Title: The effects of different regenerative agriculture practices on soil and plant microbiome: a study of its effects on grapevine defense system against downy mildew.

Objectives: The overall objective of this proposal is to prove that the application of regenerative agriculture practices in vineyards located in different Mediterranean areas, particularly in Portugal, can improve soil fertility by favoring the presence of beneficial microorganisms, that in turn will also promote a higher plant resilience to biotic stress. This main aim will be achieved through: 1) Metagenomic analyses (soil/rhizosphere) from vineyards with different agricultural practices (both from Portugal and other Mediterranean countries); 2) Metagenomic analysis plant microbiome (phyllosphere) in order to understand soil to plant microorganisms distribution and to understand if their distribution reflects the influence of vineyard management; 3) To select, from the identified microbial communities, beneficial microbes (based on their ability to form symbiosis with grapevine or to act as priming or biocontrol agents) and to isolate them; 4) To apply the isolated beneficial microorganisms either on soil (pots with grapevine cuttings) or to perform a foliar application and to infer on their impact on plant fitness and pathogen resilience.

Methodology: Regenerative agriculture can enhance soil biodiversity, some of each may be beneficial microbes. The enrichment of beneficial microbes in the soil and rhizosphere, particularly plant-growth-promoting rhizobacteria and fungi , may lead to a enhance in crop growth and yield, as well as improve the crop resilience against biotic stress, since they may trigger plant immunity by inducing the priming state. Although some information is already available, namely on the role of beneficial bacteria application or mycorrhiza inoculation in reducing the severity of grapevine grey mold and downy mildew, the characterization of microbial communities in the vineyard with different regenerative agriculture practices is largely unknown. The PhD student will characterize the soil/rhizo- and phyllosphere microbial community through a metagenomic approach based on Oxford Nanopore Long-reads Technology at BioISI genomics facility. A tailored iterative assembly scheme previously developed at BioISI Genomics will be used. Potential beneficial microorganisms will be selected, isolated and characterized (ribosomal DNA sequencing, will be performed, ability to promote plant growth testing (e.g. siderophores production, solubilization of phosphate, production of auxin, ability to fixate nitrogen). The ability of the microorganisms to hydrolyze and recycle certain substrates will also be evaluated through the analysis of their extracellular enzymatic activities (phosphatase, protease, peroxidase, phenol oxidases, sulphatase, urease, b-glucosidase activity). To evaluate the isolated microbial consortia potential on improving grapevine resilience to biotic stress, the grapevine-*Plasmopara viticola* model will be used. *Vitis vinifera* cv Trincadeira and Pinot noir will be used as models and two approaches will be considered: (1) apply the isolated microorganism into the soil (e.g., root symbionts, growth promoting bacteria); (2) perform foliar application by spraying the leaves. In the first case, growth and yield parameters will be assessed. In both cases the effect of this application will be monitored through non-invasive bio-optical methods to assess the impact on plant photosynthetic capability. To study the effects on grapevine immunity priming, grapevine plants pre-inoculated with the beneficial microbes and non- inoculated plants will be inoculated with the pathogen *P. viticola.* Disease progression will be monitored through visual inspections using the OIV descriptors 452 and expression analysis of *P. viticola* pathogenicity effectors.

This project is within the framework of the approved European project REVINE - Regenerative agricultural approaches to improve ecosystem services in Mediterranean vineyards (PRIMA funding). The disclosure of the best regenerative viticulture practices through the characterization of microbial communities will not only allow the establishment of guidelines for a more sustainable agriculture but also promote the identification of microorganisms to be used as biocontrol and priming agents.

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The members of the supervision team are participating in the approved project REVINE. Their complementary expertise covers all the aspects of this project (grapevine research, plant-pathogen interaction, OMICs data, microbiome analysis; soil restauration, plant physiology and biochemistry).

Type of fellowship (select the correct option)
☐ National
☒ Mixed (Portugal and abroad: ENEA - Centro Ricerche Trisaia, Italy)