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Structure and Function of the Soil Rhizosphere Fungal Communities in Medicinal Plants—A Preliminary Study

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Abstract: Plants regulate their rhizosphere microbiome, which partly comprises the fungal community. We conducted a study in order to determine the effect that five medicinal plant species (*Origanum syriacum*, *Salvia fruticosa*, *Teucrium capitatum*, *Myrtus communis* and *Pistacia lentiscus*) have on the fungal community in their rhizosphere. We measured abiotic parameters and used sequencing to determine the structure of the rhizosphere fungal community, both taxonomically, as phyla and genera, and functionally, as trophic modes. Our data shows that the rhizosphere fungal communities were significantly different, both taxonomically and functionally. The rhizosphere of *M. communis* had a significant relative abundance of saprotrophs and a lower relative abundance of symbiotrophs than the control soil and the rhizosphere of *T. capitatum*. The relative abundance of the genus Aureobasidium was significantly higher in the rhizosphere of *P. lentiscus* than in the control and for all other rhizospheres, but that of *S. fruiticosa*. The relative abundance of genus Alternaria was lower in the rhizospheres of *S. fruticosa* and *M. communis* than in the control soil. Our results highlight the potential use of these plants in agroforestry, as a means to influence the soil fungi population.

Keywords: soil fungi; rhizosphere; FUNGuild; microbiome

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1. Introduction

The plant rhizosphere is defined as the interaction zone between the root system and the soil environment. According to Philippot [1], the root zone region comprises a diverse community of microorganisms and invertebrates that affect the plant by direct and indirect interactions. Plants influence the rhizosphere microbiome by releasing root exudates into the soil and producing root litter [2,3].

One of the most important and abundant microbial communities in the rhizosphere are the fungi, which play a major role in carbon and nutrient cycling in ecosystems. Some of the soil fungi may create a symbiotic connection with plant roots, in which the fungi subsist on organic carbon transferred by the plant to its underground parts. In return, the plant receives nutrients and the alleviation of various forms of stress [4]. Other fungi populations act as decomposers (saprotrophs), playing a major role in nutrient cycling, thus providing nutrients for plants [5]. There are also plant pathogenic fungi, which feed off the plant and provide nothing in return, thus harming the plant [6]. As a result of their continuous effort, the scientific community has determined the function of many fungal taxa and has developed tools to analyze the function of the fungi in a community [7].

Medicinal plants produce secondary plant metabolites in one or more of their parts and affect a wide range of microorganisms [8]. These plants have been used as medicine for humans [9] for at least 5000 years [10], and even today their importance is recognized globally [11]. Many of the drugs developed over the last several decades are either defined mixtures of botanical products, unaltered natural products, or natural product derivatives; many derived from, or are, plant parts [12,13]. Interestingly, some of the medicinal compounds in plants are not synthesized by the plants themselves but rather by their microbiome [14].

Additionally, medicinal plants can affect the abundance of pathogenic fungi in the soil and their influence on newly rooted plants, such as in the study by Li et al. in 2018, that showed the suppressive effects that *Atractylodes lancea* have on Fusarium [15]. Due to these reasons and others, several previous papers described the fungal rhizosphere community of different medicinal plants. Abdul Latif Khan et al. described, in 2020, the rhizosphere fungal communities of *Adenium obesum*, *Aloe dhufarensis* and *Cleome austroarabica* [16], all of which had a high relative abundance of phyla Ascomycota. Genus Acremonium was found to be relatively abundant in the rhizospheres of *A. obesum* and *A. dhufarensis*, while genus Corynascus was abundant in the rhizosphere of *C. austroarabica.* Villalobos-Flores et al. described the rhizosphere fungal community of the medicinal plant *Bouvardia ternifolia* in 2021 [17], and found that it has a high relative abundance of class Sordariomycetes. A comprehensive list was written by Kushwaha et al. in 2020 [18].

In this study, we sampled the soil rhizosphere of the following five medical plants: *Myrtus communis*, *Origanum syriacum*, *Pistacia lentiscus*, *Salvia fruticose* and *Teucrium capitatum*, inhabiting natural systems in a Mediterranean environment. In this study, we aim to determine the effect the researched plants have on the fungal community composition in their rhizosphere. We hypothesize that the effect on the rhizosphere of each plant will be expressed by significantly different relative abundances of fungal taxa, and a relative abundance of different functional fungal modes.

2. Materials and Methods

2.1. Study Site

The study site is located in Neot-Kedumim, a nature reserve and park in Israel near the city Modi'in. The site is located at 31°56′51″ N 34°58′23″ E at 198 m a.s.l. The common basic climate of the study site is characterized by rainy winters (October–April) and prolonged dry summers (June–August). The plant-growing season commences soon after the first rains, between October and December. Average multiannual rainfall is 350 mm, and the mean multiannual temperature is 20 °C. Vegetation is dominated by shrubs, such as *Pistacia lentiscus*, *Calicotome villosa*, *Rhamnus lycioides* and *Origanum syriacum*, and large numbers of herbaceous (mostly annual) plant species. The soil at the study site is terra rosa.

2.2. Researched Plants—Ecophysiology and Medicinal Use

Origanum syriacum is an eastern Mediterranean perennial evergreen subshrub of the Lamiaceae family that is 30–50 cm tall, with a woody base and soft-wooly, glandular hairs, flowering from April to September ([19], p. 573) [20]. It is traditionally used to treat various ailments and conditions, for example, strengthening heart function, relieving toothache, and treating colds and infections in the gums, digestive tract, and urinary system. Studies show that extracts of *O. syriacum* possesses antioxidant, antibacterial, fungicidal, and nematocidal activities. Carvacrol, a major component of *O. syriacum* essential oil, has been shown to have antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, anti-inflammatory, angiogenic, antiparasitic, antiplatelet, ache inhibitory, antielastase, insecticidal, antihepatotoxic, and hepatoprotective activities [21].

Salvia fruticosa is a Mediterranean perennial evergreen shrub of the family Lamiaceae, at 1.5–1 m tall, covered with white hairs, flowering between March and June ([19], pp. 572–573) [21]. Traditionally, the plant is used for diverse medicinal purposes, which vary between different cultures, and treats a plethora of aches and ailments, including earaches, stomach aches, colds, digestive system disorders, and more [21]. Animal testing showed that *S. fruticosa* infusion has a hypoglycemic effect, probably due to its ability to reduce the intestinal absorption of glucose [22]. Extracts of the plants have been shown to have in vitro antioxidant properties and in vivo anti-inflammatory effects in mice [23].

Teucrium capitatum is a sub-shrub of the Mediterranean, Irano-Turanic, and Saharo-Arabic phytogeographic zones. It is a perennial evergreen that grows to about 40 cm high and flowers between April and August ([19], p. 554). Bedouin tribes in Jordan traditionally use it for gastrointestinal ailments, general pain, wounds, and diabetes [24],

and the general Palestinian Jordanian and Persian population use it to treat colic and diarrhea [25]. Contemporary research shows that extracts of the plant have antimicrobial properties [25,26] and wound healing potential properties [27].

Myrtus communis is a Mediterranean perennial evergreen shrub or small tree of the family Myrtaceae, growing up to 5 m tall, with small, sclerophyllous, leathery leaves, flowering between May and August ([19], p. 455) [28,29]. It has been traditionally used for a variety of medicinal purposes, including the treatment of disorders in the urinary and digestive systems. Modern studies show that the plant and its extracts have medicinal effects, which infuse anti-inflammatory, analgesic, antiproliferative, antigenotoxic, neuroprotective, anti-mutagenic, anti-diabetic, and antiviral effects [29].

Pistacia lentiscus is a Mediterranean perennial evergreen shrub or small tree of the family Anacardiaceae, 1–5 m tall, flowering between March and April ([30], p. 419) [31]. The plant's oil has been used in medicine since the 1st century AD by Pliny the Elder and Dioscorides. Different parts and extracts of the plant are used to this day in traditional human and animal medicine for ailments and illnesses such as bronchitis, digestive problems, and more. Accumulative evidence shows possible uses in modern medicine of the various plant extracts as anti-bacterial, antifungal, antihelmintic, antioxidant, and anticarcinogenic materials, although some are controversial [31].

2.3. Soil Sample Collection

During the spring, soil samples (0–10 cm, $n = 3$) were taken from the rhizospheres of the plants, *O. syriacum*, *S. fruticosa*, *T. capitatum*, *M. communis* and *P. lentiscus*, beneath their canopy, and control samples were taken from an open inter-plant space with a minimal distance of 3 m from the researched plants.

The soil samples were placed in individual plastic bags and stored in cool insulated boxes until arrival at the laboratory. Bulk soil samples were kept at 4 °C after sieving (2 mm mesh) in order to remove other organic debris, stones, and root particles. 1.5 mL of each sieved sample was placed in a sterile plastic vail in −20 °C, from which DNA was extracted.

2.4. Soil Analysis

Soil moisture (%) content was determined gravimetrically by drying soil samples for 24 h at 105 °C. The organic-matter content (%) was determined gravimetrically by placing a dry soil sample in a muffle furnace at 400 °C for 4 h and a gravimetrical measurement. Soil pH was determined by mixing 20 g of soil and 40 mL water, shaking well for 10 min at 160 rpm, and allowing the mixture to incubate overnight. The liquid was then filtered through two sheets of filter paper, and the supernatant was measured using a pH electrode. Electrical conductivity (as μS* cm^{-1}) was determined as an assessment of soil salinity. The soil was mixed with double distilled water in a 1:10 ratio and was shaken for 30 min (160 rpm). The samples were left undisturbed overnight at room temperature for the sediment to settle, and the supernatant was filtered through double filtration paper. Electrical conductivity of the supernatant was determined by an auto-ranging EC/temp.

2.5. Molecular–Taxonomy Determination

Soil DNA was extracted from 0.5 g soil using an Exgene soil DNA mini kit from GeneAll (Seoul, Korea). DNA was amplified using PCR using SimpliAmp^{TM} thermal cycler (Thermo Fisher Scientific, Walham, MA, USA), by mixing 12.5 μL HS Taq Mix Red (PCR Biosystems, London, UK), 9.5 μL ultrapure water, 1.0 μL extracted DNA, 1 μL CS1-ITS2, and 1 μL CS2-ITS4. Initial incubation was at 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s, 50 °C for 30 s, 72 °C for 30 s, and after the cycles, 72 °C for 3 min. The amplified DNA was stored at −20 °C until sequencing. Sequencing (Miseq) was performed at the Hylabs Laboratory Ltd. (Rehovot, Israel), (www.hylabs.co.il) sequencing facility using an Illumina sequencing platform (Illumina Inc., San Diego, CA, USA).

2.6. Data Processing and Analysis

Data were de-multiplexed using Basespace to generate two FASTQ files per sample. The FASTQ files were imported into CLC bio v. 12.0.3 (CLC Bio, Aarhus, DK, USA) and analyzed using the Microbiome tools in CLC, to generate abundance and OTU tables using the UNITE v7.2 database. The data sets generated in this study were deposited in the NCBI Sequence Read Archive database under accession number PRJNA788192.

After the removal of all readings of taxa that did not belong to Fungi, each repetition was normalized to 100%. Taxa registered as fungi but not identified to the level of genera were included as part of the total readings but not analyzed statistically as genera. In order to determine fungal function, we used FUNGuild [7], a tool that matches taxa with ecological guilds and trophic modes, based on contemporary knowledge. Our study used the trophic mode alone. In order to determine function relating to plants alone, we disregarded all unrelated guilds (animal pathogens, animal endosymbionts, fungal parasites, lichen parasites, lichenized fungi, and undefined Parasites). After the removal of these guilds, trophic modes were redetermined, and taxa with no relevant guilds were removed from the analysis. We normalized the data such that the sum of all readings with assigned trophic modes was 100%, and we then calculated the total from each trophic mode in each replication. Readings of taxa that belonged to multiple trophic modes were divided equally among these modes.

2.7. Statistical Analysis

Soil abiotic parameters and relative abundances of phyla, genera, and trophic modes were compared using XLSTAT (Addinsoft) (ANOVA and Duncan's multiple range test and Pearson's correlation coefficients).

3. Results

3.1. Abiotic Variables

The Mean values of the different soil physical parameters of the five plant rhizospheres and the control soil are presented in Table 1. Soil moisture percentage (SM) ranged from 7.83% in the control soil to 21.73% in the *M. communis* soil samples. SM was significantly higher ($p < 0.05$) in *M. communis* and *P. lentiscus* rhizosphere than in both the control and the *T. capitatum* soil samples. Organic matter percentage (OM) ranged from 6.37% in the control soil to 16.57% in *P. lentiscus* rhizosphere. No significant differences in OM were found between the samples.

Table 1. Mean values (±SD) of soil abiotic parameters in the rhizosphere of different plants. Different letters signify significantly different ($p < 0.05$) values.

Soil Properties	Control	Sampled Rhizosphere				
		O. syriacum	***S. fruticosa***	***T. capitatum***	***M. communis***	***P. lentiscus***
SM (%)	7.83 ± 2.99 b	13.37 ± 1.86 ab	14.23 ± 04.02 ab	10.33 ± 0.87 b	21.73 ± 03.71 a	20.07 ± 10.25 a
OM (%)	6.37 ± 10.84 a	8.40 ± 0.66 a	7.0 ± 01.57 a	7.67 ± 2.57 a	10.07 ± 5.84 a	16.57 ± 12.11 a
pH	8.0 ± 0.06 ab	8.10 ± 0.05 a	7.91 ± 0.07 bc	7.92 ± 0.06 bc	7.91 ± 0.08 bc	7.79 ± 0.09 c
EC	78.57 ± 1.18 a	84.77 ± 12.18 a	83.43 ± 04.65 a	86.3 ± 6.59 a	80.37 ± 05.06 a	83.93 ± 20.40 a

SM—soil moisture; OM—organic matter; EC—electric conductivity as μS* cm^{-1}.

Soil pH ranged between 7.79 in the rhizosphere of *P. lentiscus* to 8.10 in the rhizosphere of *O. syriacum*. The soil from the rhizosphere of *O. syriacum* was significantly ($p > 0.05$) more alkaline than in the rhizosphere of the other plants, and the control soil was significantly ($p > 0.05$) more alkaline than the rhizosphere of *P. lentiscus*.

EC ranged from 78.57 μS* cm^{-1} in the control soil to 86.30 μS* cm^{-1} in the *T. capitatum* rhizosphere without any significant differences between the samples.

3.2. Taxonomic Analysis

3.2.1. Phyla

A total of 8 phyla were identified, and all the fungi reads that were not identified to a specific phylum were designated as unidentified (Table 2, Figure 1). The percentages of each phylum's relative abundance in each plant's rhizosphere are presented in Table 2. Ascomycota was the most relatively abundant phylum in all rhizospheres and the control, ranging from 76.52% in the control soil to 88.51% in the *O. syriacum* rhizosphere. Basidiomycota was the second most abundant phylum in all rhizospheres and the control soil, ranging from 5.49% in the *T. capitatum* rhizosphere to 12.74% in the *M. communis* rhizosphere.

Table 2. Mean values (±SD) of soil fungal phyla relative abundance (%) in the rhizospheres of the different plants and the control. Different letters signify significantly different ($p < 0.05$) values.

Phyla	Control	Sampled Rhizosphere				
		O. syriacum	*S. fruticosa*	*T. capitatum*	*M. communis*	*P. lentiscus*
Asco	76.52 ± 8.54 b	88.51 ± 1.0 a	86.55 ± 6.95 ab	80.1 ± 6.3 ab	80.37 ± 05.5 ab	87.33 ± 3.89 ab
Basidio	8.87 ± 5.33 a	6.20 ± 0.72 a	7.67 ± 5.13 a	5.49 ± 1.54 a	12.74 ± 5.08 a	6.91 ± 1.41 a
Chytridio	1.36 ± 1.28 a	0.07 ± 0.06 b	0.26 ± 0.31 ab	0.25 ± 0.14 ab	0.48 ± 0.7 ab	0.40 ± 0.32 ab
Glomero	5.07 ± 3.69 a	2.11 ± 1.03 ab	1.81 ± 0.47 ab	3.98 ± 1.41 ab	1.69 ± 1.45 ab	1.32 ± 1.39 b
Kickxello	0.0 ± 0.0 b	0.01 ± 0.02 b	0.15 ± 0.02 b	0.0 ± 0.0 b	0.01 ± 0.02 b	0.55 ± 0.45 a
Mortierel	2.81 ± 2.58 a	1.67 ± 1.23 a	2.07 ± 2.4 a	4.16 ± 2.55 a	0.57 ± 0.33 a	1.83 ± 1.23 a
Mucoro	2.63 ± 3.31 a	0.03 ± 0.04 a	0.1 ± 0.14 a	3.57 ± 5.73 a	0.05 ± 0.05 a	0.51 ± 0.32 a
Rozello	0.75 ± 0.77 a	0.35 ± 0.58 a	0.12 ± 0.15 a	0.07 ± 0.06 a	0.15 ± 0.24 a	0.36 ± 0.62 a
unid	2.0 ± 1.21 ab	1.04 ± 0.55 b	1.28 ± 0.45 b	2.37 ± 1.31 ab	3.92 ± 1.98 a	0.79 ± 0.57 b

Asco—Ascomycota, Basidio—Basidiomycota, Chytridio—Chitridiomycota, Glomero—Glomeromycota Kickxello—Kickxellomycota, Mortierel—Mortierellomycota, Mucoro—Mucoromycota, Rozello—Rozellomycota, unid—unidentified.

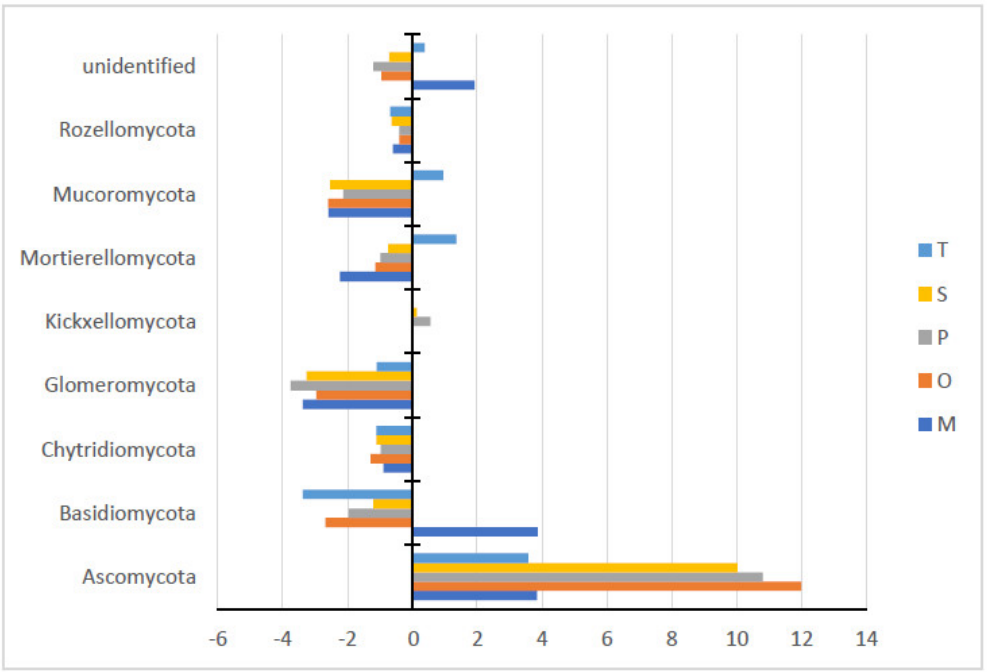

Figure 1. Differences in the relative abundance (%) of fungal phyla between control and plant rhizospheres. T—*T. capitatum*; S—*S. fruticosa*; P—*P. lentiscus*; O—*O. syriacum*; M—*M. communis*.

Our data indicated no significant ($p > 0.05$) differences in the relative abundance of the phyla Basidiomycota, Mortierellomycota, Mucoromycota, and Rozellomycota between any of the different rhizospheres or the control soil. The relative abundance of phylum Ascomycota was significantly ($p < 0.05$) higher in the rhizospheres of *O. syriacum* than in the

control soil. The relative abundance of Phylum Chytridiomycota was significantly ($p < 0.05$) higher in the control soil than in the rhizosphere of *O. syriacum*. The relative abundance of phylum Glomeromycota was significantly ($p > 0.05$) higher in the control soil than in the rhizosphere of *P. lentiscus*. The relative abundance of phylum Kickxellomycota was significantly ($p < 0.05$) higher in the rhizosphere of *P. lentiscus* than in the control soil or all other rhizospheres. The relative abundance of fungi with an unidentified phylum was significantly ($p < 0.05$) higher in the rhizosphere of *M. communis* than in the rhizospheres of *O. syriacum*, *S. fruticosa* and *P. lentiscus*.

3.2.2. Genera

A total of 230 genera were obtained. Table 3 shows the mean value and standard deviation of the relative abundance of fungal genera (%) in the rhizosphere of the different plants and the control. The most abundant genus was Aureobasidium (Table 3, Figure 2), ranging from 0.1% in the rhizospheres of *M. communis* to 20.42% in the rhizosphere of *P. lentiscus*.

Table 3. Mean values (±SD) of fungal genera relative abundance (%) in the rhizospheres of the different plants and the control. Different letters signify significantly different ($p < 0.05$) values.

Genera	Control	Sampled Rhizosphere				
		O. syriacum	*S. fruticosa*	*T. capitatum*	*M. communis*	*P. lentiscus*
Aureobasi	0.43 ± 0.22 b	0.30 ± 0.19 b	8.40 ± 13.96 ab	0.80 ± 0.42 b	0.10 ± 0.11 b	20.42 ± 20.43 a
Mycospha	4.17 ± 3.66 ab	6.31 ± 3.79 a	4.77 ± 0.62 ab	4.54 ± 1.96 ab	1.09 ± 0.5 b	3.44 ± 2.91 ab
Alternaria	4.73 ± 4.12 a	2.59 ± 1.19 ab	0.94 ± 0.35 b	3.32 ± 0.35 ab	0.21 ± 0.14 b	1.68 ± 1.59 ab
Podospora	2.27 ± 1.5 ab	1.07 ± 0.37 b	0.88 ± 0.99 b	5.55 ± 4.69 a	0.48 ± 0.71 b	1.01 ± 1.55 b
Knufia	0.3 ± 0.52 b	0.60 ± 0.95 b	0.28 ± 0.22 b	0.0 ± 0.0 b	6.74 ± 2.72 a	0.66 ± 0.77 b

Aureobasi—Aureobasidium, Mycospha—Mycosphaerella.

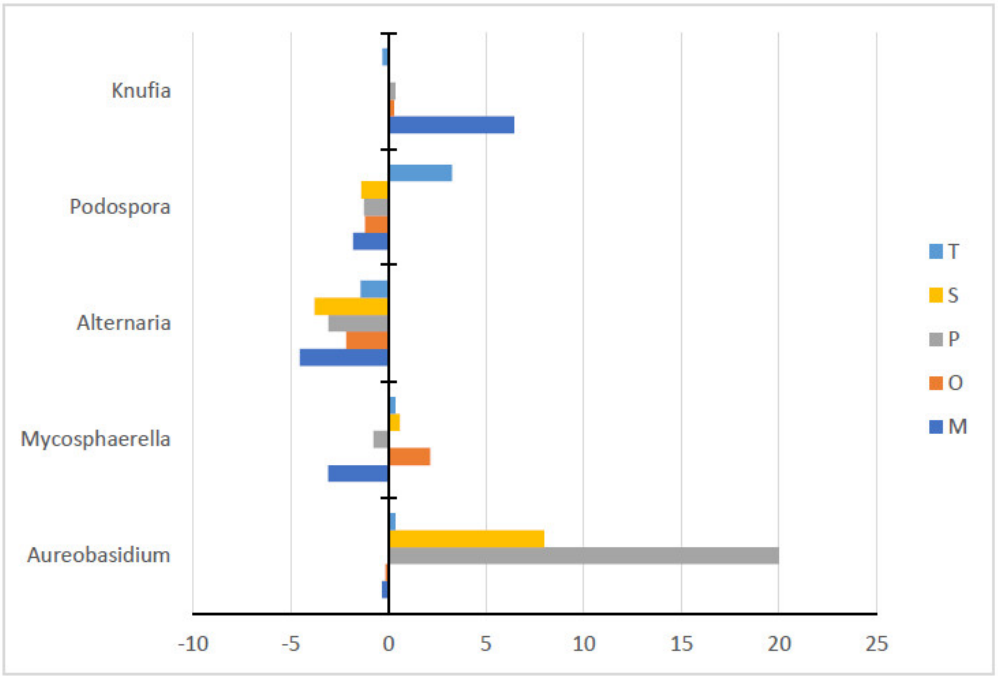

Figure 2. Differences in the relative abundance (%) of fungal phyla between control and plant rhizospheres.

The relative abundance of genus Auerobasidium was significantly ($p < 0.05$) higher in the rhizosphere of *P. lentiscus* than in the rhizospheres of *O. syriacum*, *T. capitatum*, *M. communis*, and the control soil. The relative abundance of the genus Mycosphaerella was

significantly ($p < 0.05$) higher in the rhizosphere of *O. syriacum* than in the rhizosphere of *M. communis*. The relative abundance of the genus Alternaria was significantly ($p < 0.05$) higher in the control soil than in the rhizospheres of *S. fruticosa* and *M. communis*. The relative abundance of the genus Podospora was significantly ($p < 0.05$) higher in the rhizosphere of *T. capitatum* than in the rhizospheres of *O. syriacum*, *S. fruticosa*, *M. communis*, and *P. lentiscus*. The relative abundance of the genus Knufia was significantly ($p < 0.05$) higher in the rhizosphere of *M. communis* than in any other rhizospheres and the control soil.

3.3. Fungal Functional Modes

Fungal functional modes were determined using FUNGuild [7]. Pathotrophic fungi relative abundance ranged from 13.13% in *M. communis* and 22.43% in *P. lentiscus*. The data showed no statistically significant differences between the different rhizospheres or the control soil. The relative abundance of the saprotrophic fungi ranged from 55.10% in *T. capitatum* to 77.57% in *M. communis* and was significantly ($p < 0.05$) higher in the *M. communis* rhizosphere than in both the *T. capitatum* rhizosphere and the control soil. Symbiotrophic's relative abundance ranged from 9.30% in *M. communis* to 27.17% in *T. capitatum* and was significantly higher in both the control samples and *T. capitatum* rhizosphere than in the *M. communis* rhizosphere (Table 4).

Table 4. Mean values (±SD) of fungal trophic mode relative abundance (%) in the rhizosphere of the different plants and the control. Different letters signify significantly different ($p < 0.05$) values.

Trophic Group	Control	Sampled Rhizosphere				
		O. syriacum	***S. fruticosa***	***T. capitatum***	***M. communis***	***P. lentiscus***
PA	20.9 ± 7.47 a	22.0 ± 9.53 a	16.33 ± 6.04 a	17.77 ± 2.91 a	13.13 ± 4.2 a	22.43 ± 9.79 a
SA	56.63 ± 9.15 b	61.17 ± 6.92 ab	67.73 ± 8.13 ab	55.10 ± 12.79 b	77.57 ± 6.67 a	60.83 ± 16.13 ab
SY	22.5 ± 4.95 a	16.87 ± 3.48 ab	15.93 ± 2.43 ab	27.17 ± 10.51 a	9.30 ± 2.56 b	16.73 ± 7.74 ab

PA—pathotrops; SA—saprotrophs; SY—symbiotrophs.

Using Pearson's pairwise test, we found that the relative abundance of both saprotrophic and symbiotrophic fungi correlated significantly ($p > 0.01$) with soil moisture (Table 5, Figure 3): the saprotrophic fungi's relative abundance correlated positively (r = 0.59), whereas the symbiotrophic fungi's relative abundance correlated negatively (r = −0.71) (Table 5, Figures 3 and 4).

Table 5. Pearson's correlation of abiotic factors and trophic mode relative abundance.

	SM (%)	**OM (%)**	**pH**	**EC (µS* cm^{-1})**	**SA**	**PA**	**SY**
SM (%)	-						
OM (%)	0.75 **	-					
pH	NS	NS	-				
EC (µS* cm^{-1})	NS	0.58	NS	-			
SA	0.59 *	NS	NS	NS	-		
PA	NS	NS	NS	NS	−0.79 **	-	
SY	−0.71 *	NS	NS	NS	−0.83 ***	NS	-

NS—non-significant | * $p < 0.01$ | ** $p < 0.001$ | *** $p < 0.0001$.

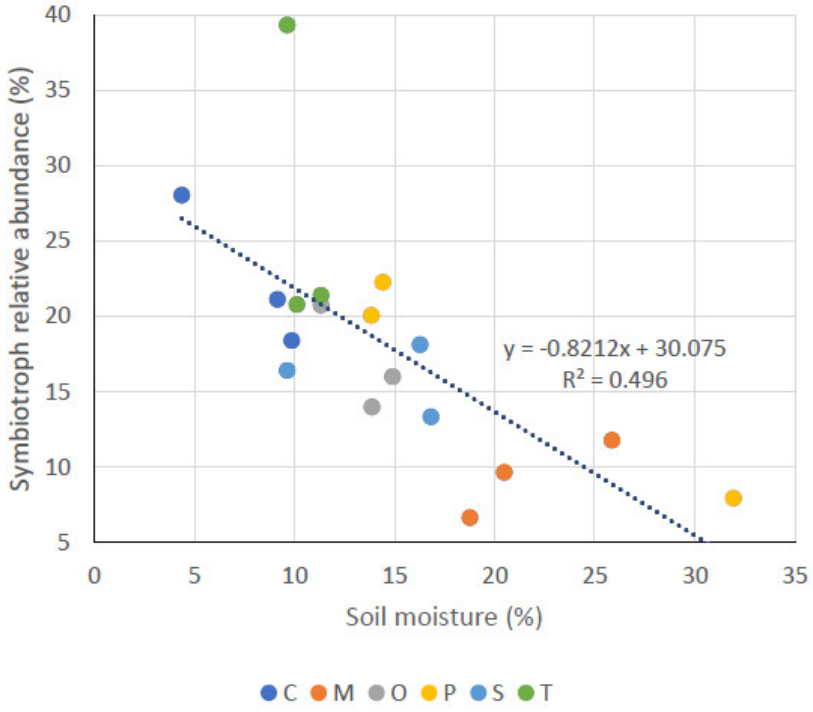

Figure 3. Correlation plot of symbtiotroph relative abundance (%) and soil moisture levels.

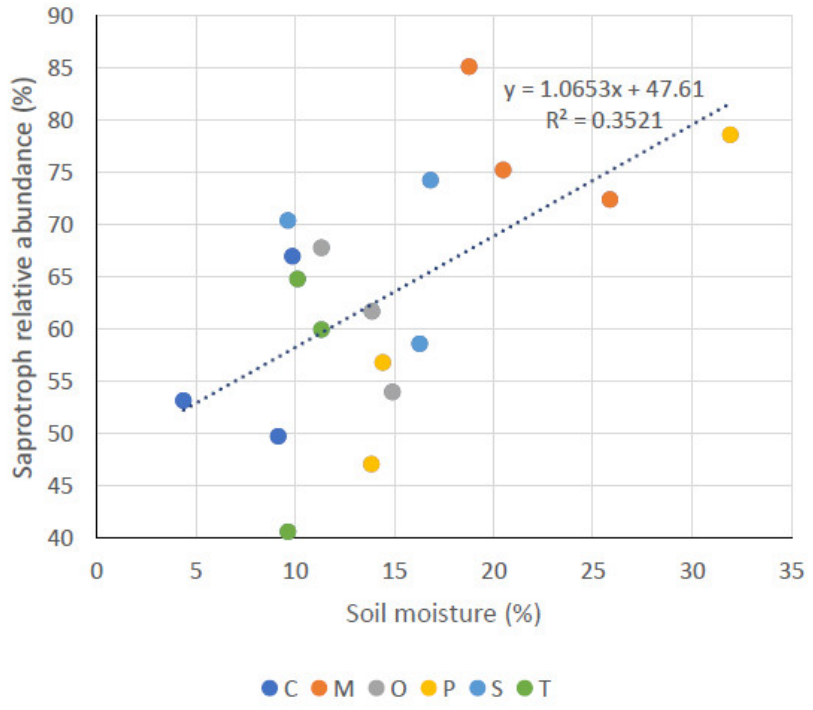

Figure 4. Correlation plot of saprotroph relative abundance (%) and soil moisture levels.

4. Discussion

The results show that the researched plants affected the fungal community structure in their rhizosphere on both functional and taxonomic levels. Statistically significant differences were found between the different plant rhizospheres and the control soil in the relative abundance of different fungal phyla and genera, and the relative abundance of the different functional groups.

The most relatively abundant fungal phylum in all of the samples was Ascomycota, and the second most abundant was Basidiomycota, which is found to be a common pattern

in soils globally [32]. Ascomycota is not only the most abundant fungal phylum but also the largest in terms of number of species [33]. The phylum Glomeromycota is entirely comprised of photoautotroph symbiotic fungi [4]. The findings showing that the relative abundance of the phylum is higher in the control soil, and the inter-shrub soil is higher than in the rhizosphere of *P. lentiscus* raise questions regarding the physical distribution of their mycelia, which are beyond the scope of this study.

The fungal genus with the highest relative abundance in this study was Aureobasidium. It was significantly ($p < 0.05$) more abundant in the rhizosphere of *P. lentiscus* than in the control and all other rhizospheres but that of *S. fruticosa*, reaching the relative abundance of 20.42% in the rhizosphere of *P. lentiscus*. According to Funguild, Aureobasidium are pathotrophs, saprotrophs, and symbiotrophs. Although it may be pathogenic to some plants, multiple studies of different crops show that Aureobasidium functions as an antagonist to several plant pathogens [30,34]. It can increase the soil availability of several nutrients and plant growth [35].

The genus Alternaria consists of saprotrophic-pathogenic fungi [36]. Our results show that the relative abundance of Alternaria was significantly lower in the rhizospheres of *S. fruticosa* and *M. communis* compared to the control. Previous studies found that extracts from *S. fruticosa* [37] and *M. communis* [38] reduce the growth of some Alternaria species, which might explain our findings.

The relative abundances of both saprotrophic and symbiotrophic fungi correlated with soil moisture. Saprotrophic fungi's relative abundance correlated positively, while symbiotrophic relative abundance correlated negatively. Research regarding the effect of different soil moisture content on the saprotrophic and symbiotrophic fungi seems lacking [39], so we are unable to place this finding in a broader context.

This study is one of the first in which fungal communities in the soil rhizosphere of the researched plants have been described. Previously published studies described arbuscular mycorrhizal fungi in *P. lentiscus* rhizosphere [40–42] and the fungal community in the soil rhizosphere of *M. communis* [43]. However, their methods and focus differ from those in the current study, and as such, a comparison between the results showing no similarities is expected.

Previous studies have shown that intercropping medicinal plants can benefit soil microbial health and crop productivity [15]. Further research that includes the intercropping of *M. communis* and *S. fruticosa* might result in lower Alternaria infections in crops.

5. Conclusions

The different plant rhizospheres hosted fungal communities with different structures. Symbiotroph relative abundance and Saprotroph relative abundance seems to corelate negatively and positively (respectively) with soil moisture across samples. We found that the rhizosphere of *P. lentiscus* had a significantly higher relative abundance of the genus Aureobasidium than in the control soil, and the rhizospheres of *M. communis* and *S. fruticosa* had a significantly lower relative abundance of genus Alternaria than in the control soil. We suggest further research should be undertaken investigating the potential use of these plants in agroforestry to effect fungal populations.

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References

1. Philippot, L.; Raiijmakers, J.M.; Lemanceau, P.; Van der Putten, W.H. Going back to the roots: The microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [CrossRef] [PubMed]
2. Broeckling, C.D.; Broz, A.K.; Bergelson, J.; Manter, D.K.; Vivanco, J.M. Root Exudates Regulate Soil Fungal Community Composition and Diversity. *Appl. Environ. Microbiol.* **2020**, *74*, 738–744. [CrossRef] [PubMed]
3. Veen, G.F.; Fry, E.L.; ten Hooven, F.C.; Kardol, P.; Morriën, E.; De Long, J.R. The Role of Plant Litter in Driving Plant-Soil Feedbacks. *Front. Environ. Sci* **2019**, *7*, 168. [CrossRef]
4. Smith, S.; Read, D. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: Amsterdam, The Netherlands, 2008; pp. 1–8.
5. van der Wal, A.; Geydan, T.D.; Kuyper, T.W.; de Boer, W. A thready affair: Linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiol. Rev.* **2013**, *37*, 477–494. [CrossRef]
6. Doehlemann, G.; Ökmen, B.; Zhu, W.; Sharon, A. Plant Pathogenic Fungi. *Microbiol. Spectr.* **2017**, *5*, 703–726. [CrossRef]
7. Nguyen, N.H.; Song, S.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [CrossRef]
8. Musilova, L.; Ridl, J.; Polivkova, M.; Macek, T.; Uhlik, O. Effects of Secondary Plant Metabolites on Microbial Populations: Changes in Community Structure and Metabolic Activity in Contaminated Environments. *Int. J. Mol. Sci.* **2016**, *17*, 1205. [CrossRef]
9. Prajapati, N.D.; Purohit, S.S.; Sharma, A.K.; Kumar, T. *A Handbook of Medicinal Plants a Complete Source Book*; Jodhpur (India) Agrobios: Jodhpur, India, 2003; pp. 1–3.
10. Petrovska, B.B. Historical review of medicinal plants' usage. *Pharmacogn. Rev.* **2012**, *6*, 1–5. [CrossRef]
11. World Health Organization. *WHO Traditional Medicine Strategy: 2014–2023*; World Health Organization: Geneva, Switzerland, 2013.
12. Newman, D.J.; Cragg, G.M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, *83*, 770–803. [CrossRef]
13. De Smet, P.A.G.M. The Role of Plant-Derived Drugs and Herbal Medicines in Healthcare. *Drugs* **1997**, *54*, 801–840. [CrossRef]
14. Köberl, M.; Schmidt, R.; Ramadan, E.M.; Bauer, R.; Berg, G. The microbiome of medicinal plants: Diversity and importance for plant growth, quality and health. *Front. Microbiol.* **2013**, *4*, 400. [CrossRef] [PubMed]
15. Li, X.; de Boer, W.; Zhang, Y.; Ding, C.; Zhang, T.; Wang, X. Suppression of soil-borne Fusarium pathogens of peanut by intercropping with the medicinal herb Atractylodes lancea. *Soil Biol.* **2018**, *116*, 120–130. [CrossRef]
16. Khan, A.L.; Asaf, S.; Abed, R.M.M.; Chai, Y.N.; Al-Rawahi, A.N.; Mohanta, T.K.; Al-Rawahi, A.; Schachtman, D.P.; Al-Harrasi, A. Rhizosphere Microbiome of Arid Land Medicinal Plants and Extra Cellular Enzymes Contribute to Their Abundance. *Microorganisms* **2020**, *8*, 213. [CrossRef] [PubMed]
17. Villalobos-Flores, L.E.; Espinosa-Torres, S.D.; Hernández-Quiroz, F.; Piña-Escobedo, A.; Cruz-Narváez, Y.; Velázquez-Escobar, F.; Süssmuth, R.; García-Mena, J. The Bacterial and Fungal Microbiota of the Mexican Rubiaceae Family Medicinal Plant Bouvardia ternifolia. *Microb. Ecol.* **2021**. [CrossRef]
18. Kushwaha, R.K.; Rodrigues, V.; Kumar, V.; Patel, H.; Raina, M.; Kumar, D. Soil Microbes-Medical Plants Interactions: Ecological Diversity and Future Prospects. In *Plant Microbiome Symbiosis*; Varma, A., Tripathi, S., Prasad, R., Eds.; Springer: Cham, Switzerland, 2020; pp. 263–286.
19. Feinburn-Dothan, N.; Danin, A. *Analytical Flora of Eretz-Israel*, 2nd ed.; CANA Publishing House Ltd.: Jerusalem, Israel, 1998; p. 583.
20. Mayer-Chissick, U.; Lev, E. Wild Edible Plants in Israel Tradition Versus Cultivation. In *Medicinal and Aromatic Plants of the Middle-East*; Yaniv, Z., Dudai, N., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 12–14.
21. Dudai, N.; Yaniv, Z. Endemic Aromatic Medicinal Plants in the Holy Land Vicinity. In *Medicinal and Aromatic Plants of the Middle-East*; Yaniv, Z., Dudai, N., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 37–44.
22. Perfumi, M.; Arnold, N.; Tacconi, R. Hypoglycemic activity of Salvia fruticose Mill. From Cyprus. *J. Ethnopharmacol.* **1991**, *34*, 135–140. [CrossRef]
23. Boukhary, R.; Raafat, K.; Ghoneim, A.I.; Aboul-Ela, M.; El-Lakany, A. Anti-Inflammatory and Antioxidant Activities of *Salvia fruticosa*: An HPLC Determination of Phenolic Contents. *Evid. Based Complement. Altern. Med.* **2016**, *2016*, 7178105. [CrossRef]
24. Nawash, O.; Shudiefat, M.; Al-Tabini, R.; Al-Khalidi, K. Ethnobotanical study of medicinal plants commonly used by local Bedouins in the badia region of Jordan. *J. Ethnopharmacol.* **2013**, *148*, 921–925. [CrossRef]
25. Maccioni, A.; Falconieri, D.; Porcedda, S.; Piras, A.; Gonçalvese, M.J.; Alves-Silva, J.M.; Salgueiro, L.; Maxia, A. Antifungal activity and chemical composition of the essential oil from the aerial parts of two new *Teucrium capitatum* L. chemotypes from Sardinia Island, Italy. *Nat. Prod. Res.* **2020**, *35*, 6007–6013. [CrossRef]
26. Ghareeb, B.A.; Weld-Ali, D.H. Assessment of antibacterial virtues of *Teucrium capitatum* (Ja'da) on pathogenic bacteria (From Folk to Complementary Medicine). *IJMPAM* **2015**, *3*, 1–9.
27. Chabane, S.; Boudjelal, A.; Keller, M.; Doubakh, S.; Potterat, O. *Teucrium polium*—Wound healing potential, toxicity and polyphenolic profile. *S. Afr. J. Bot.* **2020**, *137*, 228–235. [CrossRef]
28. Yaniv, Z.; Dudai, N. (Eds.) Myrtle (*Myrtus communis* L.)—A Native Mediterranean and Cultured Crop Species. In *Medicinal and Aromatic Plants of the Middle-East*; Springer: Dordrecht, The Netherlands, 2014; pp. 253–267.
29. Alipour, G.; Dashti, S.; Hosseinzadeh, H. Review of Pharmacological Effects of *Myrtus communis* L. and its Active Constituents. *Phytother. Res.* **2014**, *28*, 1125–1136. [CrossRef] [PubMed]

30. Di Francesco, A.; Milella, F.; Mari, M.; Roberti, R. A preliminary investigation into Aureobasidium pullulans as a potential biocontrol agent against Phytophthora infestans of tomato. *Biol. Control.* **2017**, *114*, 144–149. [CrossRef]
31. Landau, S.; Muklada, H.; Markovics, A.; Azaizeh, H. Traditional Uses of *Pistacia lentiscus* in Veterinary and Human Medicine. In *Medicinal and Aromatic Plants of the Middle-East*; Yaniv, Z., Dudai, N., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 163–197.
32. Egidi, E.; Delgado-Baquerizo, M.; Plett, J.M.; Wang, J.; Eldridge, D.J.; Bardgett, R.D.; Maestre, F.T.; Singh, B.K. A few Ascomycota taxa dominate soil fungal communities worldwide. *Nat. Commun.* **2019**, *10*, 2369. [CrossRef] [PubMed]
33. Schoch, C.L.; Sung, G.H.; López-Giráldez, F.; Townsend, J.P.; Miadlikowska, J.; Hofstetter, V.; Robbertse, B.; Matheny, P.B.; Kauff, F.; Wang, Z.; et al. The Ascomycota tree of life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Syst. Biol.* **2009**, *58*, 224–239. [CrossRef] [PubMed]
34. Bozoudi, D.; Tsaltas, D. The Multiple and Versatile Roles of Aureobasidium pullulans in the Vitivinicultural Sector. *Fermentation* **2018**, *4*, 85. [CrossRef]
35. Di Francesco, A.; Sciubba, L.; Becivenni, M.; Marzadori, C.; Baraldi, E. Application of Aureobasidium pullulans in iron-poor soil. Can the production of siderophores improve iron bioavailability and yeast antagonistic activity? *Ann. Appl. Biol.* **2021**. [CrossRef]
36. Thomma, B.P.H.J. *Alternaria* spp.: From general saprophyte to specific parasite. *BSPP* **2003**, *4*, 225–236.
37. Soković, M.; Tzakou, O.; Pitarokili, D.; Couladis, M. Antifungal activities of selected aromatic plants growing wild in Greece. *Mol. Nutr. Food Res.* **2002**, *46*, 317–320.
38. Kordali, S.; Usanmaz, A.; Cakir, A.; Komaki, A.; Ercisli, S. Antifungal and herbicidal effects of fruit essential oils of four *Myrtus communis* genotypes. *Chem. Biodivers.* **2016**, *13*, 77–84. [CrossRef]
39. Rousk, J.; Bååth, E. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiol. Ecol.* **2011**, *78*, 17–30. [CrossRef]
40. Azcón-Aguilar, C.; Palenzuela, J.; Roldán, A.; Bautista, S.; Vallejo, R.; Bera, J.M. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Appl. Soil Ecol.* **2003**, *22*, 29–37. [CrossRef]
41. Marouf, B.K.; Ighilhariz, Z.; de Lajudie, P.; Duponnois, R.; Bekki, A. Assessing the Native Arbuscular Mycorrhizal Symbioses to Rehabilitate a Degraded Coastal Sand Dune In Algeria. *Int. J. Agric. Crop Sci.* **2015**, *8*, 194–202.
42. Alguacil, M.M.; Torres, M.P.; Torrecillas, E.; Díaz, G.; Roldán, A. Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land. *Soil Biol. Biochem.* **2011**, *43*, 167–173. [CrossRef]
43. Vaz, A.B.M.; Sampedro, I.; Siles, J.A.; Vasquez, J.A.; García-Romera, I.; Vierheilig, H.; Roas, C.A.; Ocampo, J.A. Arbuscular mycorrhizal colonization of Sorghum vulgare in presence of root endophytic fungi of *Myrtus communis*. *Appl. Soil Ecol.* **2012**, *61*, 288–294. [CrossRef]