EFFECT OF ONE-YEAR COVER CROP AND ARBUSCULAR MYCORRHIZA INOCULATION IN THE MICROBIAL SOIL COMMUNITY OF A VINEYARD

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Background

The "terroir" and the organoleptic properties of wine are influenced by the soil microbial composition. Climate, biogeography and crop management have the potential to modify the composition and functionality of the soil microbial community. Different agronomic techniques, like maintaining green covers or direct application of beneficial microorganisms as Arbuscular Mycorrhizal Fungi (AMF) to the soil have also been used to increase soil microbial diversity. However, their effectiveness is context dependent since soil characteristics and clime can determine final soil microbial composition.

The aim of this study was to analyse the impact on the soil and its microbiota of two agricultural techniques: inoculation with AMF and the establishment of a leguminous cover crop.

Experimental design

The study has been carried out in a 'Callet' cultivar vineyard in Binissalem (Mallorca, Spain). Three different treatments were applied: control (normal management); inoculation with a commercial mycorrhizae complex of *Rhizophagus irregularis* and *Funeliformis mosseae* applied by irrigation; and establishment of a winter legume crop of *Vicia sativa* and *Vicia faba,* used as green cover. Soil samples were collected close to roots of the plants in sterile tubes and sent for molecular analysis to Biome Makers laboratory in Sacramento, US. The 16S rRNA and ITS marker regions were selected for bacteria and fungi, respectively. In the same soil sampling points, 700 g of soil were collected for physicochemical analysis (texture, pH, Electrical conductivity, OM, Organic Carbon, Nitrogen and Phosphorus).

Control with a usual management

Leguminous cover crop.

Inoculation of commercial mycorrhizae
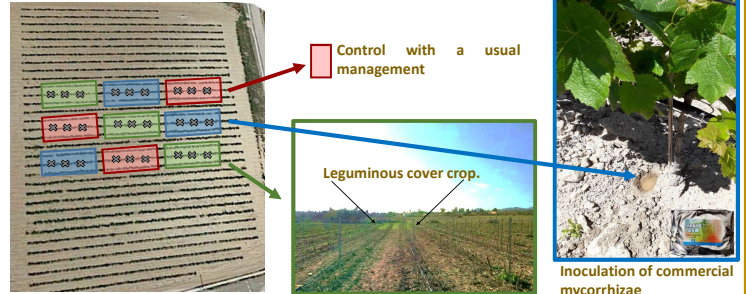
Bioinformatics workflow

Libraries were prepared following a two-step PCR Illumina protocol using custom primers for the 16S rRNA V4 and the ITS1. Pair-end sequencing (2×300bp) was conducted in an Illumina MiSeq. The bioinformatics processing of reads included the merging of forward and reverse paired reads to create robust amplicons, using Vsearch with minimum overlaps of 100 nucleotides and merge read sizes between 70 and 400 nucleotides. OTU clustering was performed at 97% sequence identity, followed by quality filtering through *denovo* chimera removal using the UCHIME algorithm. Taxonomic annotation was performed using the SINTAX algorithm, which uses k-mer similarity to identify the top taxonomy candidate, after which we retained results where the species level had a score of at least 0.7 bootstrap confidence. We used the SILVA database version 132 and UNITE database version 7.2 as taxonomic references.

Do different treatments lead to different soil physicochemical characteristics?
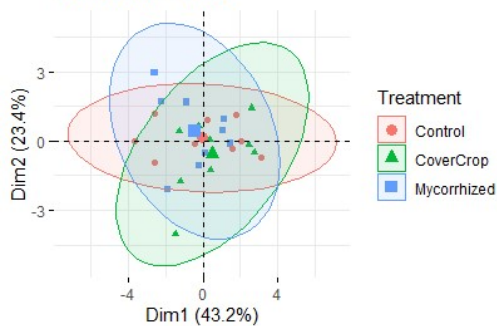
No. The PCA considering pH, conductivity, organic material, organic C, N, P, and % of texture classes (clay, silt and sand), did not show any variation among samples depending on the treatment.

Is the diversity of bacteria/fungi affected by the treatments applied?

There were not significant differences between treatments for Shannon index both for bacterial and fungi (P>0.05 GLMM).

Do different soil characteristics affect microbial community composition?

Soil content of Organic Matter significantly decreased fungi diversity P=0.02 GLMM. Meanwhile, non soil characteristics appeared to have a statistically significant effect on bacterial diversity (p>0.05 GLMM).

Does soil treatment or soil characteristics have an effect on the composition of microbial communities?

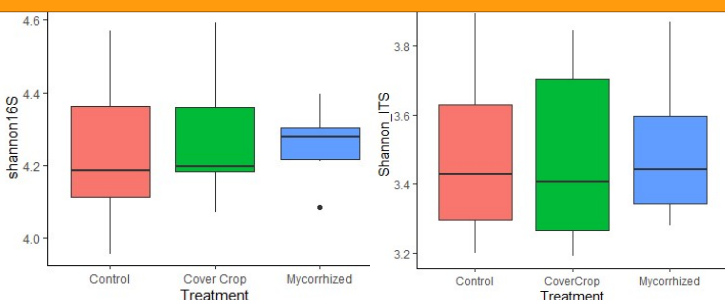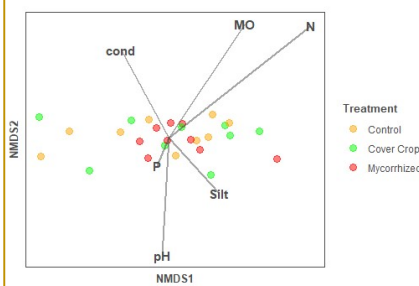### 16S BACTERIAL COMMUNITY

No significant effect of treatments was detected on bacterial community composition (p=0.790 permutational ANOVA (PERMANOVA; function adonis2 in package "vegan")). Meanwhile, soil organic matter and nitrogen content significantly influenced the bacterial community composition.
(OM p = 0.015; N p = 0.013 PERMANOVA).

ITS FUNGY COMMUNITY

No significant effect of treatments was detected on fungi community composition(p=0.940; PERMANOVA). However, soil pH significantly influenced the fungi community composition (pH p = 0.009; PERMANOVA).

Ordination by nonmetric multidimensional scaling (NMS) based on Bray–Curtis dissimilarity (function metaMDS in package "vegan") did not show a variation in bacterial community composition dissimilarity between treatments. Nonetheless, we observed a significant correlation between organic matter (p= 0.054) and nitrogen (p= 0.033) with the NMDS scores, showing the influence of this environmental factors on the bacterial community.
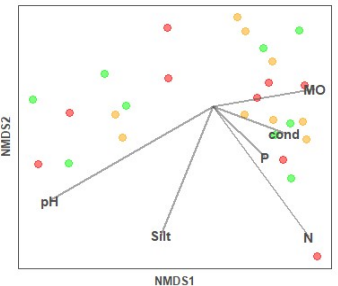
NMS did not show a variation in fungi community composition dissimilarity between treatments. However, there was a significant correlation of pH (p= 0.013) with the NMDS scores (the envfit function in the vegan package) confirming the influence of this environmental factor on fungi community.

TAKE HOME MESSAGES

✓ **Key soil characteristics to maintain productivity, like Organic Matter, Nitrogen content and pH properties, significantly affect soil microbiome.**
✓ **Cover crop and mycorrhizas inoculation, described as potential activators of microbial diversity, did not show a significant effect on it in the first year of application.**
✓ **Further research is needed to find conditions denoting positive impact of treatment on microbial soil diversity.**