olive4 climate

CLIMATE CHANGE MITIGATION THROUGH A SUSTAINABLE SUPPLY CHAIN FOR THE OLIVE OIL SECTOR

ACTION D2

BOOKLET ON THE ESTIMATES OF THE EFFECTIVENESS OF TREATMENTS IN TERMS OF SOIL PRESERVATION, MICROBIOME RICHNESS, PERCENTAGE OF SOIL ORGANIC MATTER COMPARING DATA BEFORE AND AFTER THE IMPLEMENTATION

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Introduction

The goal of this activity was that of verifying how different types of olive orchard management may affect the interactions of fungi and bacteria with the olive plants and may select different microorganisms. Experiments have been conducted on soil and root samples collected from trees under irrigated and dry conditions of an arid area of Israel.
A plethora of fungi and bacteria were identified through microbiome sequencing analysis.

Irrigated and dry olive trees where root and soil samples were collected for the microbiome analysis.

Material and methods

Randomized samples of soil and olive roots of the two selected orchards, were bulked together for a single DNA extraction using the kit PowerSoil (MO BIO, Qiagen) and Qiagen Plant mini kit (Qiagen, Hilden, Germany), respectively. DNA from both kind of samples was then PCR amplified and amplicons were then used for metabarcoding analysis of Internal

Transcribed Spacer (ITS) by using the ITS2 region of ribosomal DNA (rDNA) amplified by fits7 (GTGARTCATCGAATCTTTG) and its4 (TCCTCCGCTTATTGATATGC) primers. Within the 16S ribosomal RNA, able to distinguish the bacteria composition of our samples, the amplified hypervariable region was between the V5 and V6 using the v5f (ATTAGATACCCNGGTAG) as forward primer and the reverse primer v6R (CGACAGCCATGCANCACCT). As described for ITS region, also for the 16S a tag of 9-11 bp was added at 5' of the primer forward in order to be able to identify the reads for each sample. The library was built with modified primers. The libraries were run in the Ion Chef System and then the chips were charged in the Ion PGM Dx System. Raw sequences from the Ion Torrent PGM system were processed and analyzed using MG-RAST server v4.0.3 (http://metagenomics.anl.gov/). Raw data were uploaded as FASTAQ files after demultiplexing of paired-end reads. Reads generated after quality processing and deduplication by MG-RAST pipeline analysis were subjected to taxonomic analysis. MG-RAST pipeline made available an estimation of bacterial and fungi presences and abundances in dry and irrigated conditions highlighting the differences between samples.

Results

Bacteria analysis

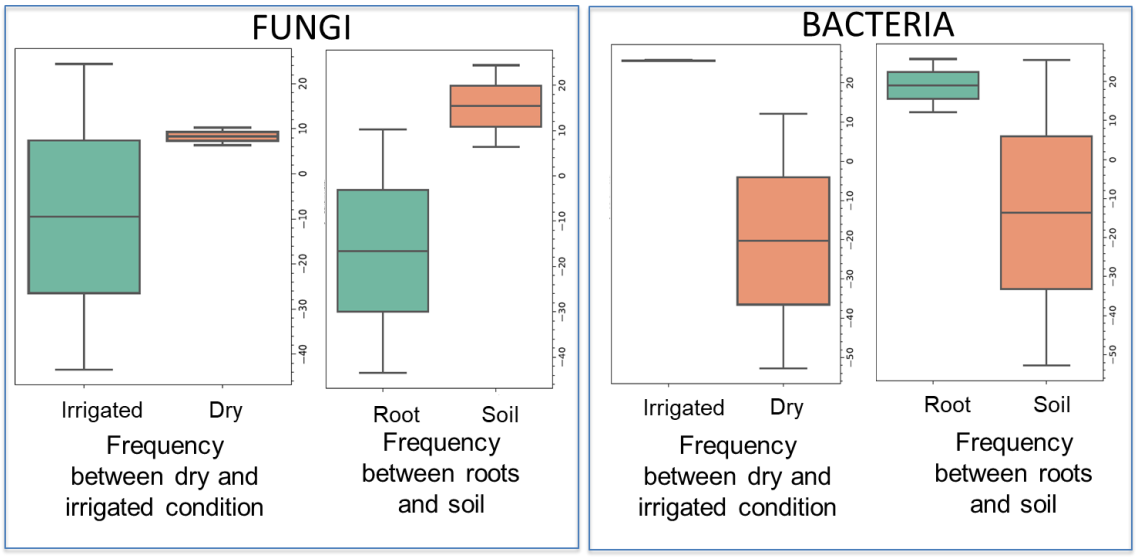
The sequencing analysis performed on the 16S fragment revealed a total of 819,081 sequences equal to 264,330,650 bp. Within the four samples the average of predicted features was 124,405.75 equal to 62.27%, while 64,091.50 were unknown features (29.98% of total). The sequences were blasted against three databases: RDP, SSU and Greengenes. The highest number of features were found for the sample related to roots under irrigation (143,261), while the lowest for the dry soil sample (105,980). The *Phyla* detected by the databases evidenced the presence of *Acidobacteria* and *Verrucomicrobia* only in soil both dry and irrigated. At genus level two hits were found only in the irrigated soil sample, *Actinoplanes*, *Micromonosporas*. Only in irrigated roots the *Amycolatopsis* genus was observed, while in dry roots five genera were revealed: *Kibdelosporangium*, *Kribella*, *Mesorhizobium*, *Mycobacterium* and *Planctomyces*. *Geodermatophilus* genus was found only in dry soil.

Fungi analysis

For what concern the ITS analysis, a total of 416,545 sequences equal to 125,801,719 bp were found. The predicted features of the four analyzed samples were in average 88,809.25

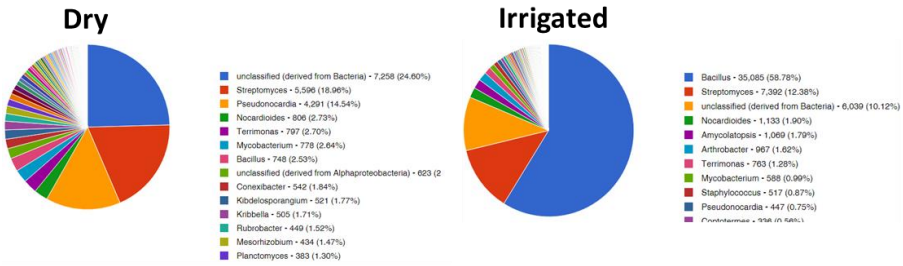
equal to 85.04% of total sequences. The unknown features were in average 13,889.50 (13.63%) of total sequences. The database used to check the similarity with already submitted ITS sequences was RDP.

At *Phylum* level *Basidiomycota* were absent only in dry roots. *Chytridiomycota* were observed only in dry soil sample as well as *Glomeromycota*, which seemed to be related to dry condition and were found in dry soil and roots. Botryosphaeriaceae (33.35% on total) were characteristic of dry roots. Lasiospheriaceae, Massarinaceae, Sclerotinaceae and Sordariaceae were observed only in irrigated soil. Dothioraceae, Malassenciaceae and Mortierellaceae were detected in dry soil. At genus level, in dry root samples were observed *Botryosphera* (34.12% on total), *Sarcinomyces* and *Tetracladium*. *Auricolaria*, *Dendryphiella* and *Meyerozyma* were present only to irrigated roots. *Diaporthe*, *Fusarium*, *Humicola* and *Podospora* were detected in irrigated soil. On the contrary, in dry soil *Aureobasidium*, *Guehomyces*, *Malassezia*, *Mortierella* and *Preussia* were found, not present in the other analyzed samples.

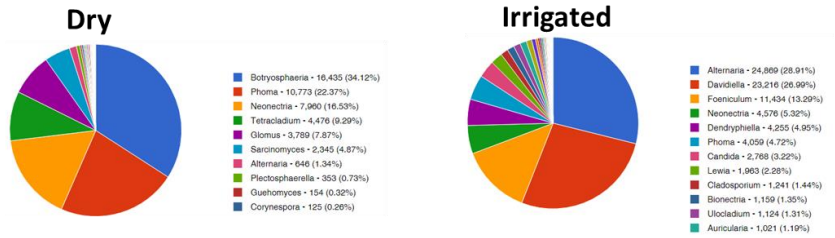


Fungi and Bacteria frequency in roots and soil under dry and irrigated conditions.

Bacteria occurrence in olive roots under dry and irrigated condition

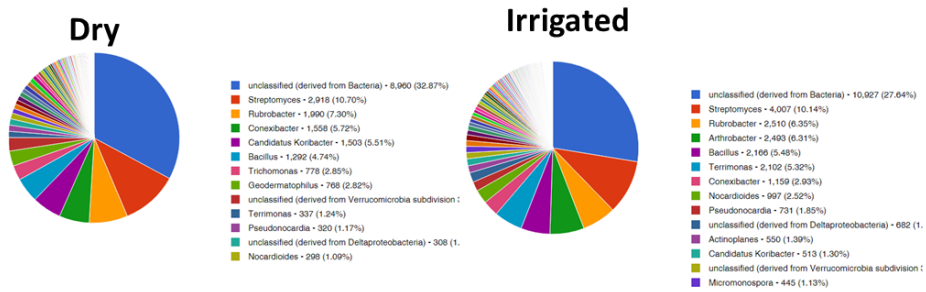


Fungi occurrence in olive roots under dry and irrigated condition

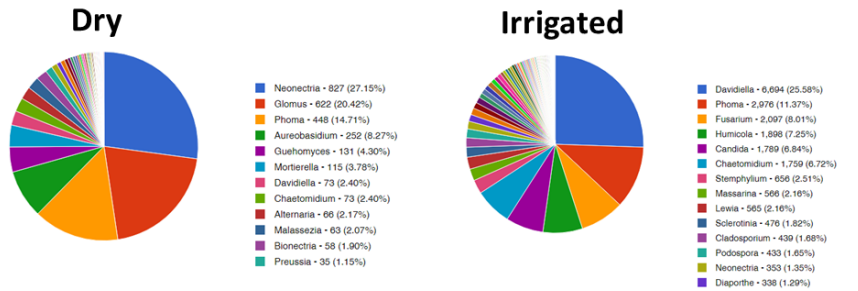


Bacteria and fungi detected in the olive root system under dry and irrigated conditions.

Bacteria detected in the soil under dry and irrigated condition



Fungi detected in the soil under dry and irrigated condition



Bacteria and fungi detected in the olive soil under dry and irrigated conditions.

Discussion

Bacteria were in general more abundant in the soil then in roots. Most of them were in common among samples, in three out of four samples exceeded 50% of total *Bacteria*, as in the case of *Actinobacteria*.

Differences of the bacterial composition in the soil and inside the olive roots between dry and irrigated condition were observed. *Microbacteriaceae* family, largely found in association with animals, fungi and plants, was only found in dry condition both in root and soil. This family contained pathogen agents, which often may affect plants. The well-known family of *Solanaceae* parasites, *Conexibacterium*, was present in the olive root under dry condition but it was never observed in irrigated root even if it was present in the soil. Inside roots under drought condition several promoters of plant growth were found, such as *Kribella* and *Mesorhizobiu* together with *Kibdelosporangium,* which is known for the production of siderophores (ion chelate) and also as plants pathogens defender.

The presence of positive microorganisms for the well-being of plants was confirmed also by the presence of some fungi, especially in the dry soil, with a peculiar attendance of genera as *Mortierella*, which works against plants diseases, and *Preussia*. The phyla *Glomeromycota*, found only in dry condition, may generate mycorrhizas, which may help plants to absorb soil nutrients. On the contrary, *Botryosphaeriaceae* are plant pathogens able to generate branch cancers. In the irrigated olive orchard some specific families were found, such as *Massarinaceae*, considered beneficial for plants, because they produce some bioactive compounds, dihydrobenzofurans and xanthenes, effective against *Microbotryum violaceum* and *Bacillus subtilis*. On the contrary, the *Sclerotinaceae* are described as one of the worst family of plants pathogens', such as *Botrytis* spp. and *Monilinia laxa*.

Concluding remarks

Plants growing under dry or irrigated conditions are characterized by a different occurrence of bacteria and fungi in their root system and in the surrounding soil at different taxa levels.

It is still unknown if the microorganisms specifically selected under dry conditions may be helpful or not in improving plant tolerance to drought and arid climates in general. Further steps in this research are currently in progress.

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Table 4. Cultivars mostly originated from arid zones and cultivated in different environmental conditions. They were included in the analysis together with reference international cultivars.

Cultivar	Country of origin	Cultivation site
Abbadi abou gabra	Syria	
Abou kanania	Syria	
Abou satl mohazama	Syria	
Barri	Syria	
Chemlal de kabylie	Algeria	Env2
Jabali	Syria	
Maarri	Syria	
Majhol-1013	Syria	
Majhol-152	Syria	
Chemlali	Tunisia	
Chetoui	Tunisia	
Coratina	Italia	
Khalkali	Syria	
Kadesh	Israel	
Massahabi	Syria	Env1
Meski	Tunisia	
Sari Hasebi	Turkey	
Sigoise	Algeria	
Sourani	Syria	
Zaituna	Italy	
Berri meslal	Morocco	
Menara	Morocco	Env3
Meski	Tunisia	
Picual	Spain	
Barnea	Israel	
Kadesh	Israel	
Massahabi	Syria	Env4
Shatqui	Syria	
Sourani	Syria	
Souri	Israel	

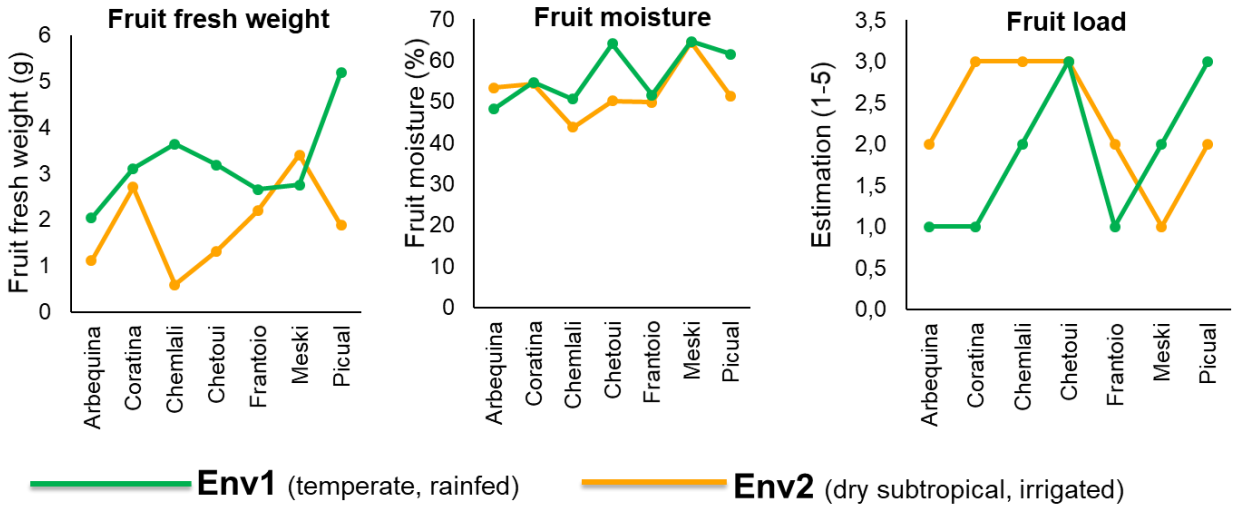

Figure 1. Fruit traits and estimated fruit load in two environments.

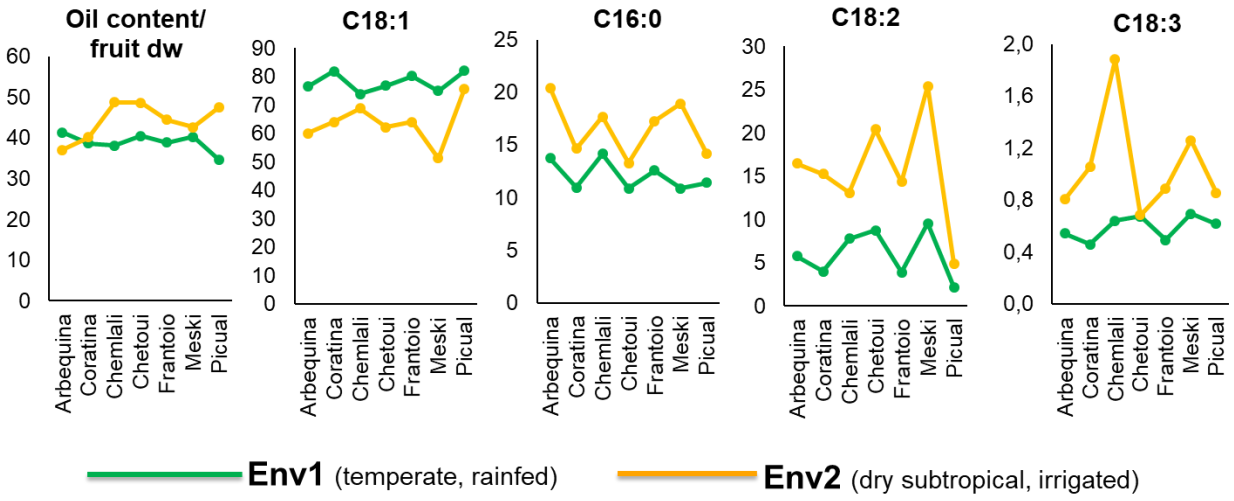

Figure 2. Fruit oil content and fatty acid composition in two environments.

plant (third algorithm