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MICROBIAL BIODIVERSITY AND ITS ROLE ON SOIL HEALTH AND AGRICULTURE RESILIENCE IN MEDITERRANEAN DRYLANDS

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Abstract

In Mediterranean ecosystems, agriculture faces significant challenges. Understanding the dynamics and diversity of microorganisms in arid lands is essential for improving soil quality and resilience to climate change. This study involves sampling 460 paired sites (two nearby locations with different land uses) across Spain, Croatia, Turkey, Greece, Tunisia, Morocco, France, and Italy, collecting topsoil samples (0–20 cm). While the study is still in its early stages, initial analyses have been conducted on a subset of samples, focusing on enzymatic activities. Microbiological analyses include: i) DNA-based massive sequencing, ii) phospholipid characterization (PLFA) for taxonomic identification, and iii) enzymatic activity assays to assess functional groups. Additional- ly, pesticide levels will be analyzed. The results show higher activities of β-glucosidase, acid phosphatase, and L-leucine aminopeptidase in woodland soils, possibly linked to a more diverse and active microbial community, less impacted by pesticide application compared to the olive tree farm. Conversely, lower arylsulfatase activity in woodland soils may be influenced by specific soil properties, such as the presence of rocks, which could affect sulfur cycling and enzyme function. This integrated approach will provide valuable insights into soil health across the Mediterranean basin.

Keywords*: Mediterranean drylands; soil; microorganisms; enzymatic activity*

1. Introduction

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Mediterranean ecosystems are characterized by a climate with warm, humid winters and hot, dry summers. Much of their soils are drylands, defined by a severe lack of water, where precipitation is counteracted by surface evaporation and plant transpiration.

In these regions, agriculture faces significant challenges due to climate change intensifying water scarcity, primarily dedicated to agriculture, and the use of unsustainable practices, such as excessive fertilization or intensive plowing, which compromise soil quality and agricultural productivity [1].

Agricultural soil degradation is the greatest threat to the sustainability of Mediterranean arid soils and is likely the factor that most negatively affects the capacity to adapt to climate change. Major issues faced by these soils include high erosion rates, decline in organic matter, vulnerability in organic carbon reserves, and a decrease in biodiversity, water quality, and quantity [1].

Soil organic carbon (SOC) is a crucial indicator of soil fertility and an important sink for greenhouse gases. It directly supports agricultural productivity and soil biodiversity and provides many ecosystem services. SOC contributes to the soil's resistance to erosion (an important factor in soil degradation in arid areas) and increases water retention capacity and nutrient availability. Land sequesters a third of the anthropogenic carbon released into the atmosphere, with soil being a key player [2]. Arid soils are a reservoir of inorganic carbon (SIC), which can release greenhouse gases. Both SOC and SIC depend on environmental conditions (e.g., pH, mineral content, moisture, etc.) and management practices, especially soil tillage, which has been scarcely considered in Mediterranean drylands [3].

Another negative factor affecting agricultural soils is the use of pesticides, as they have a direct impact on soil biodiversity loss and represent a global threat to health. Therefore, in the past two decades, pesticide use in agricultural soils has been subject to several regulations by the European Union (EU), and currently, the sustainable use of pesticides is a priority action within the European Commission's Green Deal. However, they remain a concern today, as they persist in the soil for extended periods. Thus, the study and determination of pesticide presence in the Mediterranean basin and their impact on soil health is highly relevant.

On the other hand, soil harbors large populations of microorganisms that play a crucial role in the biogeochemical processes occurring within it. These microorganisms are closely related to factors affecting soil degradation, such as SOC decomposition, carbon storage regulation in the soil, and nutrient cycling. Moreover, those in contact with plant roots (rhizospheric microorganisms) that can benefit and promote plant growth are called plant growth-promoting microorganisms (PGPM). These mechanisms can be direct, through the production of phytohormones, siderophores, phosphate solubilization, and atmospheric nitrogen fixation, or indirect, where they compete with other microorganisms, including pathogens, through the production of antibiotics, lytic enzymes, toxic substances, and can increase induced systemic resistance in plants [4]. Therefore, microbial communities in the soil play a crucial role in soil health and fertility, directly influencing plant growth and productivity.

Understanding the dynamics and diversity of microorganisms in arid lands is essential to comprehend and improve soil quality in these agricultural systems and is a key parameter for understanding soil resilience to climate change.

2. Materials and methods

Soil Sampling Campaign. This work is part of a PRIMA Project called SHARInG-MeD, this project comprises a sampling soil campaign in countries of mediterranean drylands, which participate in the consortium, including an approximately total of 460 locations, distributed in Spain, Croatia, Turkey, Greece, Tunisia, Algeria, Morocco, France, and Italy following the (Table 1).

Table1. Number of established locations per partner country

Participant country	Number of samples
Spain	40
Croatia	20
Turkey	120
Greece	20
Tunisia	90

Morocco	80
France	10
Italy	80

A distinctive feature of this work, compared to LUCAS, Soil4Africa, and RECSOIL, is the implementation of sampling in paired sites. This strategy allows for the elimination of variation due to environmental conditions, ensuring that differences in soil properties, including the rhizosphere, are primarily caused by variations in land use and soil types. Sampling will be conducted at paired sites with different land uses, such as agricultural, pastoral, or forest, or between two different agricultural uses, as shown in (Fig. 1). Biological samples will be collected from the topsoil layer (0–20 cm).

Subsequently, microbiological analyses will be performed, including enzymatic activity measurements, metataxonomic analysis, and phospholipid characterization (PLFA). Additionally, pesticide determination analyses will be conducted. These analyses will be carried out on the total number of samples, there are two samples per location, approximately 920, collected by all groups participating in the project. To this end, samples will be shipped from the different countries to Spain under specific preservation conditions, except for sequencing analyses, which, due to their cost, will be performed on only 10% of the samples, approximately 90 samples.

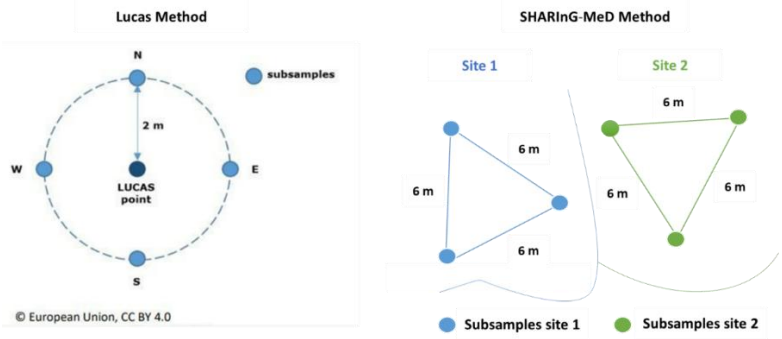

Fig. 1. Sampling design compared to the LUCAS project

Clustering procedure made for biological samples. The biological analyses require at least three replicates per cluster that, while not identical, share similar characteristics. Therefore, a clustering process was required to enable meaningful comparisons. Additionally, metadata is essential for analyzing results in biological analyses such as massive sequencing.

As previously mentioned the project determined that 10% of the total locations, equating to forty-five locations, would be used for biological analyses, fifteen clusters -with each cluster containing three locations- were established for accomplishing the forty-five sites.

The first step in the clustering process was to organize all country locations, provided by the *Conditioned Latin Hypercube Analysis* (cLHS). After compiling these locations, a filtration was applied to include only one land use class: agricultural fields, considered most relevant for biological analyses. For clustering, K-means algorithm, suitable for quantitative variables, was applied with mean temperature, annual rainfall, and the aridity index selected as clustering variables.

Once the clusters were generated, an additional filtration step was per- formed to meet two essential conditions: ensuring each cluster contained three suitable locations and adhering to the predefined number of biological locations per country. A cluster could consist of locations from different countries or a single country, as long as similar characteristics within the cluster were maintained.

Physicochemical parameters. As part of the Sharing-Med project, data obtained by participating research groups will include various analyses and physicochemical parameters, such as pH, salinity, SOC (soil organic carbon), total capacity, wilting point, and bulk density. Additionally, 15% of the samples will undergo analysis for metals, minerals, ^{13}C in CO_2, SOC, and SIC (soil inorganic carbon). In 5% of the samples, pyrolysis Rock Eval® (thermal soil rock analysis) will be conducted, along with an analysis to trace SOC transformation into SIC and CO_2. Approximately 300 samples will have hyperspectral data collected, ranging from the visible wavelength to near-infrared spectroscopy (NIR).

These data will serve as metadata for the previously described microbiological analyses.

Microbiological analyses; soil enzyme activity analysis. To study the functionality of microbial groups in soil, one enzyme representing each nutrient cycle will be selected: β-glucosidase for the carbon cycle, acid phosphatase for the phosphorus cycle, and arylsulfatase for the sulfur cycle, along with three enzymes for the nitrogen cycle - proteases (L-leucine aminopeptidase), ureases, and nitrogenases.

The activities of β-glucosidase, protease (determined by L-leucine aminopeptidase), acid phosphatase, and arylsulfatase will be measured using fluorometry. This method employs a specific substrate for each enzyme, which, when hydrolyzed, releases a fluorochrome (7-amino-4-methylcoumarin (AMC) for L-leucine peptidase, or 4-methylumbelliferone (MUF) for the others) that fluoresces when excited under ultraviolet light at 330–380 nm, emitting fluorescence in the 440–480 nm range. Urease activity will be determined colorimetrically with a spectrophotometer using the salicylate-cyanurate reaction method, as proposed by, based on the production of 5-aminosalicylate in the presence of salicylate, cyanurate, and ammonia. Finally, nitrogenase activity will be determined through the acetylene reduction assay (C_2H_2 to ethylene C_2H_4), following and analyzed using gas chromatography-mass spectrometry (GC-MS) to quantify ethylene production [5].

Microbiological analyses; massive sequencing analysis of microbial communities. DNA extraction will be performed on selected samples using the FastDNA Spin kit for soil (MP Biomedicals, Palex, Spain). The bacterial 16S rRNA V4 region will then be amplified and sequenced using the 515F–806R primer pair; for fungi, the internal transcribed spacer (ITS2) of the 5.8S large subunit will be amplified using the ITS1f and ITS2 primers; and for eukaryotes, the 18S rRNA V4 region of the small 40S subunit will be amplified using the F-574 and R-952, primer pair, all sequenced on the Illumina MiSeq platform. Bioinformatics analyses will compare these sequences with databases using MOTHUR, remove chimeras with VSEARCH, and apply a quality filter (denoising) with DADA2, working with ASVs (Amplicon Sequence Variants). Databases such as SILVA (for bacteria) and UNITE (for fungi) will be employed. Raw sequences will be deposited in the GenBank SRA database.

Microbiological analyses; quantification of fungal and bacterial communities via phospholipid analysis (PLFA). Phospholipid profiles will be determined using gas chromatography equipped with a mass spectrometer (GC-MS, Agilent 7890A) following. This process involves lipid extraction with chloroform, separation using silica-based solid-phase extraction (SPE) columns, and separation of phospholipids from total lipids and glycolipids[6].

Microbiological analyses; pesticide determination. The presence of pesticides in the soil will be analyzed using liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS QqQ, Agilent 6400). This will follow an optimized method developed by the RNM-270 research group, which includes extraction and cleanup using the QuEChERS method and separation with an SPE column. Around 30 pesticides, including herbicides, fungicides, insecticides, and other pesticides, will be analyzed.

3. Results and discussion

This work is part of a larger ongoing project. However, some samples have already been collected and a reduced part analyzed, providing preliminary insight.

Location of the soil samples. The location with coordinates (+37.0242116, -5.174833), shown in the satellite view in (Fig. 2), consists of olives trees and woodland area made up of wild-type olive trees. These two samples will be compared in the subsequent paragraphs.

Preliminary information. Information was gathered from the agricultural land thought a form, with the farm owner's consent, to collect relevant data. Based on the provided information, the olives trees farm follows a rainfed cultivation system with no tillage practices implemented for the past two years. The olives trees are treated with nitrogen fertilizer (urea) annually, typically in February, and pesticides are applied every 6 months. However, for the adjacent woodland, no information could be gathered. To the best of our knowledge, no land management practices are applied there.

Fig. 2. Satellite view from Google Earth showing the study location; the blue triangle marks the olive tree zone, while the red triangle highlights the woodland area composed of wild-type olive trees

Physicochemical parameters. The parameter of pH, moisture percentage, and bulk density were determined for both samples. The pH values were similar, with 8.12 recorded for olive trees farm and 8.05 for woodland. The moisture percentage was slightly higher in the woodland compared to the olive trees farm, across both depths (0-20 cm and 20-30 cm), measuring 26.44% and 29.48% for the woodland, and 25.20% and 27.81% for the olive trees farm, respectively. In contrast, bulk density showed an opposite trend, being the lower in the woodland than in the olive trees farm, measuring 0.95 g/cm³ and 1.05 g/cm³ for the woodland, and 1.56 g/cm³ and 1.49 g/cm³ for the olive trees farm, respectively.

Microbiological analyses; soil enzyme activity analysis. The determination of enzyme activity by using the fluorometry method resulted higher activity levels for the enzymes β-glucosidase, acid phosphatase and L-leucine aminopeptidase in the woodland compared to the olive tree farm. Conversely, arylsulfatase exhibited a decreasing trend in the woodland compared to the olive tree farm. This information is showed in the (Fig. 3).

The low activity of acid phosphatase in alkaline soils is due to its pH sensitivity, with the enzyme showing optimal activity in acidic conditions and negligible activity in higher pH ranges [7].One plausible explanation for the higher activity levels of β-glucosidase, acid phosphatase, and L-leucine aminopeptidase in the woodland compared to the olive tree farm could be the greater microbial presence in the woodland, potentially influenced by the application of pesticides in the olive tree farm, which might reduce microbial diversity and activity. In contrast, the lower arylsulfatase activity in the woodland may be attributed to the effect of rock presence, which can impact sulfur cycling dynamics and enzyme activity [8].

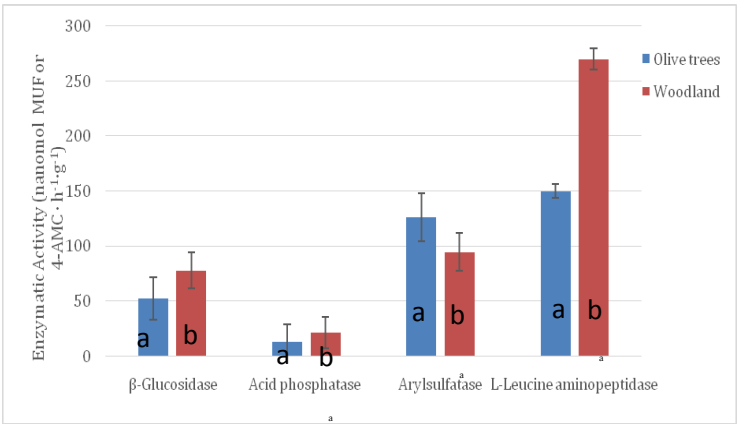

Fig. 3. Graphic bar forenzymatic activities; different letters in each column indicate significant differences between treatments (P\0.05)within each sampling;
Mean values ± SE (n=12)

4. Conclusions

These findings highlight differences in soil enzyme activity between agricultural and natural ecosystems, which may reflect variations in soil health and microbial dynamics.The higher activities of β-glucosidase, acid phosphatase, and L-leucine aminopeptidase in the woodland could be linked to a more diverse and active microbial community, less impacted by pesticide application compared to the olive tree farm. On the other hand, the lower arylsulfatase activity in the woodland might be influenced by soil properties such as the presence of rocks, which can alter sulfur cycling and enzyme function. However, this work is still in its early stages, and further analysis is needed to confirm these preliminary findings and explore additional factors influencing enzyme activity.

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References

[1] Iglesias A., Mougou R., Moneo M., Quiroga S. Towards adaptation of agriculture to climate change in the Mediterranean. *Reg Environ Change.* 2011 Mar 1;11(1):159–66.

[2] Friedlingstein P., Jones MW., O'Sullivan M., Andrew RM., Bakker D.C.E., Hauck J. et al. Global Carbon Budget 2021 [*Internet*]. 2021 [cited 2024 May 28]. Available from: https://essd.copernicus.org/preprints/essd-2021-386/essd-2021-386.pdf

[3] Scharlemann JP., Tanner EV., Hiederer R., Kapos V. Global soil carbon: understanding and managing the largest terrestrial carbon pool. *CarbonManag.* 2014, Feb;5(1):81–91.

[4] Molina-Romero D., Bustillos M., Rodríguez-Andrade O., Morales-García Y., Santiago Saenz Y., Castañeda M. et al. Mecanismos de fitoestimulación por rizobacterias, aislamientos en América y potencial biotecnológico. *BIOLOGICAS.* 2015 Dec 1;17:24–34.

[5] Sinsabaugh RL., Reynolds H., Long TM. Rapid assay for amidohydrolase (urease) activity in environmental samples. *Soil Biol Biochem*. 2000 Dec 1;32(14):2095–7.

[6] Lewe N., Hermans S., Lear G., Kelly LT., Thomson-Laing G., Weisbrod B. et al. Phospholipid fatty acid (PLFA) analysis as a tool to estimate absolute abundances from compositional 16S rRNA bacterial metabarcoding data. *J Microbiol Methods.* 2021 Sep 1;188:106271.

[7] Turner BL. Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. *Appl Environ Microbiol.* 2010 Oct;76(19):6485–93.

[8] Zwikel S., Lavee H., Sarah P. The spatial differences in arylsulfatase enzyme activity on hillslopes along a climatic transect. *Aust J Soil Res - AUST J SOIL RES.* 2007 Jan 1; 45.