
Kinetics

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



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)



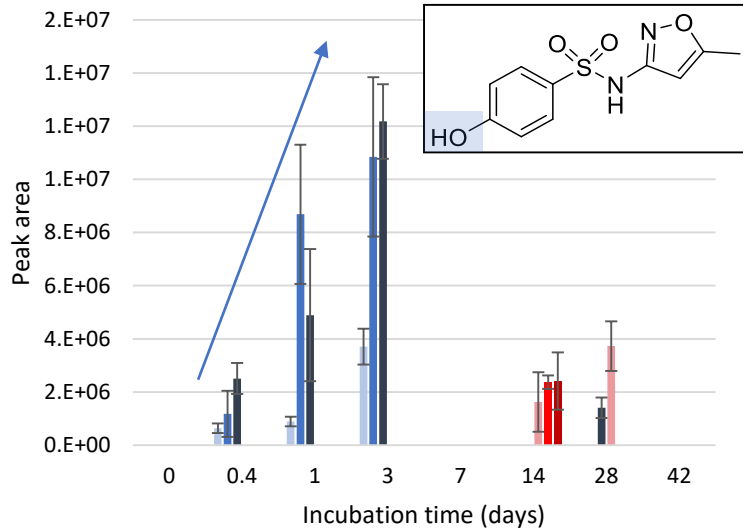
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*J. Bengtsson-Palme, D.G.J. Larson, *Environmental International*. 2016, 86, 140-149

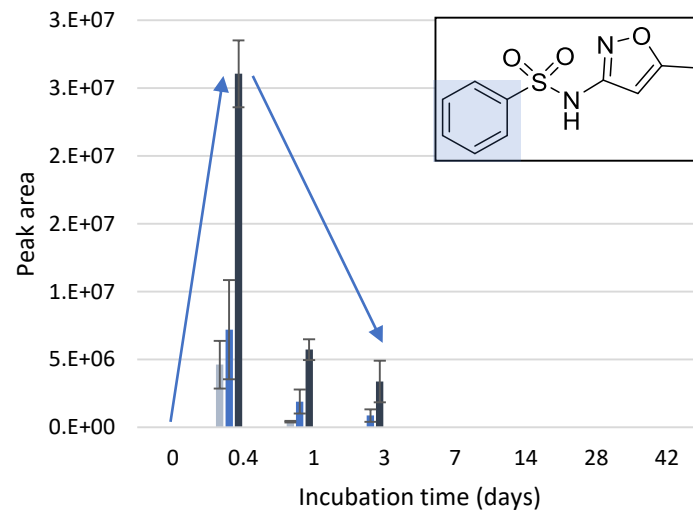
► Sulfamethoxazole biodegradation

Suspected identification of sulfamethoxazole transformation products (LC-HRMS)

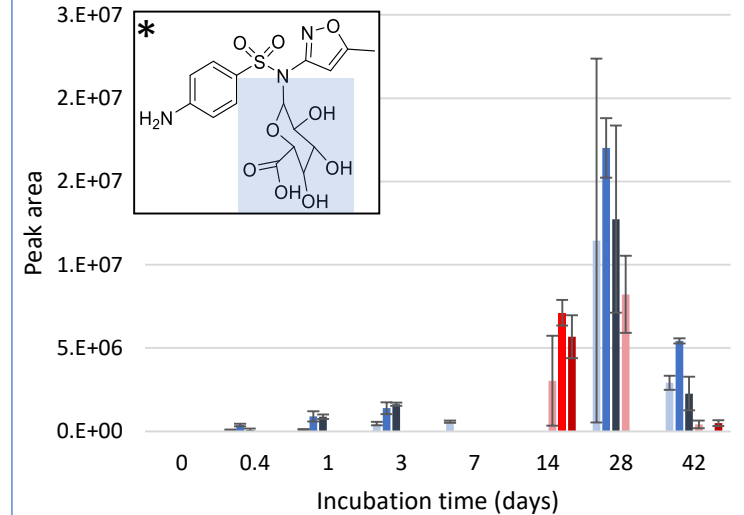
Oxidation



Desamination



Glucuronidation



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)

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



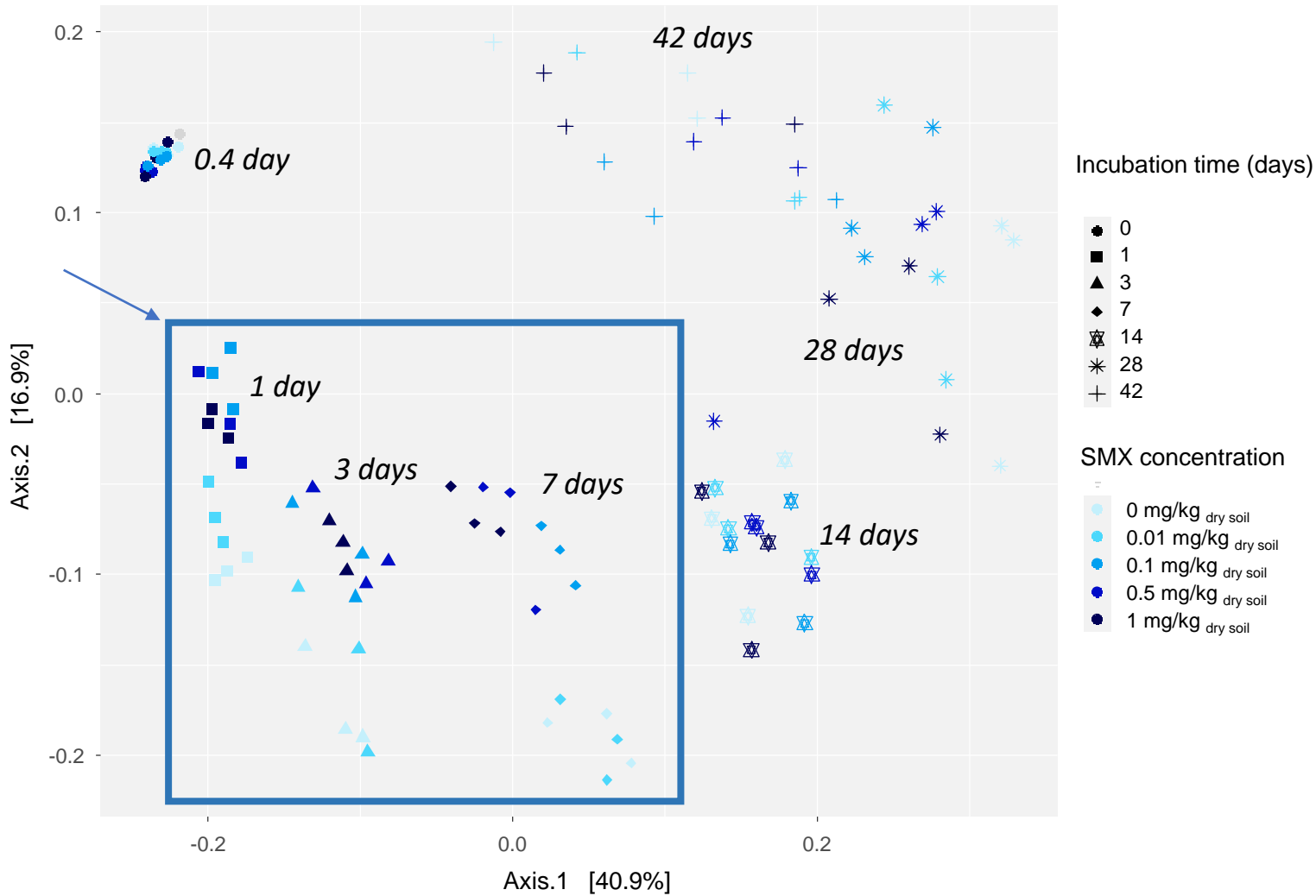
¹E. Müller et al., *Chemosphere*. **2013**, 92(8), 969-978

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



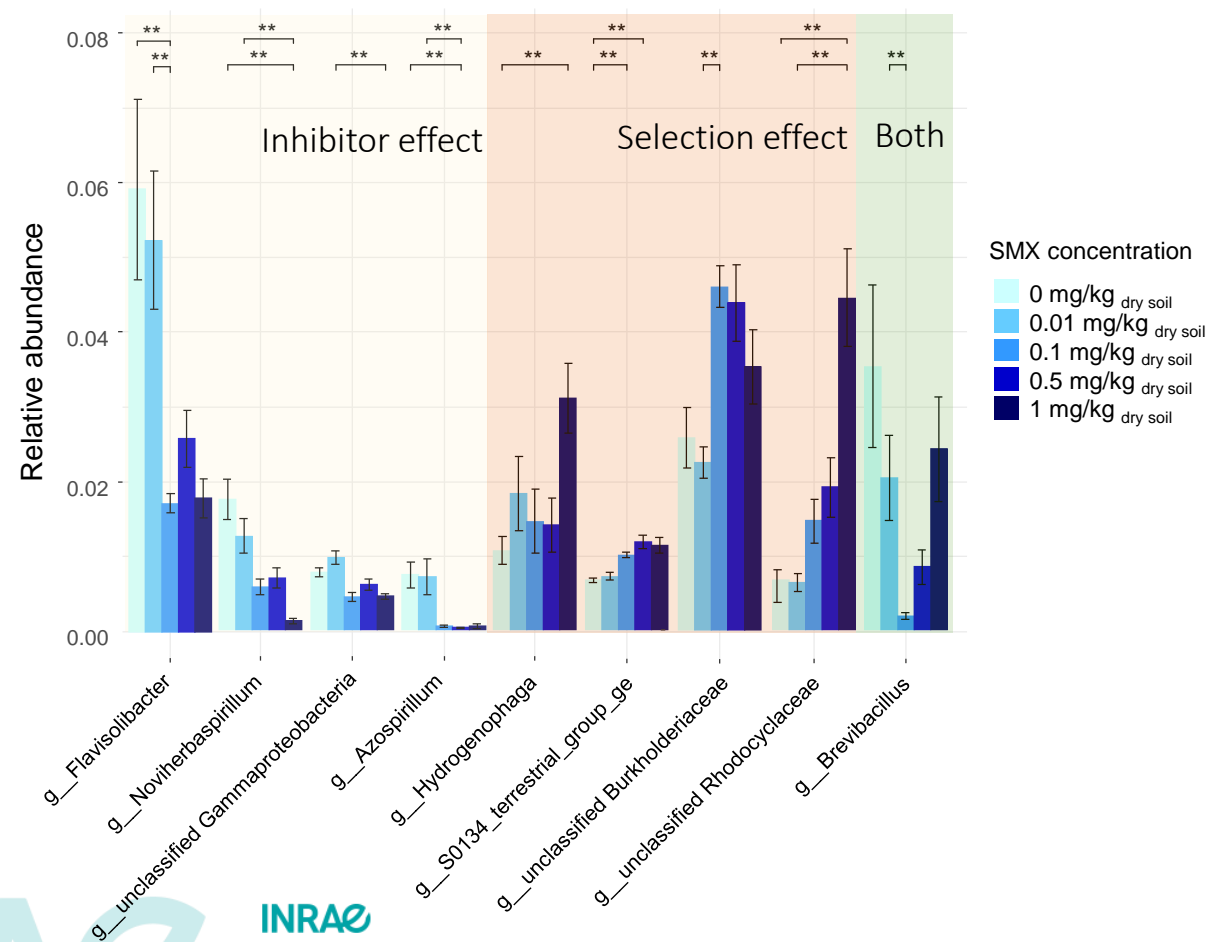
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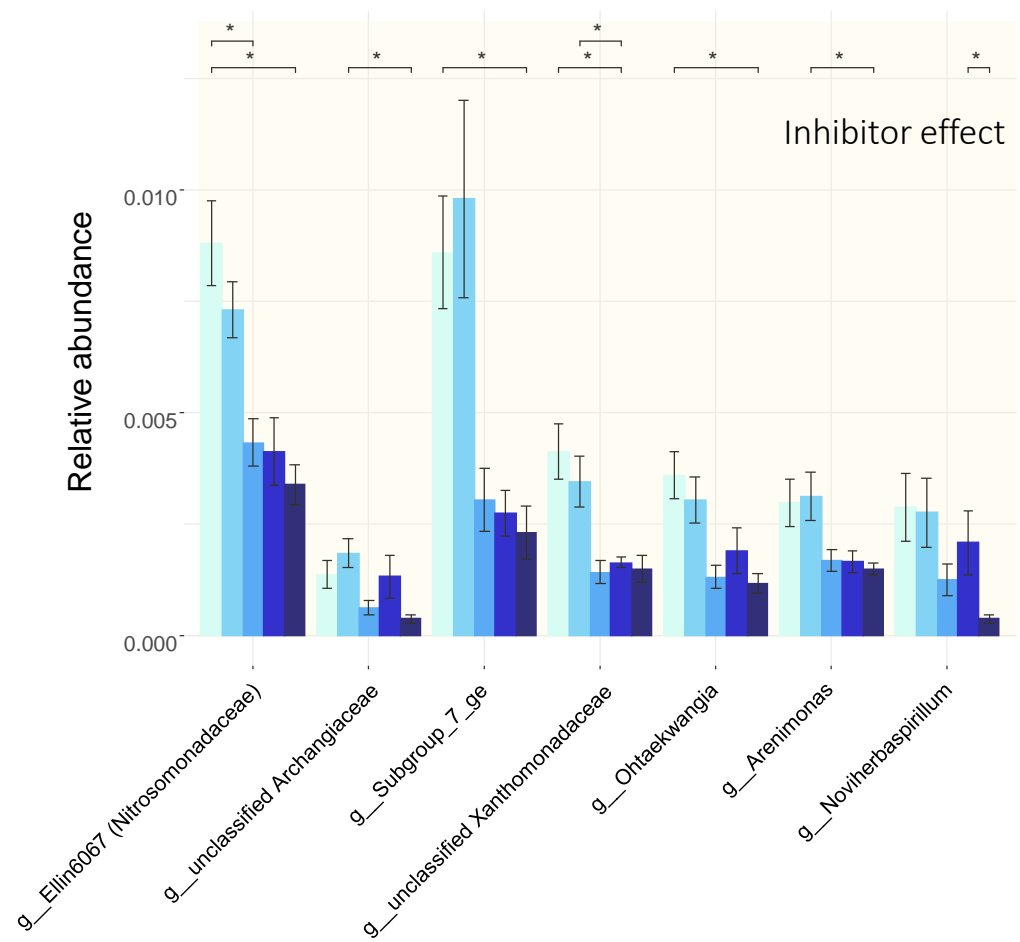
► Biomarkers of sulfamethoxazole concentrations

LEfSe

LEfSe on times 1,3,7 days

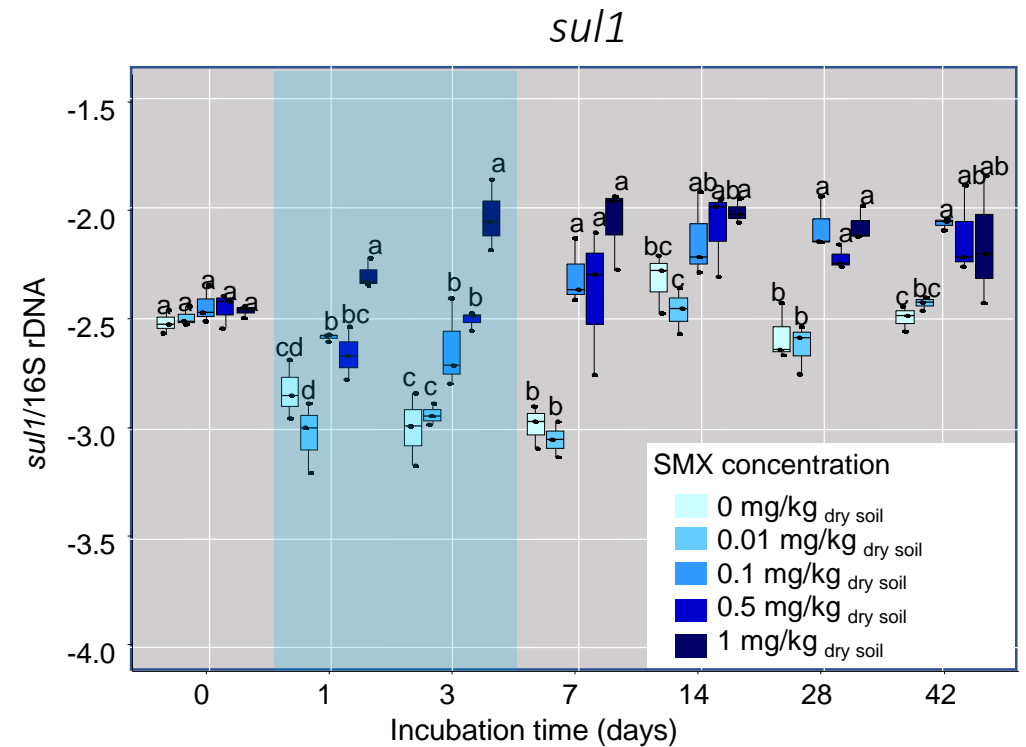
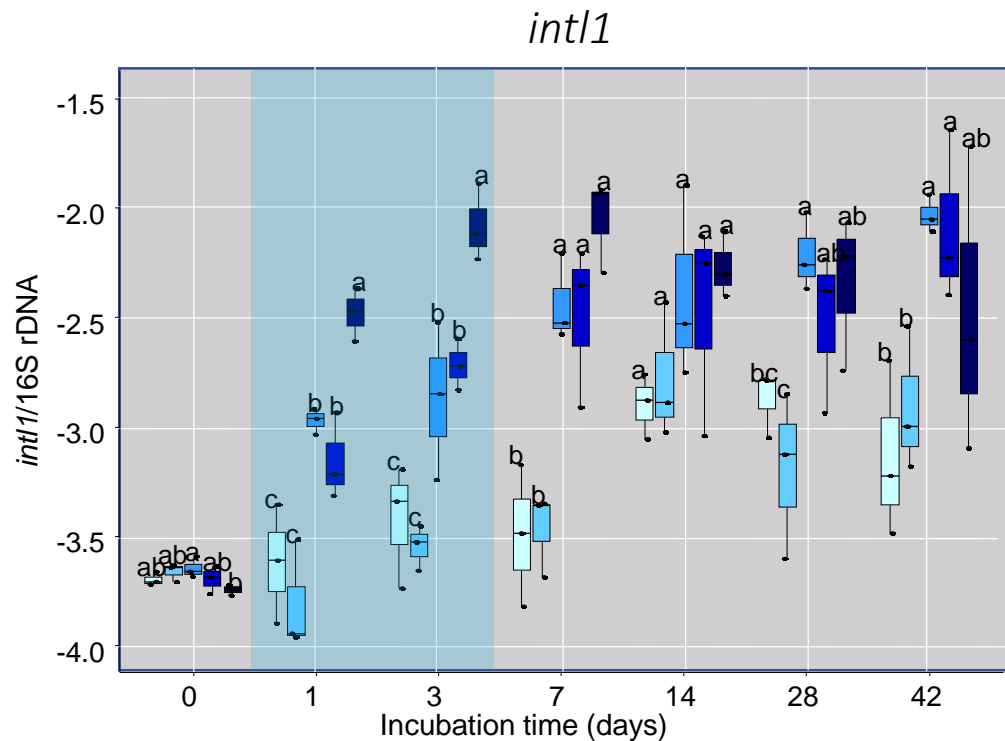


LEfSe on times 14,28,42 days



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

➤ 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)

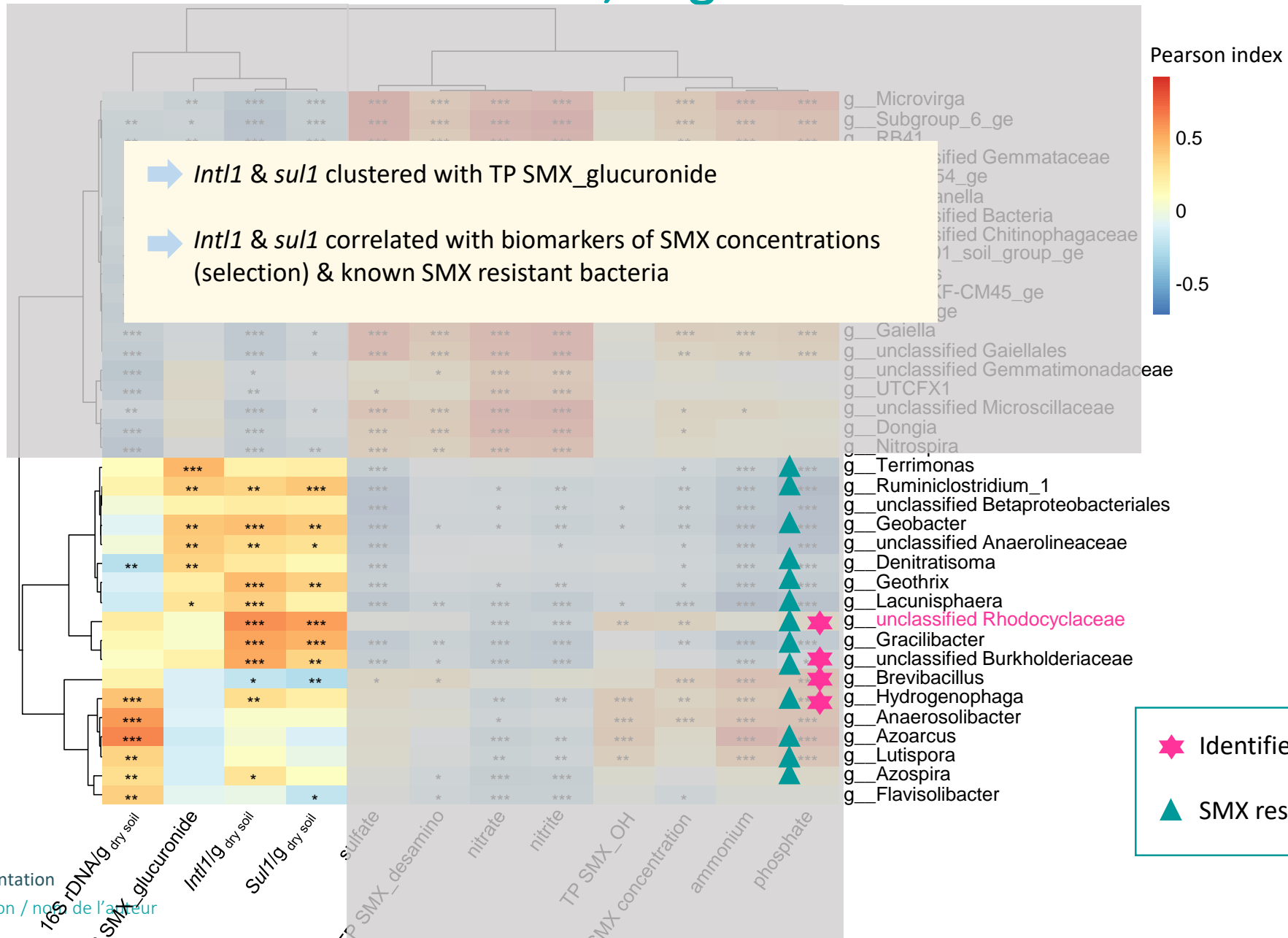
➔ Concentration effect: **3 levels** between 1 & 3 days (0, 0.01 // 0.1, 0.5 // 1 mg/kg dry soil), then **2 levels** (> 0.01 mg/kg dry soil)

➔ 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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