Influence of grapevine rootstock-scion combination on rhizosphere and root endophyte microbiomes

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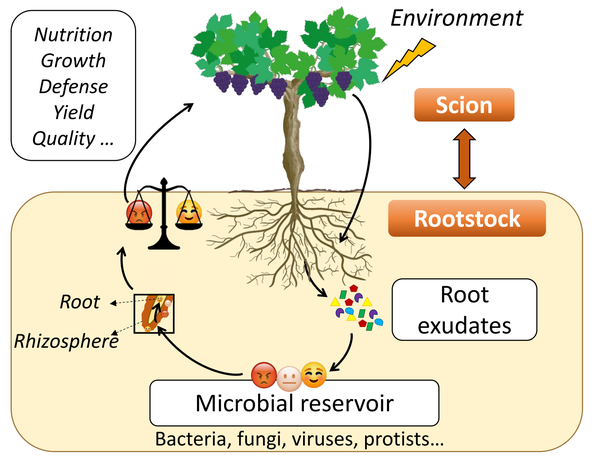
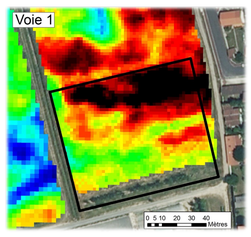
Grapevine interacts with soil microorganisms

Soil is a reservoir of microorganisms playing important roles in biogeochemical cycles and in interactions with plants in the rhizosphere (*Darriaut et al., 2022*). The rhizosphere is the volume of soil that sticks to the roots, which influences its biology and chemistry. The rhizosphere is the gateway of various microorganisms in the root endosphere. In both compartments, microbial communities impact the plant health. For example, arbuscular mycorrhizal fungi improve grapevine growth and tolerance to biotic and abiotic stresses (*Trouvelot et al., 2015*). Rhizodeposits (such as sugar, organic and amino acids, secondary metabolites, dead root cells ...) are released by the roots and influence the community of rhizospheric microorganisms. They act as signaling compounds or carbon sources for microbes. Root exudates composition depends on several factors including genotype. Recent studies showed that grapevine rootstock-scion combination regulates the bacterial and fungal microbiomes in the rhizosphere and the root endosphere (*Marasco et al., 2022; Vink et al., 2021*). The molecular mechanisms involved in this regulation are still misunderstood as well as the impact of microbiomes composition on grapevine health. The aim of this study is to understand the effects of rootstock and scion genotypes on rhizosphere and root endophyte microbiomes. Correlation between microbiomes composition and phenotypic data (plant vigor, berry yield and mineral nutrition) will be explored in order to better understand the links between the microbiomes and plants physiology.



Experimental design

GreffAdapt plot:
- **3 blocks according** to the **soil resistivity**
- **Annual phenotypic analysis** on **plants** & **yield**



10 rootstock-scion combinations were **studied:**

❊ **6 rootstocks grafted with the same scion**
→ **Rootstock impact on the microbiomes**

❊ **5 scions grafted on the same rootstock**
→ **Rootstock impact on the microbiomes**

One combination is common!

Sampling in May 2021

3 roots samples with rhizosphere on **2 blocks** for **each combination** at a **depth** of **20-30 cm** in **sterile** condition

Roots with rhizosphere

Rhizosphere

Sterilized roots

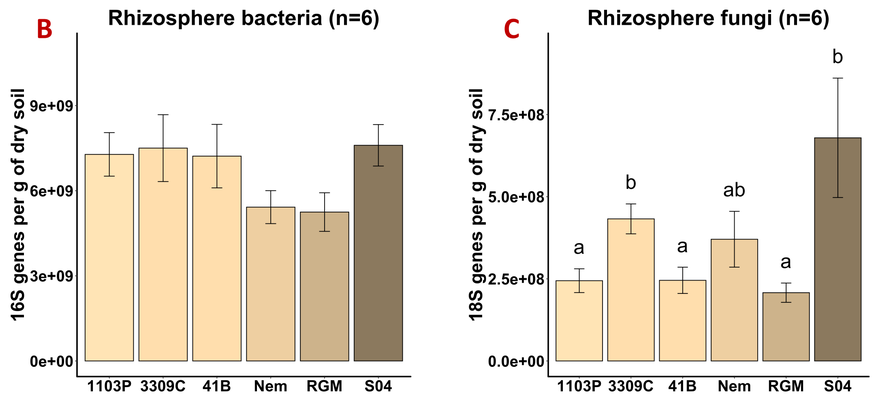
- **Quantitative PCR** (**bacteria, fungi** & **archaea**)
- **Cultivable approach** (**bacteria** & **fungi**)
- **Targeted metagenomics**:
  - **Bacteria**: 16S rRNA gene
  - **Fungi**: ITS region
  - **Arbuscular mycorrhizal fungi**: 28S rRNA gene
- **Measurement** of **macro-/micro-elements**

Effect of the rootstock genotype on the quantity of bacteria and fungi in the rhizosphere

Fig.1: Rootstock effect on the quantities of bacteria and fungi measured by the cultivable and the qPCR approaches (A). n=3 individual plants. **Results obtained by qPCR for the bacteria (B) and the fungi (C).** Data are presented as means ±SE, n=6 individual plants. Letters indicate significant differences between genotypes as determined by Pairwise Wilcoxon Rank Sum Tests.

A

	Cultivable		qPCR	
	Bacteria	Fungi	Bacteria	Fungi
Test	Two-way ANOVA	Two-way ANOVA	Two-way ANOVA	Scheirer-Ray-Hare
Rootstock (R)	ns	ns	ns	**
Block (B)	ns	ns	ns	ns
R x B	ns	ns	ns	ns



❊ **No effect** of the **rootstock** on the **amount** of **rhizospheric bacteria** & **fungi** with the **cultivable approach**

❊ **Effect** of the **rootstock** on the **amount** of **fungi** with the **qPCR approach**

❊ **SO4** has the **highest level** of **fungi** in the **rhizosphere**

Effect of the rootstock on the proportion of bacterial and fungal phyla in the rhizosphere and the root endosphere

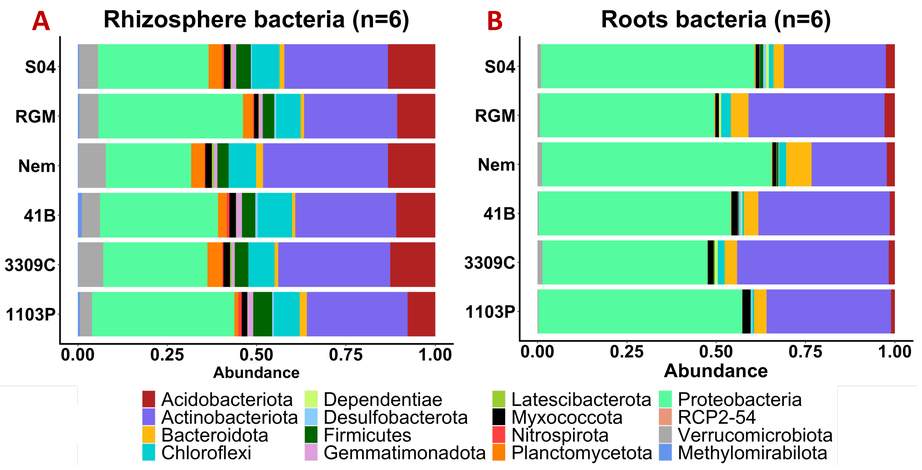


Fig.2: Bacterial community presented at phylum level in the rhizosphere (A) and the roots (B)

❊ **Rhizosphere**: **domination** of **proteobacteria** & **actinobacteriota,** followed by **acidobacteriota**

≠

❊ **Roots**: **domination** of **proteobacteria**, followed by **actinobacteriota** & **bacteroidota**

❊ **Proportion of bacterial phyla** in **both microbiomes differs between rootstocks**

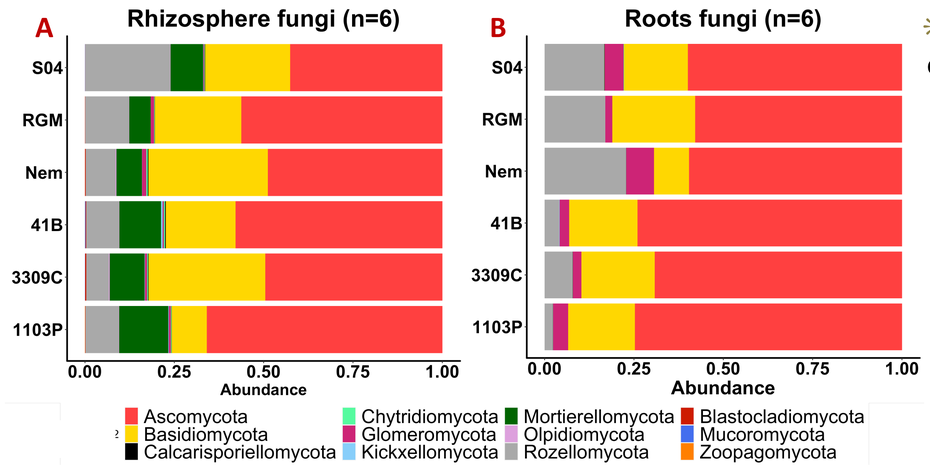


Fig.3: Fungal community presented at phylum level in the rhizosphere (A) and the roots (B)

❊ **Rhizosphere: domination** of **ascomycota,** followed by **basidiomycota, rozellomycota** & **mortierellomycota**

≠

❊ **Roots**: **domination** of **ascomycota**, followed by **basidiomycota, rozellomycota** & **glomeromycota**

❊ **Proportion** of **fungal phyla** in **both microbiomes differs between rootstocks**

Effect of the rootstock genotype on the α-diversity of bacteria and fungi in the rhizosphere and the root endosphere

Chao1: Number of observed species (richness) + number of unobserved one (estimated)

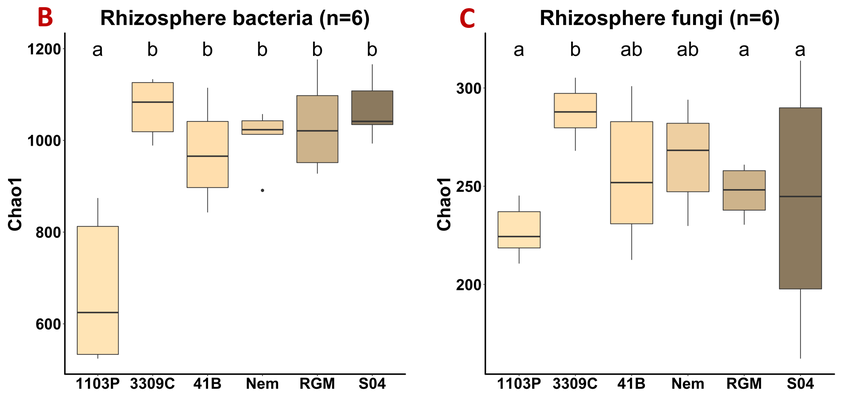
Fig.4: Rootstock effect on the α-diversity of bacteria and fungi (A). n=3 individual plants. **Comparison of Chao1 index for bacteria (B) and fungi (C) in the rizosphere between rootstock genotype.** Data are presented as means ±SE, n=6 individual plants. Letters indicate significant differences between genotypes as determined by Pairwise Wilcoxon Rank Sum Tests.

A

	Bacteria		Fungi	
	Rhizosphere	Roots	Rhizosphere	Roots
Test	Two-way ANOVA	Two-way ANOVA	Two-way ANOVA	Two-way ANOVA
Rootstock (R)	***	ns	*	ns
Block (B)	ns	ns	ns	ns
R x B	***	*	ns	ns

❊ **Roots: no effect** of the **rootstock** on the **α-diversity** of **bacteria** & **fungi**

❊ **Rhizosphere: effect** of the **rootstock** on the **α-diversity** of **bacteria** & **fungi**



❊ **1103P** has the **lowest α-diversity** of **bacteria** in the **rhizosphere**

❊ **3309C** has a **higher α-diversity** of **fungi** than **1103P, RGM** & **SO4**

Effects of rootstock genotype on the rhizosphere and root endophyte microbiomes:

➢ **Impact on the quantity of rhizospheric fungi but not on bacteria**

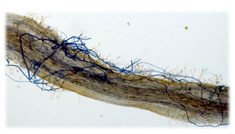
➢ **Impact on the proportion of bacterial and fungal phyla in both microbiomes**

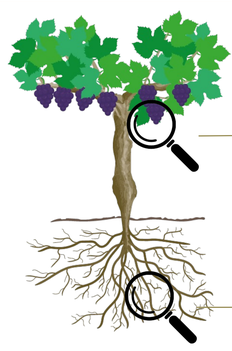
➢ **Impact on α-diversity of bacteria and fungi in the rhizosphere but not in the roots**

Conclusion and future directions

What is the contribution of the scion genotype ?

What is the influence of grapevine combination on the AMF?





- **Phenotypic analysis** (vigor and yield) & **Content of micro-/macro-elements**
- **Microbiomes analyses**

→ **Data integration: Impacts of the microbiomes on plant health?**

Bibliography

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