

# EFFECT OF THE COMMERCIAL INOCULUM OF ARBUSCULAR MYCORRHIZA IN THE ESTABLISHMENT OF A COMMERCIAL VINEYARD OF THE CULTIVAR "MANTO NEGRO"

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## TAKE-HOME MESSAGES

- ✦ We found non-favourable effects of mycorrhizal inoculation.
- ✦ We question the in-field application of mycorrhizal inoculation.
- ✦ This type of biofertilizer should only be used when the cultivation conditions require it (e.g. very low previous microbial diversity, foreseeable stress due to drought, salinity, or lack of nutrients) and not as a general fertilization practice.

## BACKGROUND

The favorable effect of symbiosis with arbuscular mycorrhizal fungi (AMF) has been known and studied since the 60s. Nowadays, many companies sell commercial inoculants of AMF, in order to be used as biofertilizers and encourage sustainable biological agriculture. However, the positive effect of these commercial biofertilizers on plant growth is not always demonstrated, especially under field conditions. In this study, we used a commercial inoculum on newly planted grapevines of a local cultivar Manto Negro grafted on a common rootstock R110.

### GROWTH PARAMETERS

Inoculated plants presented less maximum plant height (cm), from the surface of the soil to the tip of the vine, measured 42 days, 72 days and 170 days after inoculation. Also, less number of shoots were measured on September 2020, 513 days after inoculation (Figure 1A). However, the shoot length (cm), and shoot diameter were not different between treatments.

### PHYSIOLOGICAL PARAMETERS

Overall, mycorrhization did not have any influence on plant physiological state during the first two months after inoculation. However, contrary to what one would expect, after the summer (177 day), the inoculated plants reached a lower rate of photosynthesis of mycorrhized plants compared to controls in autumn (Figure 1B). Although, these differences disappeared over time, and we did not observe an effect one year after the inoculation.

### PRODUCTION AND QUALITY

Overall, inoculation did not affect total harvest weight (Figure 2E) or number of clusters per vineyard (Figure 2F), however, negatively affects the average weight per cluster (Figure 4D) grape production. Regarding quality grapes did not show differences in acidity (Figure 2A), however, both brix and pH were lower in inoculated plants (Figures 2B and C).

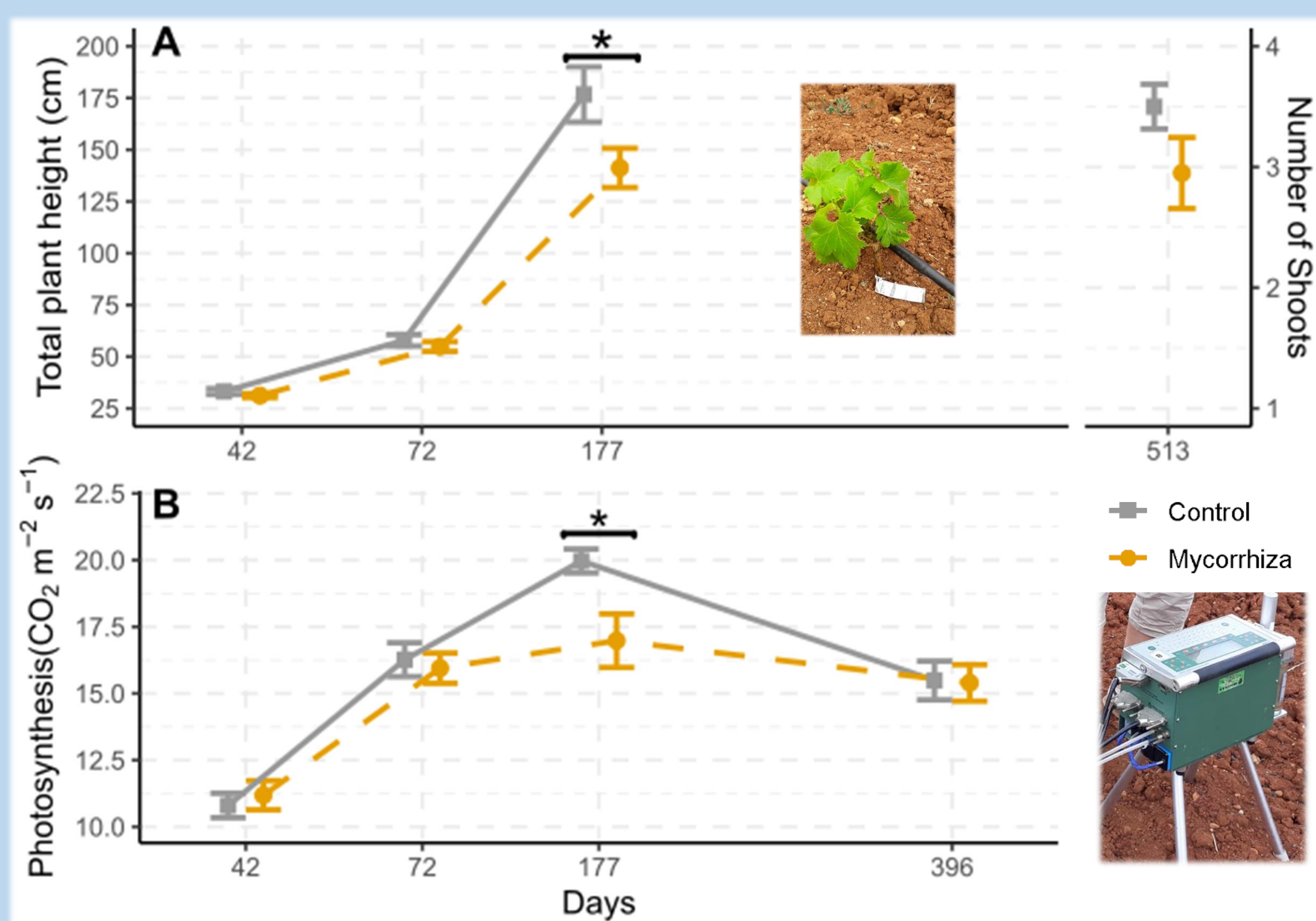


Figure 1. Total plant height, number of shoots (A) and photosynthesis (B). Data are means and standard error (SE) of 20 plants per treatment. Asterisks show significant differences between mycorrhiza treatment and control (\* p<0.01) for a post-hoc test by Bonferroni's pairwise comparisons.

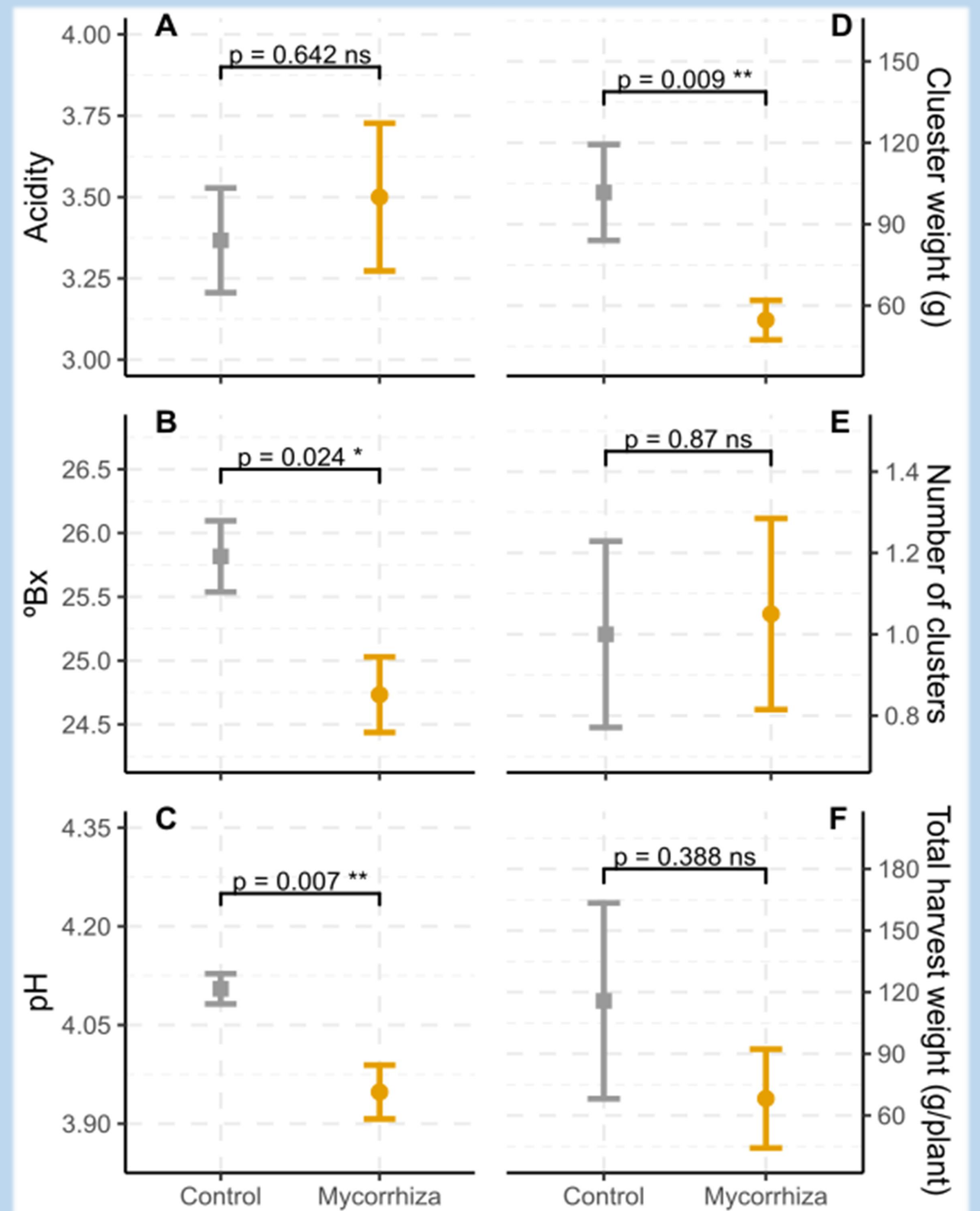


Figure 2. Total acidity (A), total soluble solids (°Brix) (B), pH (C), cluster weight (D), number of clusters (E) and total harvest (g/plant) (F). Data are means and standard error (SE) of 20 plants per treatment. Asterisks show significant differences between mycorrhiza treatments (\* p<0.05; \*\* p<0.01) for a post-hoc test by Bonferroni's pairwise comparisons.

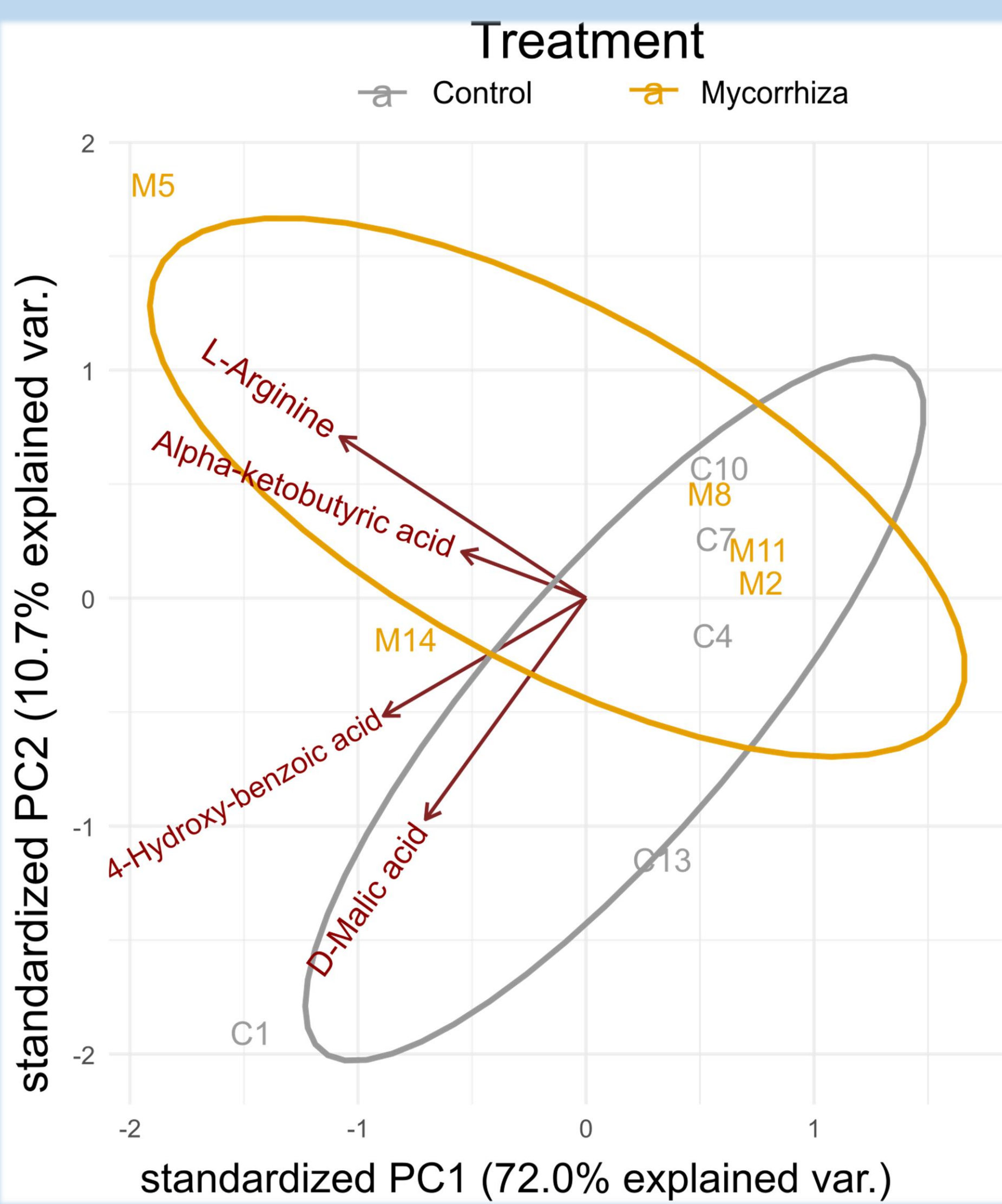


Figure 4. Biplot of the first two principal components (PC1 and PC2) from a PCA using OD at 590 nm for Biolog EcoPlate™ 31 carbon substrates after 168h of incubation. Substrates with contributions >1% are shown.

### SOIL BACTERIA FUNCTIONAL DIVERSITY

The community-level physiological profiles were analyzed by Biolog EcoPlate™ (Figure 3). The average value of the absorbance of three wells of each substrate was used for the calculation of the Shannon index (H) and the Average Well Color Development (AWCD). In both cases we did not find significant differences between treatments. However, the two plates per treatment with more microbial activity showed different substrate oxidation (Figure 4). Control samples presented higher degradation of 4-Hydroxy-benzoic Acid and D-Malic Acid. Meanwhile, microbial communities from soils of mycorrhized plants showed higher activity in other substrate as L-Arginine and Alpha-ketobutyric Acid (Figure 4).

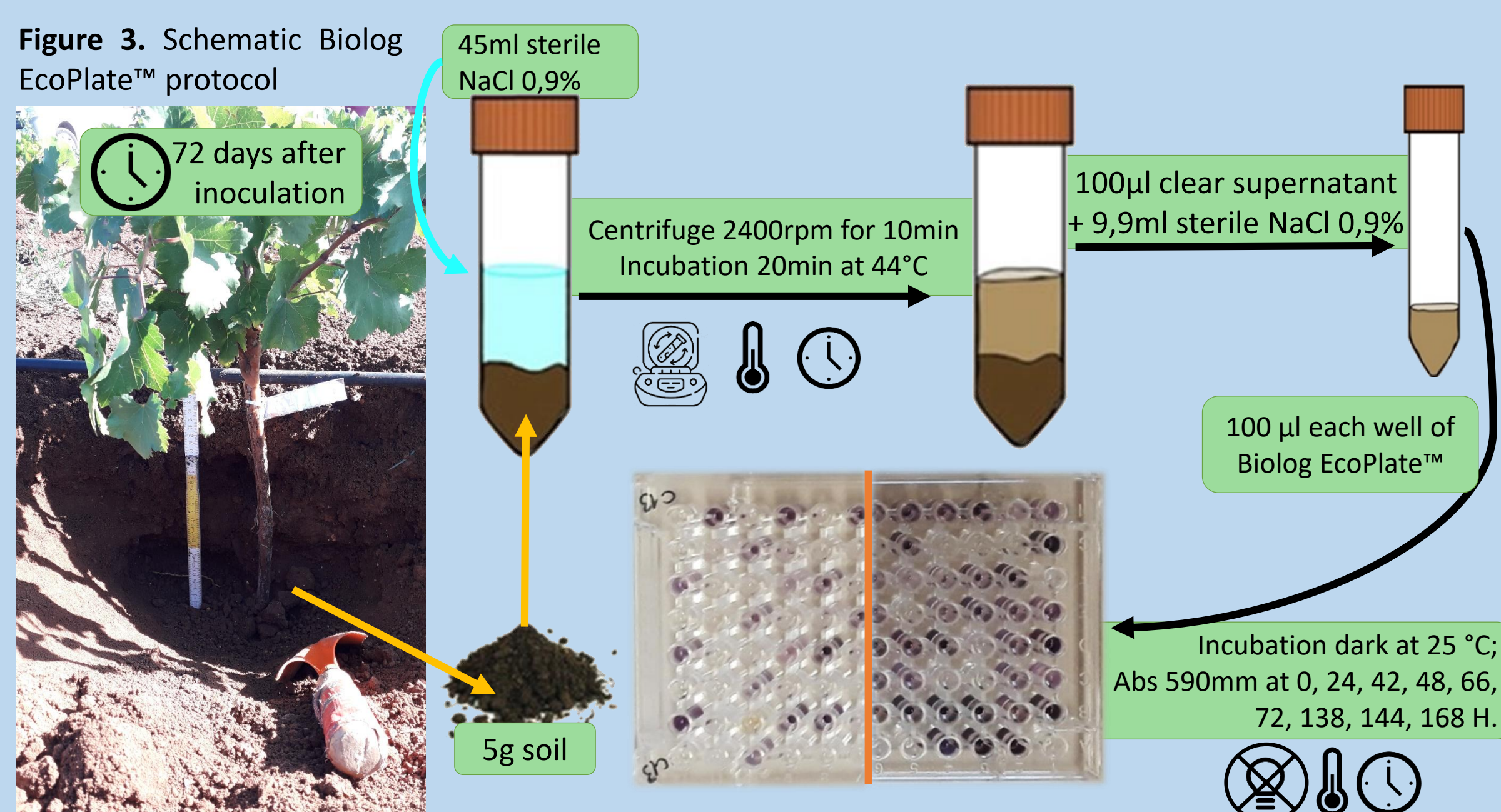


Figure 3. Schematic Biolog EcoPlate™ protocol. 45ml sterile NaCl 0,9%. 72 days after inoculation. Centrifuge 2400rpm for 10min. Incubation 20min at 44°C. 100µl clear supernatant + 9,9ml sterile NaCl 0,9%. 100 µl each well of Biolog EcoPlate™. Incubation dark at 25 °C; Abs 590nm at 0, 24, 42, 48, 66, 72, 138, 144, 168 H.



### EXPERIMENTAL SITE AND PLANT MATERIAL AND TREATMENTS

The experiment was carried out in an ecological commercial vineyard in Sencelles. The plantation was settled on fifth of April 2019. 20 plants were mycorrhized by adding 4 grams of inoculum (AEGIS SYM ©) in a hole of 15 cm of depth as close as possible to the plant roots. The inoculum used contained 25 spores/g of *Rhizoglyphus irregularis* (before known as *Glomus intraradices*) and 25 spores/g of *Funneliformis mosseae* (before known as *Glomus mosseae*).

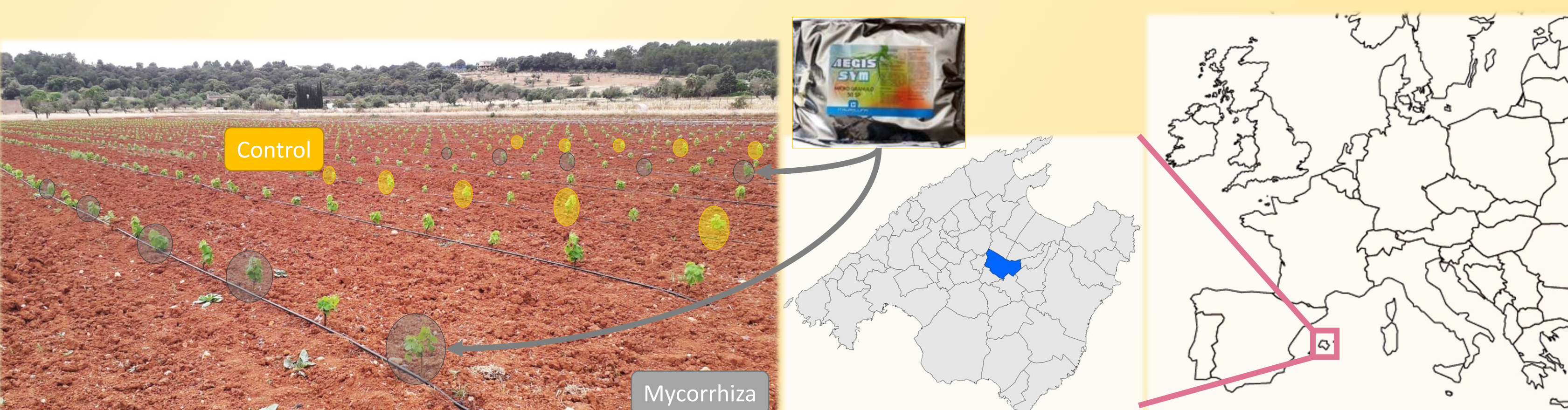


Table 1. Soil physico-chemical properties.

Parameter	Mean ± SE (n=6)
pH	8.38 ± 0.016
Conductivity (sS/m)	0.15 ± 0.002
Organic Matter (Walkley-Black %)	1.35 ± 0.072
N (Kjeldahl g/kg)	0.143 ± 0.007
P (Olsen mg/kg)	9.95 ± 3.48
Sand (%)	13.6 ± 0.723
Silt (%)	15.7 ± 0.510
Fine slime (%)	25.6 ± 0.4381
Clay (%)	45.01 ± 1.183

### SOIL PHYSICO-CHEMICAL CHARACTERISTICS

The farm has a basic soil with a high proportion of clays and low levels of organic matter, high nitrogen content and great variability in phosphorus content (Table 1). Plants probably did not present nutritional stress.



Acknowledgements: This work has been funded through project RTI2018-094470RC22 Thanks to Biel Torrens for facilitating our work in their commercial plantation

