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Phytobeneficial Traits under the Control of Multiple Crosstalks' Belowground

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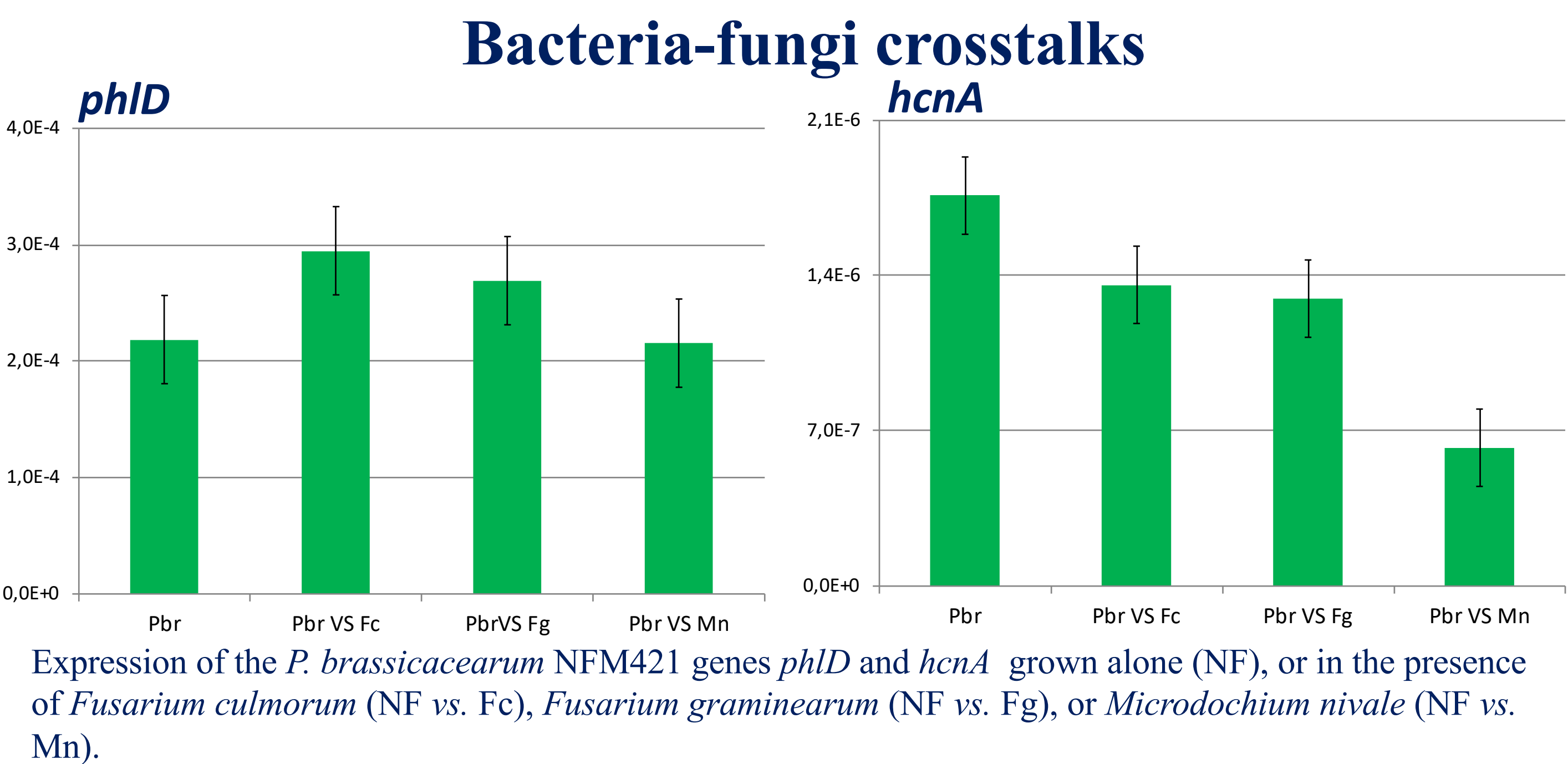
Introduction

Pseudomonads play crucial roles in plant growth promotion and the control of plant diseases. Specifically, many fluorescent pseudomonads with biocontrol abilities protect plants from soil-borne diseases by producing antimicrobial secondary metabolite(s) such as hydrogen cyanide (HCN) and 2,4-diacetylphloroglucinol (DAPG) (Haas & Défago, 2005). Among them, *Pseudomonas brassicacearum* described as the major root-associated bacterium in the rhizosphere of *Arabidopsis thaliana* and *Brassica napus* (Achouak *et al.*, 2000; 2004).

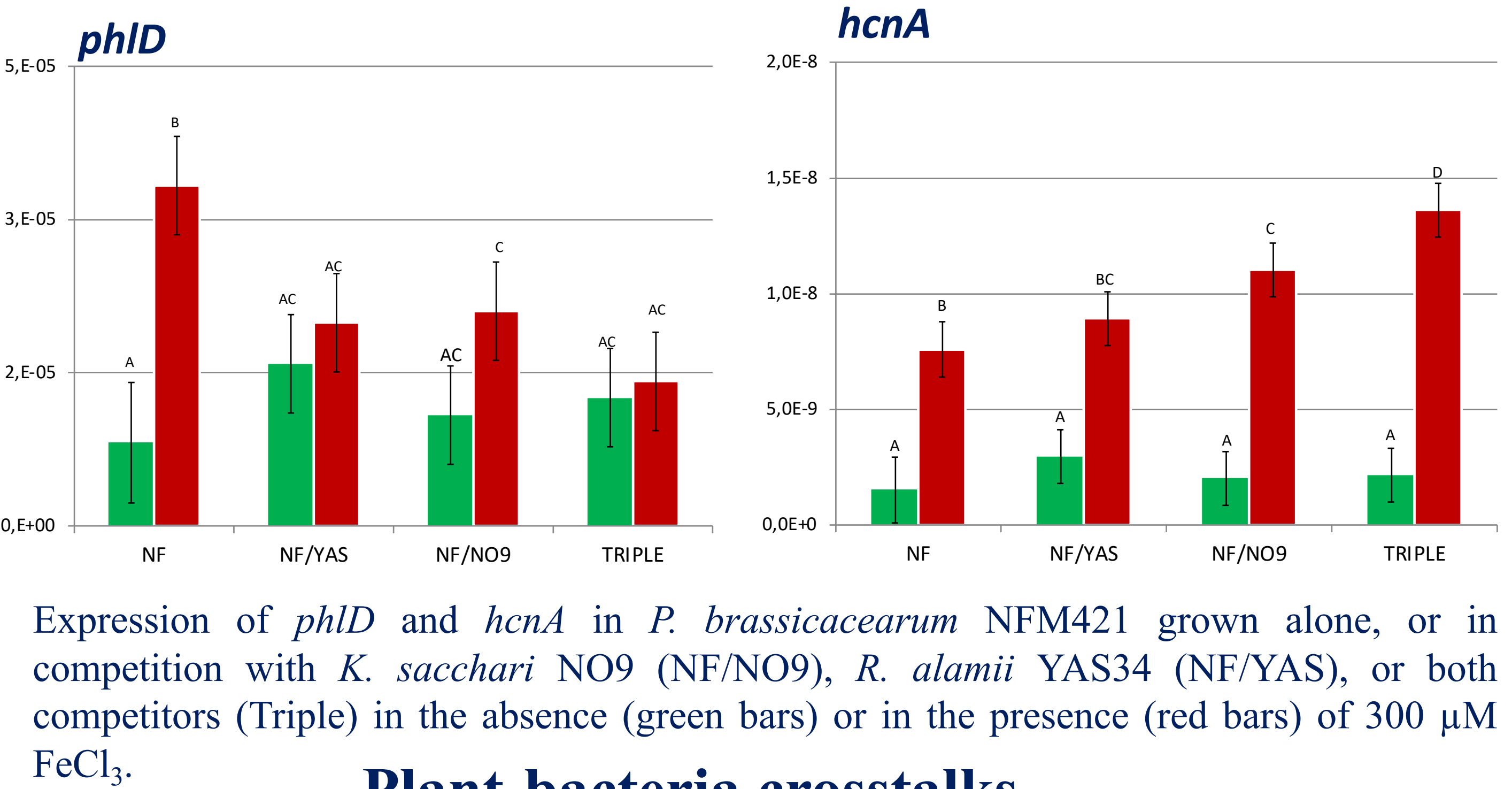
Aim

Various living organisms (*i.e.* other rhizobacteria, fungi and plants) might interfere with the efficiency of biocontrol agents. In this work, we assessed the expression of phytobeneficial genes, *phlD* and *hcnA*, in *P. brassicacearum* co-cultivated with three phytopathogenic fungi, *Fusarium culmorum*, *Fusarium graminearum* and *Microdochium nivale*, and two other rhizobacteria, *Kosakonia sacchari* NO9 and *Rhizobium alarii* YAS34 in the presence or absence of plant, as well as in relation to iron availability. We also investigated the impact of the introduction of these rhizobacteria on the assembly and network of the plant root-associated microbiota in a natural soil.

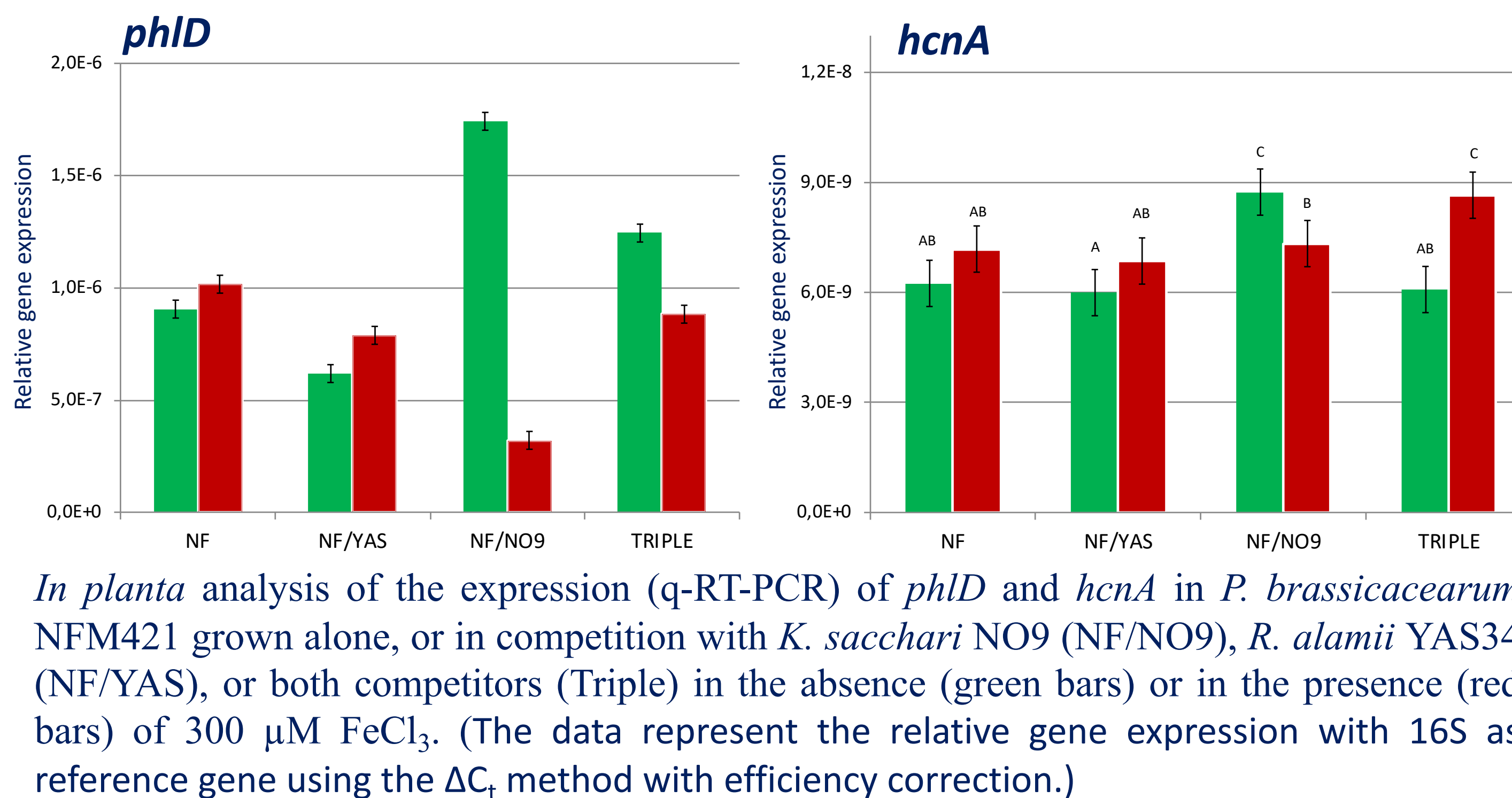
Antifungal activity under control



Bacteria-bacteria crosstalks

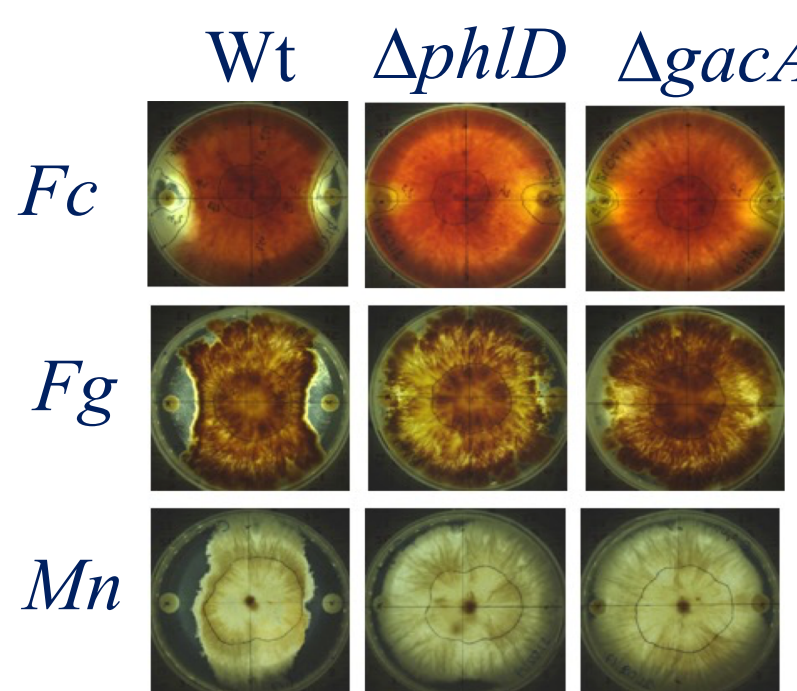


Plant-bacteria crosstalks

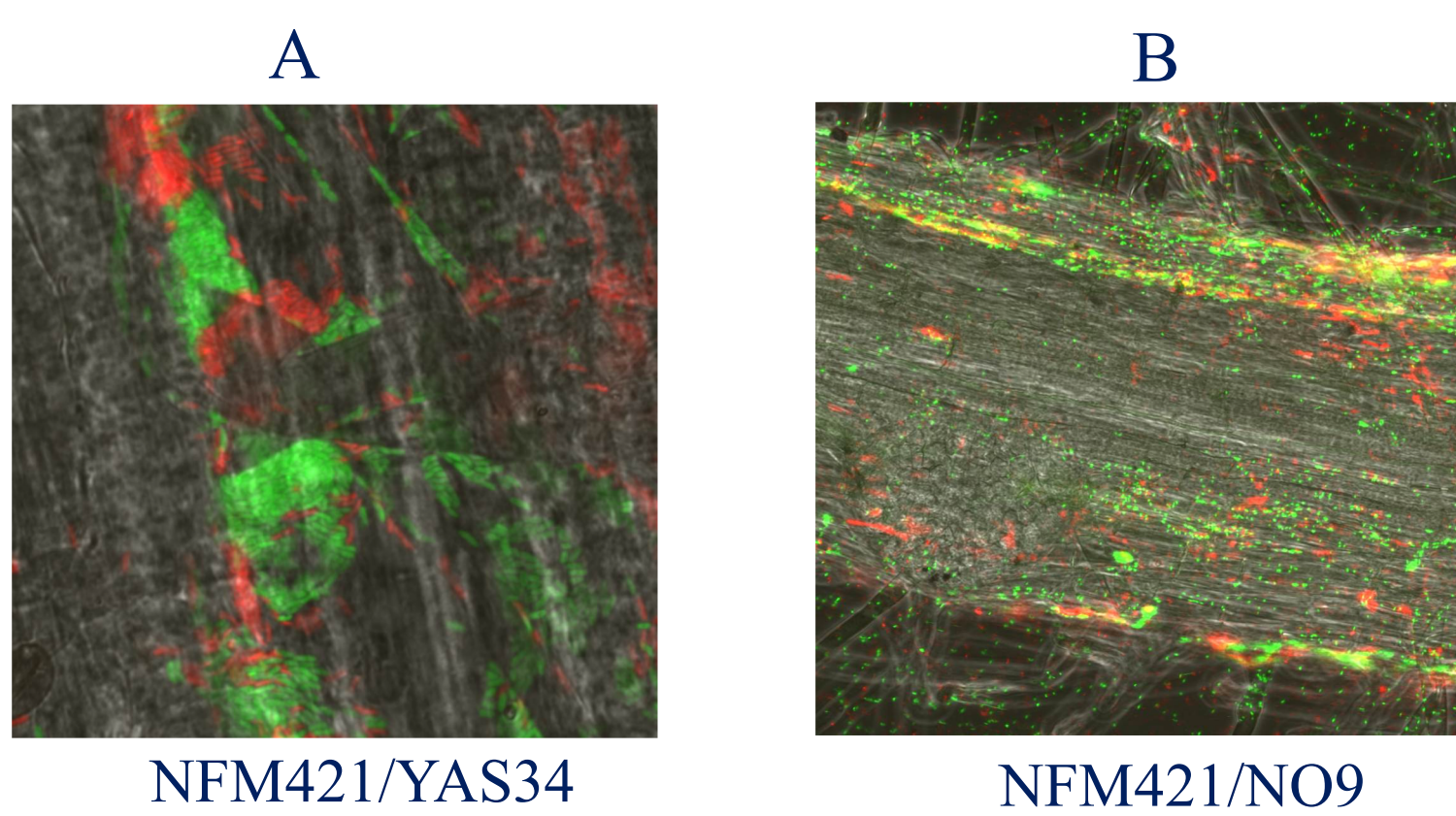


Conclusion

- Antifungal activity of NFM421 strain depends only on DAPG. HCN production is suppressed by fungi.
- In the presence of NO9 and YAS34 the antifungal activity of NFM421 is modulated in relation to iron availability.
- In planta*, NFM421 increases the expression of *phlD* in the presence of NO9 strains with which it shares the same microniche on the root surface.
- Inoculation of the plant with these bacteria led to a shift of microbiota assemblage, hubs and ecological network.



Antifungal activity of *P. brassicacearum* NFM421 Wt, $\Delta phlD$ and $\Delta gacA$ towards *Fusarium culmorum* (Fc), *Fusarium graminearum* (Fg), *Microdochium nivale* (Mn).



In planta colocalization of *rfp*-tagged *P. brassicacearum* NFM421 with: *gfp*-tagged *R. alarii* YAS34 (A), and *gfp*-tagged *K. sacchari* NO9 (B).

Mat & Methods

Microbial cultures and growing conditions

Fungal-bacteria interaction experiments were performed using potato dextrose agar (PDA) medium. Bacterial competitions: pure culture and co-culture in CAA medium +/- 300 μ M FeCl₃.

phlD and hcnA gene expression

RNA extracted from bacteria, retro-transcribed using *phlD* or *hcnA* primers. Real-time PCR was performed in triplicate, and mRNA relative expression was normalized to the 16S reference gene.

In planta phlD and hcnA gene expression

Surface sterilized seeds of *Brassica napus* were inoculated with one or a mixture of the three bacterial species.

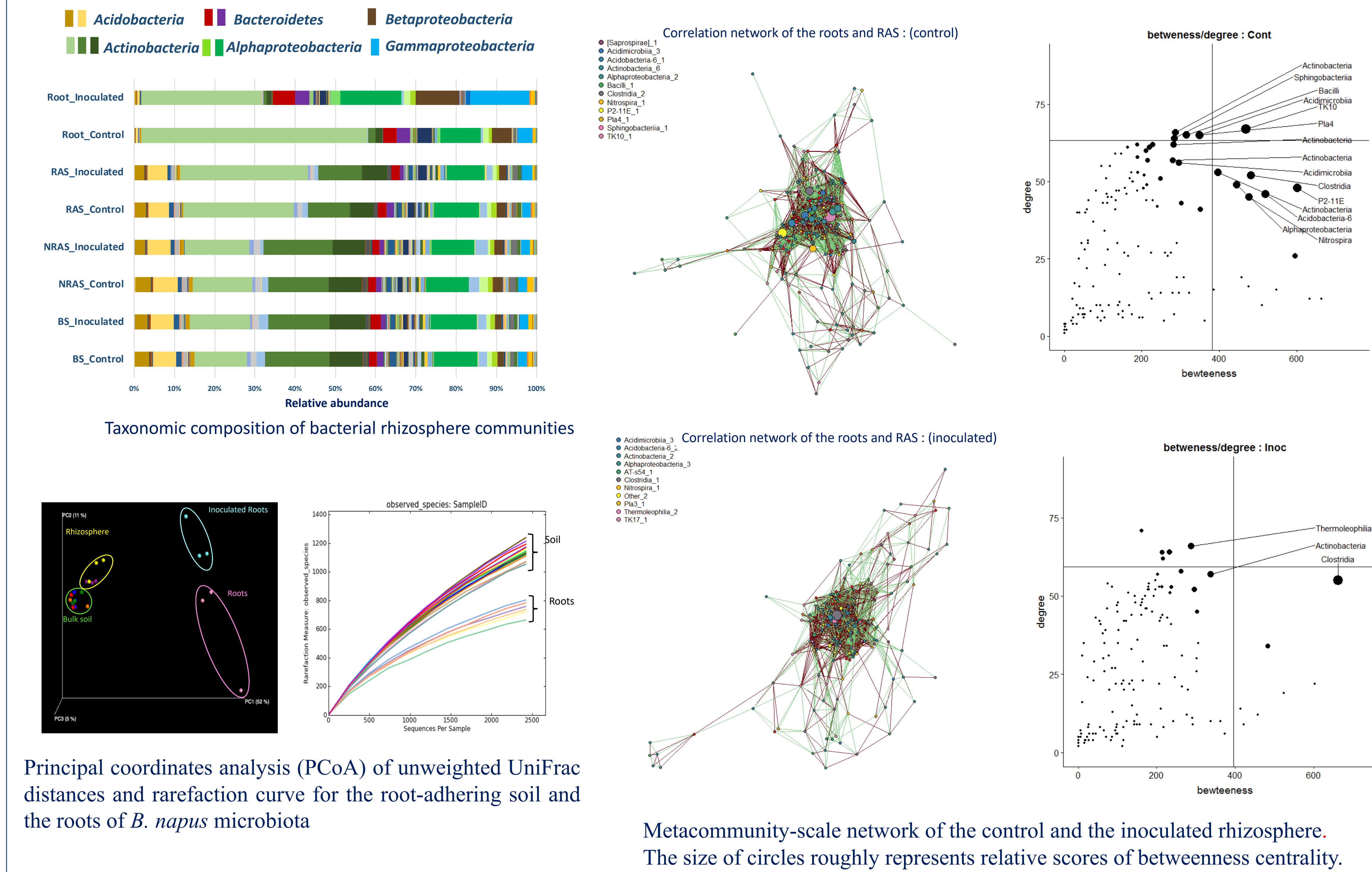
Colocalization of competing bacteria

Bacteria were tagged by plasmids encoding fluorescent proteins. YAS34 strain pHc60::gfp (Santaella *et al.*, 2008); NO9 strain pBBR1MCS-2::mkate; NFM421::rfp or NFM421::gfp (Achouak *et al.*, 2004). Root colonization was imaged by CLSM.

Microbiota profiling & microbial network

Comparison of microbiota from the rhizosphere of inoculated plants vs control plants by DNA extraction from the root-adhering soil and the roots and bulk soil; 16S *rrs* amplicon sequencing on a MiSeq instrument in paired-end 2 x 300 bp mode (Illumina) (Achouak *et al.*, 2019). Correlations with a p-value >0.05 (Spearman correlation test) were selected to build networks (package igraph 1.2.2 using R 3.4.3). The determination of the betweenness and degree for every nodes and their size made it possible to identify the hubs.

Shift in microbiota assemblage and ecological network upon bacterial inoculation



Principal coordinates analysis (PCoA) of unweighted UniFrac distances and rarefaction curve for the root-adhering soil and the roots of *B. napus* microbiota

Schnider-Keel *et al.*, 2000 Autoinduction of 2,4-diacetylphloroglucinol biosynthesis in the bio-control agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial 194. metabolites salicylate and pyoluteorin. J. Bacteriol. 182:1215–1225. Santaella C., Schœue M., Berge O., Heulin T., & W. Achouak (2008) Role of exopolysaccharide produced by *Rhizobium* sp. YAS34 in the colonization of *Arabidopsis thaliana* and *Brassica napus* and biofilm formation on roots. Environ. Microbiol 10(8):2150–63 Achouak W., Conrod S., Cohen V., and Heulin. (2004) Phenotypic variation of *Pseudomonas brassicacearum* as a plant root colonization strategy. Mol Plant Microbe Interact. 17:872–9. Achouak W., L. Sutra, T. Heulin, J-M. Meyer, N. Fromin, S. Degraeve, R. Christen, and L. Gardan. (2000). Description of *Pseudomonas brassicacearum* sp. nov. and *Pseudomonas thivervalensis* sp. nov., two root-associated bacteria isolated from *Arabidopsis thaliana* and *Brassica napus*. Int. J. Syst. Evol. Microbiol 50:9–18. Achouak W, Abrouk D, Guyonnet J, Barakat M, Ortet P, Simon L, Lerondelle C, Heulin T, Haichar Fez (2018) Root exudates mediated host plant selection of microbiota. *FEMS Microbiol Ecol*