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Hydration and evaporation effects on bacterial horizontal transfer of antibiotic resistance genes in porous media under sub-inhibitory antibiotic selection

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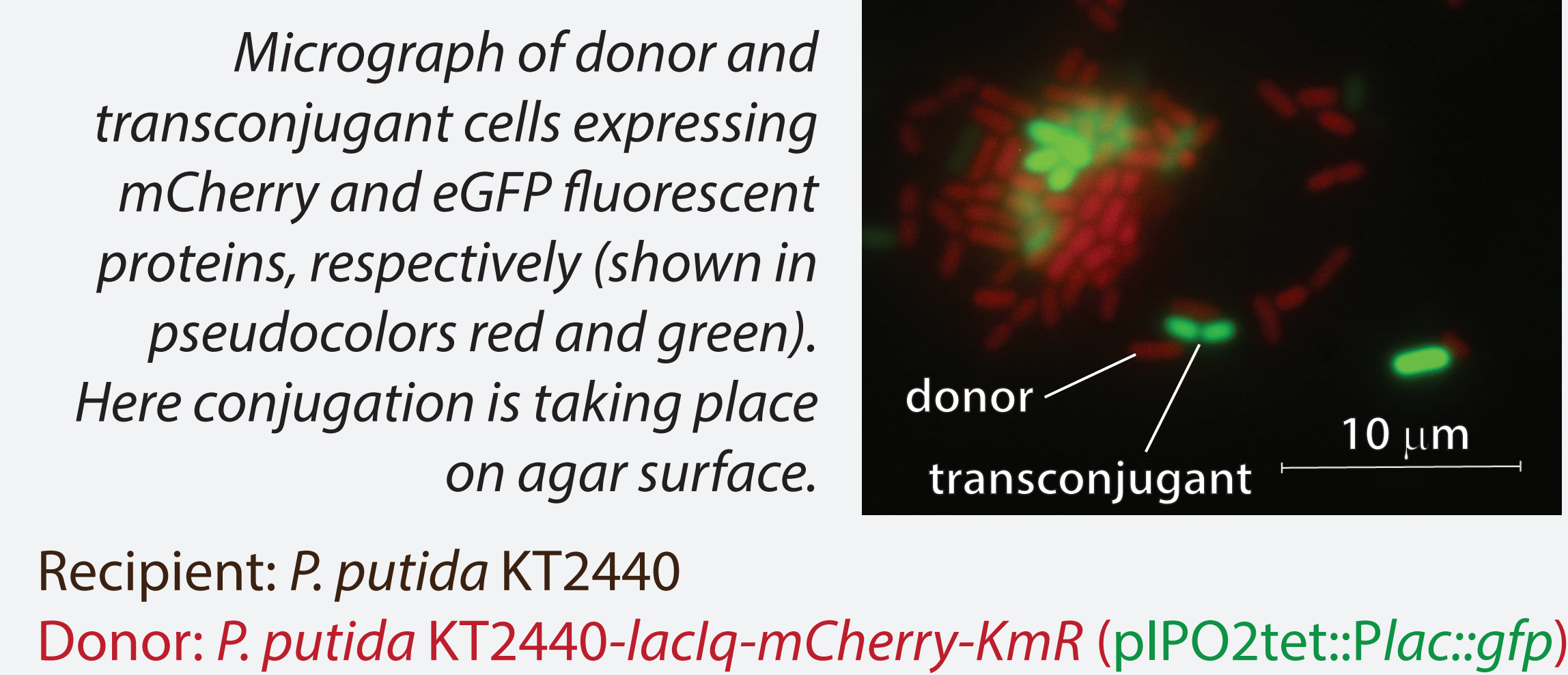
Introduction

Horizontal gene transfer (HGT) is a key factor in the emergence and dissemination of antibiotic resistance among bacterial populations. The factors influencing HGT and the propagation of resistance genes in natural environments such as soil are not yet fully understood. Here, we focused on HGT by bacterial conjugation in porous media that mimic unsaturated conditions in soil in order to examine the effects of hydration parameters.

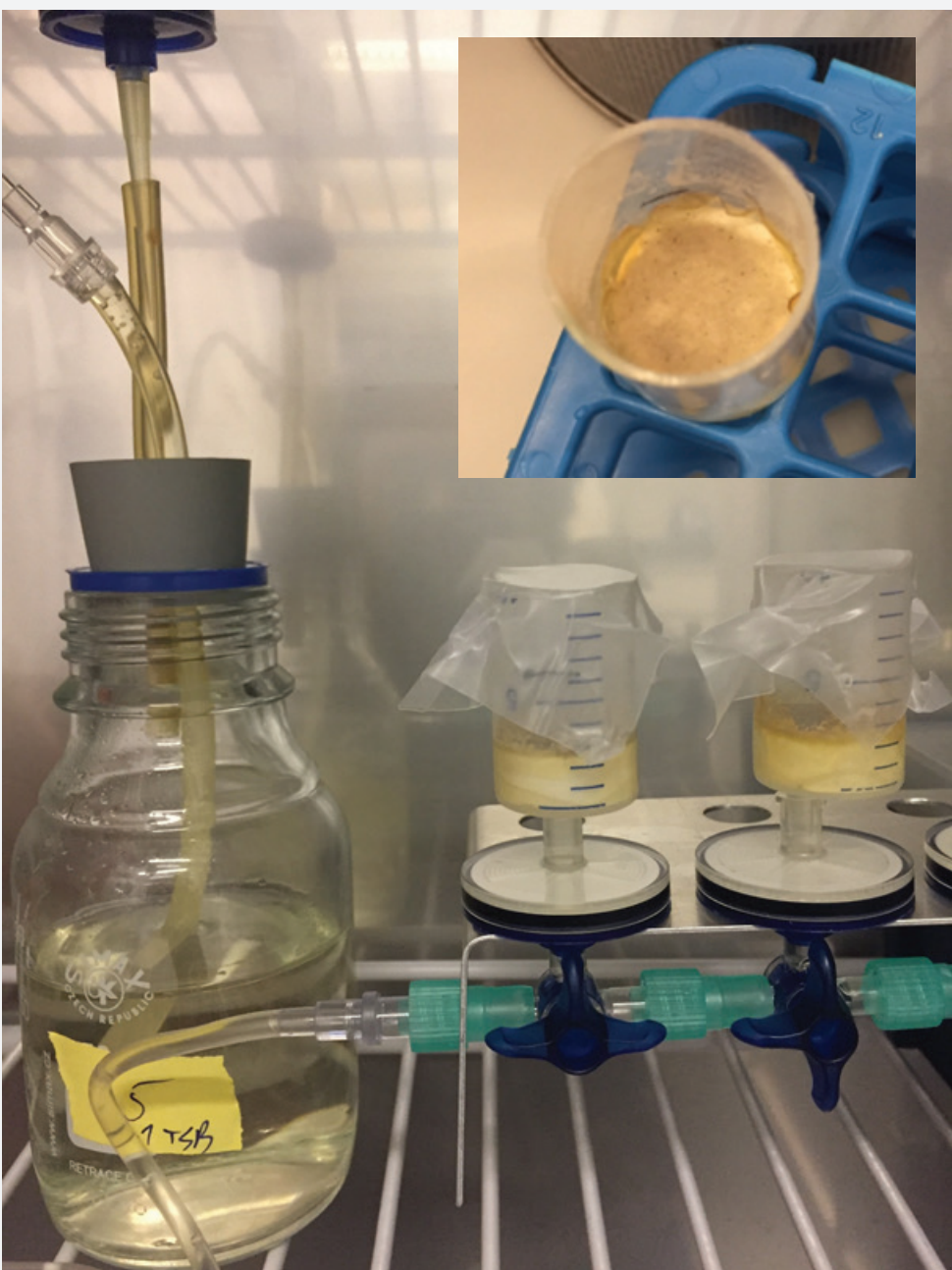
Objective: To quantify conjugation events in porous media as function of hydration conditions, and to determine whether variations in sub-inhibitory antibiotic concentrations (e.g., due to soil evaporation) would affect the distribution of resistance in bacterial populations.

Methods

Pseudomonas putida served as donor and recipient of the conjugative plasmid pIPO2 carrying a tetracycline resistance gene, while a tagging system with fluorescent proteins allowed us to visually discriminate donors and transconjugants (for details see Klümper *et al.*, 2015).



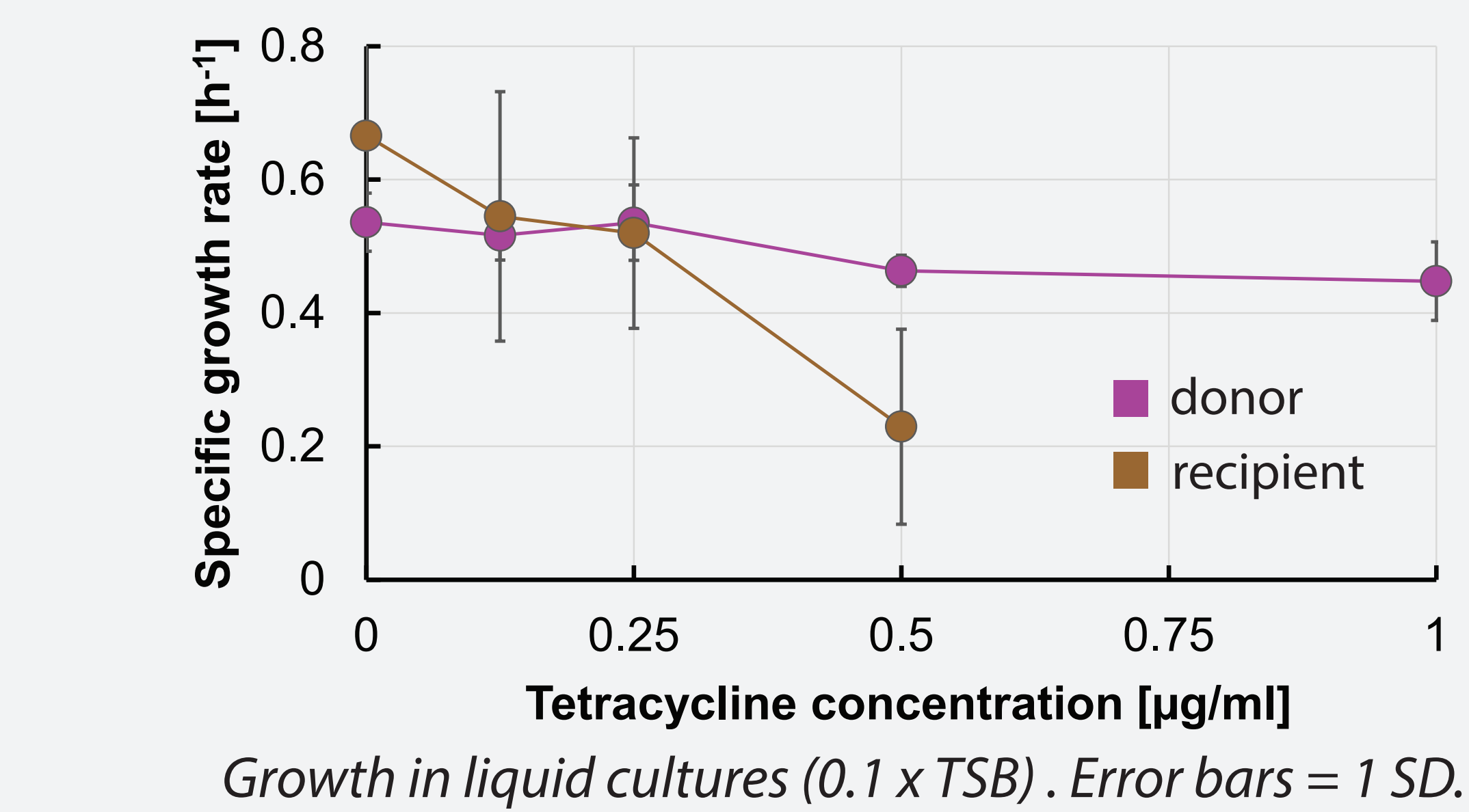
Recipient and donor cells (initial ratio 10:1) were incubated on agar surfaces or in sand microcosms with controlled hydration conditions mimicking the effects of soil water matric potential. Each microcosm contained 0.5 g of quartz sand and was hydrated with 0.1 x tryptic soy broth (TSB). Tetracycline was provided at 0.5 μg/ml (below its minimal inhibitory concentration). After 20 hours of incubation at 25 °C, cells were collected and resuspended in buffer medium for plating on selective media and CFU enumeration.



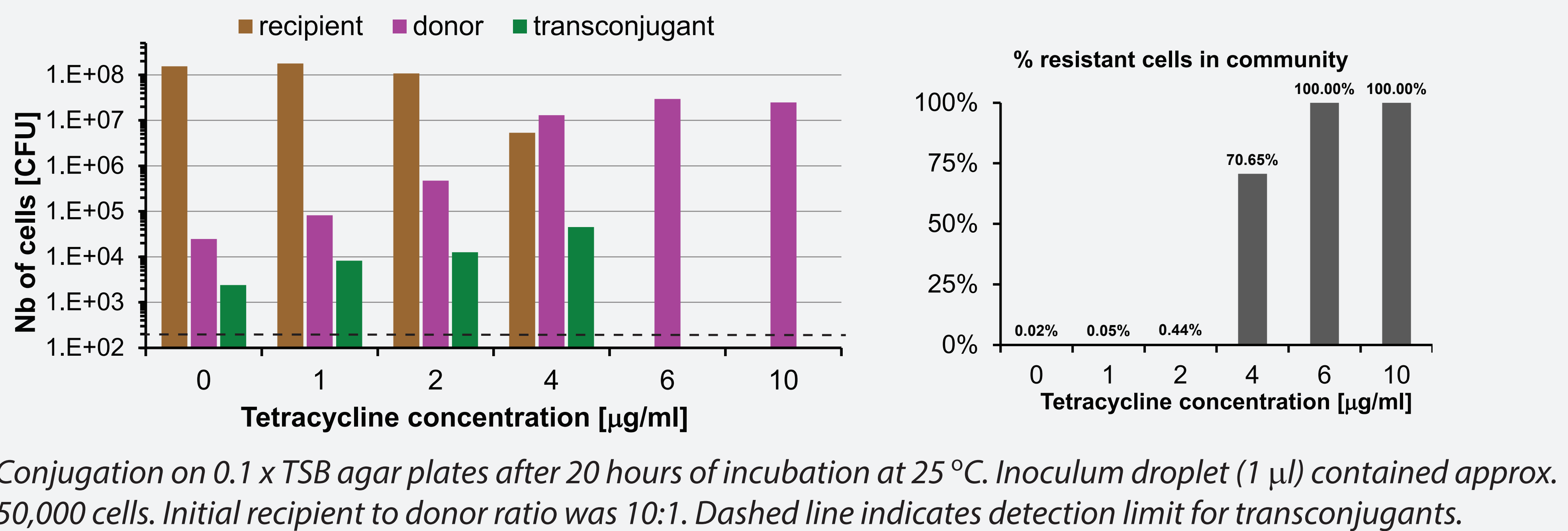
Sand microcosms with controlled hydration conditions

Results

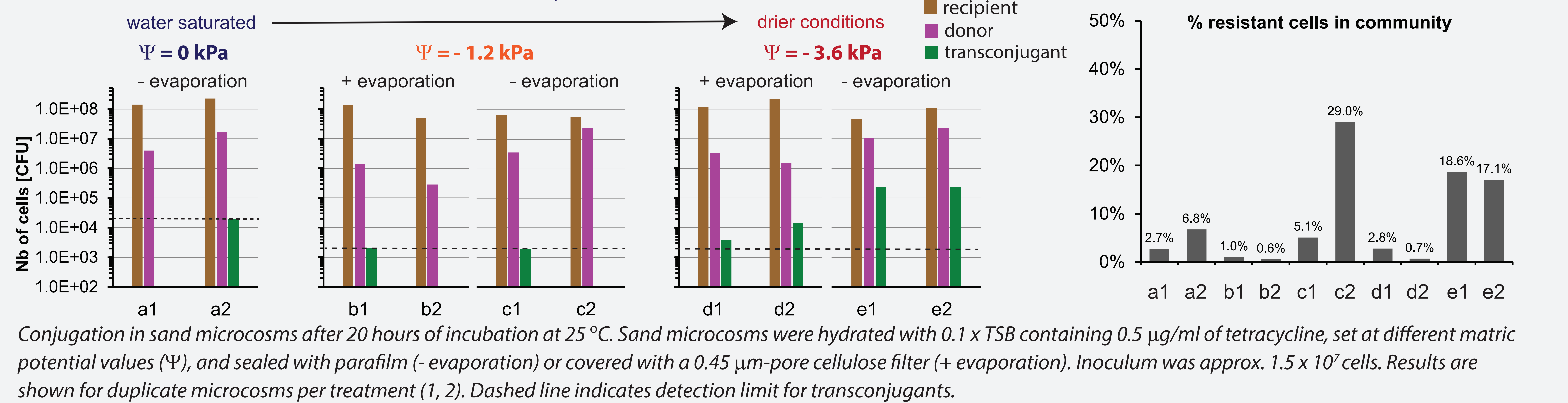
Growth at sub-inhibitory antibiotic concentrations



HGT and resistant/sensitive ratios on agar surface



HGT and resistant/sensitive ratios in hydrated porous media



Conclusions

- ★ HGT (conjugation) was observed in hydrated porous media mimicking unsaturated soil, and lower matric potential values (drier conditions) resulted in higher numbers of transconjugants. This suggests that thinner aqueous films may promote HGT, possibly by limiting cell dispersion.
- ★ Reduced evaporation (parafilm sealing) appeared to result in more conjugation events and higher proportion of resistant cells in the community. This suggests competing effects of increased drying and of higher antibiotic concentrations (both due to evaporation) on HGT and on the distribution of resistance in the community.

Reference: Klümper U., Riber L., Dechesne A., Sannazzarro A., Hansen L. H., Sorensen S. J., and B. F. Smets (2015) Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. *The ISME Journal* 9:934-945.

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