Specific Microbiome Signatures under the Canopy of Mediterranean Shrubs

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Abstract

Background: Shrub encroachment (SE) is a phenomenon in which grasses and herbaceous vegetation are replaced by woody shrubs. The progressive spread of shrubs represents a form of land cover change that is widespread in arid and semi-arid grassland ecosystems. Many previous studies have highlighted the effects of SE on soil respiration rates and nutrient storage, but little is known about its belowground effects. While previous work considered shrubs to be non-species specific or as a single intervening species, we selected an *Ampelodesmos mauritanicus* grassland and six coexisting shrubs (i.e. *Pistacia lentiscus* L., *Juniperus phoenicea* L., *Myrtus communis* L., *Rosmarinus officinalis* L., *Olea europaea* L., and *Euphorbia dendroides* L.) to investigate the effects of their encroachment on soil microbiota. We used high-throughput sequencing, coupled with soil chemical analyses and litter using ¹³C CPMAS NMR spectroscopy.

Results: Results showed a strong influence of shrub canopy on bacterial and fungal community diversity, species richness and overall community composition in the soil. Litter chemistry was dominated by O-alkyl-C, with the highest content in *Ampelodesmos* and *Euphorbia*, but richer of aromatic C in *Pistacia* and *Rosmarinus*. Bacterial diversity was highest under *Juniperus* and *Euphorbia*, while lowest under *Rosmarinus* and grassland. Conversely, fungal diversity was highest under *Olea* and *Euphorbia*, while lowest under *Myrtus* and grassland. Moreover, soil C and N contents were highest under *Olea*, *Pistacia* and *Myrtus* compared to the other canopies. In addition, grassland and *Rosmarinus* had the highest Fe content. Furthermore, increased co-occurrence network size and connectivity were recorded under shrubs compared to the grassland matrix.

Conclusions: Our results suggest that the individual effect of each shrub on the grassland matrix depends mainly on the chemical properties of the shrub litter, which alters the chemical profile of the soil and, in cascade, shapes the associated microbiota.

Introduction

Grassland ecosystems occupy approximately 41% of the Earth's land surface (Reynolds et al., 2007), and play an important role in global biogeochemical, hydrological, and energy cycles (Huang et al., 2018). However, in 10–20% of these ecosystems, shrub encroachment (SE) is occurring (Reynolds et al., 2007). SE is a phenomenon in which grasses and herbaceous vegetation are replaced by woody shrubs (Sankaran et al., 2004). SE is a form of land cover change that is widespread in arid and semi-arid grassland ecosystems (Eldridge et al., 2011). This phenomenon has been shown, thus, to significantly alter the landscape, microclimate, and above- and belowground biological processes (Dong et al., 2014). Several studies have suggested many possible causes for SE (Ratajczak et al., 2012), including the increase in atmospheric CO₂ (Wigley et al., 2010), climate change (D'Odorico et al., 2010), nitrogen deposition (Kochy & Wilson, 2001), changes in fire regime (Van Auken, 2000), and overgrazing (Briggs et al., 2005).

The global encroachment at the expense of grasses is predicted to increase in the next years, as woody plants have multiplied, in many parts of the world, over the past 100 years (Kulmatiski & Beard, 2013). Woody plant encroachment is often a conservation concern (Van Auken, 2000) because it alters open canopy ecosystem functions and processes (Eldridge et al., 2011), such as total primary productivity, decomposition rates, nutrient availability, and soil carbon dynamics (Guido et al., 2017). The resulting resource distribution, also called “islands of fertility” (Schlesinger et al., 1990), could favour the growth of other woody species because of the shrub nurse effect (Callaway, 2007), thus creating a positive feedback that could lead to an irreversible woody encroachment process (Du et al., 2016). The formation of fertility islands under woody plant canopies involves several ecological processes. First, the extensive root system of woody plants is able to extract nutrients from the depth of the subsoil and the interspaces that are deposited under the canopy through litterfall (Gherardi et al., 2013). The formation of island of fertility is especially evident under nitrogen-fixing woody species that accumulate large amount of N and P in soil through litterfall (Facelli & Brock, 2000). The formation of island of fertility is dependent on litterfall amount, litter chemical traits, litter decay rate and associated accumulation of soil

organic carbon. For example, Stinca et al., (2015) reported that colonisation of the nitrogen fixing *Genista aetnensis* over bare volcanic soil resulted in a sharp increase in C, N and P stocks in the topsoil in few decades. On the other hand, invasion of *Juniperus virginiana* into lower-lying grasslands in the western USA has been shown to alter the amount and distribution of C and N stocks in soil and plants (McKinley & Blair, 2008). As a result, the soil under the canopy of woody plants become the preferred site for plants, animals and microorganisms, whose metabolism increases soil C and N and further enriches soils in the understory (Dean et al., 1999). In addition, shrub canopy reduce wind speed, allowing atmospheric dust, wind- and water-transported nutrients, detritus, and seeds to accumulate beneath woody canopies (Eldridge et al., 2011), resulting in further enhancing fertile island effect (Reynolds et al., 1999).

Understanding the consequences of SE is important (Ratajczak et al., 2012). However, most studies on the effects of SE have focused mainly on vegetation (Hu et al., 2015), soil chemical cycling (Eldridge et al., 2015), and microbial biomass and enzymatic activities (Eldridge et al., 2015). Instead, little attention has been paid to the effects on soil microbiota composition and diversity (Yannarell et al., 2014). Plants are known to influence soil microbial communities both through the quantity and quality of their aboveground litter and through root exudates released into the soil that feed heterotrophic soil microorganisms, leading to overall shifts in the community composition of soil (Wallenstein et al., 2007). In this context, SE has been shown to significantly increase bacterial biomass (Yannarell et al., 2014) and alter fungal community composition (Bragazza et al., 2015). Therefore, dynamic and complex feedback mechanisms exist between aboveground vegetation and the belowground microbial community (Xiang et al., 2014). Moreover, microbial species are actors in the plant-soil feedback that can alter the outcome of plant competition and drive the process of plant community succession (Bever, 2003; Idbella et al., 2021). For this reason, SE may have lasting consequences for grassland ecosystem restoration and management, as shifts in microbiota composition may facilitate long-term succession from grassland to shrub/forest ecosystems (Yannarell et al., 2014).

As for the study of the influence of SE on the composition of the soil microbial community, very little evidence was found in the literature, since they all consider shrubs as non-species specific or as a single encroached species. For example, Xiang et al., (2019) showed that the encroachment of *Caragana microphylla* into a grassland dominated by *Cleistogenes songorica* induced significant changes in soil bacterial community composition. Similarly, Ding et al., (2020) showed that the encroachment by *Vaccinium fragile* into a grassland dominated by *Eulalia pallens* significantly restructured the diversity and composition of soil bacterial and fungal communities. On the other hand, Yannarell et al., (2014) showed that the encroachment by four different species on the grassland Remnant Hill Prairies significantly altered both bacterial and fungal communities without selecting species-specific signatures under the canopy of each woody plant. However, most of the previous studies compared only a single woody species with the surrounding open vegetation. Here, our study aimed to investigate the influence of six coexisting Mediterranean shrubs (i.e. *Pistacia lentiscus*, *Juniperus phoenicea*, *Myrtus communis*, *Rosmarinus officinalis*, *Olea europaea*, and *Euphorbia dendroides*), over *Ampelodesmos mauritanicus* grassland, on the soil microbiota under their canopies. Specifically, we combined soil and litter chemistry analyses with next-generation sequencing techniques to determine how the soil microbiota is shaped by the canopy of Mediterranean shrubs. Our hypothesis states that litter chemistry and canopy structure are evidently different between shrubs, resulting in a specific microbial fingerprint. This specific effect is thought to be derived from the specific chemical characteristics of the litter of the shrubs as it falls and decomposes (De Marco et al., 2011), leading to different changes in soil chemistry and also microbial composition. The aim of this study was therefore to provide basic information and novel insights into the environmental selection of soil microbial communities by each of the most abundant species-specific shrubs in a Mediterranean SE ecosystem. Specific aims were:

1. to assess the “island of fertility” effect under the canopy of different Mediterranean shrubs;
2. to describe the bacterial and fungal microbiota associated with the different shrub species;
3. to explore the link between soil chemistry and bacterial and fungal microbiota.

Material And Methods

Study site description

The study was conducted in Cape Palinuro shrubland site (40°01'35 "N 15°16'30 "E), located in southwestern Italy, about 40 miles southwest of the city of Salerno (Fig. 1). This area is located in a hot Mediterranean/dry summer subtropical climate zone characterised by mild with moderate seasonality. Summers are dry and hot due to the dominance of high-pressure subtropical systems, while winters have moderate temperatures and changeable, rainy weather due to the polar front. This climate is typical of the western sides of continents between 30° and 45° latitudes. Vegetation is adapted to dry summers and it is fragrant and oily, making it susceptible to fire (<http://www.cape-palinuro.climatemps.com>). The site is characterized geomorphologically with limestone rocks overlying clay soils with abundant rock outcrops. The elevation of the study area is 185 m a.s.l., and the average annual temperature is 16.7°C. In winter, temperatures average is 13.3°C during the day and drop to 7.9°C overnight; in spring, temperatures reach 17.6°C, mostly in the afternoon, while during the night-time it drops to 11.2°C; in summer, average maximum temperature is 27.1°C and average minimum is 19.7°C. The average annual precipitation is 789.8 mm.

Soil sampling

Within the study area, six shrubs were selected for soil sampling, including five evergreens: *Pistacia lentiscus* L., *Juniperus phoenicea* L., *Myrtus communis* L., *Rosmarinus officinalis* L. and *Olea europaea* L., and one deciduous: *Euphorbia dendroides* L. (Fig. 1). Soil samples were also collected in the grassland soil dominated by *Ampelodesmos mauritanicus* L., a perennial, fire-prone tall grass that dominate in the matrix between shrubs (Incerti et al., 2013). For each shrub, three replicates were randomly selected for soil sample collection. In April 2019, 21 samples were collected independently: 7 samples (6 samples under the canopy of each shrub + one sample for the grassland) with 3 replicates. Shrub's size was 135 ± 22 cm for *Euphorbia*, 163 ± 74 cm for *Olea*, 173 ± 59 cm for *Pistacia*, 126 ± 9 cm for *Juniperus*, 164 ± 8 cm for *Rosmarinus*, and 117 ± 11 cm for *Myrtus*. Soil samples were collected by a 5 cm diameter soil corer, at a depth of 10 cm after removal of above-ground litter, in four randomly selected points under each canopy. Subsequently, soil was pooled and sieved (2 mm mesh) on site resulting in a single composite sample for each canopy replicate. The samples were stored in sterile plastic bags and labelled. Before every sampling operation, the soil corer was thoroughly cleaned and sterilised to avoid between samples contamination. After collection, samples were then divided into two fractions: one fraction was kept at 4°C to investigate soil chemical properties; the other fraction was stored at -20°C and used for molecular analysis.

Litter collection and chemical analysis

Under each canopy, leaf litter was collected with net traps during the period of maximum leaf fall from three randomly selected individuals of each shrub species. Freshly collected litters were dried in a ventilated chamber at 30°C until they reached a constant weight and then stored at room temperature. Litters were then characterized for total C and N content by flash combustion of microsamples (5 mg of litter) using a CN soil elemental analyser (Flash EA2000 Thermo). Proximate cellulose and lignin content were quantified as acid-hydrolysable fraction and acid-unhydrolyzable materials, respectively (Gessner, 2005). In addition, leaf materials were characterized by ^{13}C cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) (Kögel-Knabner, 2002) obtained in the solid-state and under the same conditions, which allowed a comparative analysis of the resulting spectra. The spectrometer used was a Bruker AV -300 equipped with a 4 mm wide-bore MAS probe (Bonanomi et al., 2013). The spectral ranges and corresponding C types were identified as described by Bonanomi et al., (2013): 0–45 ppm = alkyl-C (characteristic of lipid waxes, cutins and microbial products); 46–60 ppm = methoxyl- and N-alkyl-C (characteristic of amino acids and lignin components); 61–90 ppm = O-alkyl-C (characteristic of carbohydrates and polysaccharides); 91–110 ppm = di-O-alkyl-C (anomeric C1 of celluloses, tannin and lignin components); 111–140 ppm = H- and C-substituted aromatic C (mainly associated with polyphenols,

lignin and tannin components); 141–160 ppm O-substituted aromatic C (phenolic and O-aryl-C, characteristic of phenols, lignin and tannin components); 161–190 ppm carboxyl-C (characteristic of organic acids, amides, esters).

Soil chemical analyses

Soil samples were dried in a ventilated chamber at room temperature until a constant weight was reached. The soil was analyzed for 16 parameters that included the most important factors for root development, i.e., total organic carbon, pH, total nitrogen, and macro- and micronutrients important for plant growth. Specifically, the following parameters were measured: soil electrical conductivity (EC) and pH, were determined in 1:5 and 1:2.5 soil-water suspensions, using a conductivity meter and a pH meter, respectively (Czekala et al., 2016). Total nitrogen was determined by the Kjeldhal method (Czekala et al., 2016), while phosphorus was assessed by the molybdovanadate phosphate method (AOAC, 1990). Water content and organic matter content were determined by weight loss at 105°C for 24 h and 550°C for 8 h, respectively (Silva et al., 2014). Potassium, magnesium, iron, manganese, calcium, sodium, copper and zinc were determined by flame atomic absorption spectroscopy (Peters et al., 2003). Total limestone is determined by the weight method against a strong acid, the attack of the limestone leads to a gas release of CO₂, the volume of which is measured (LANO: NF ISO 10693). Finally, the chloride content (Cl) in the soil was determined by the volumetric method described by Meldrum and Forbes (1928).

Soil DNA extraction and amplification

The microbiome of homogenized soil samples under each canopy was analyzed by Illumina high-throughput sequencing. The DNeasy PowerSoil kit (Qiagen) was used to extract the microbial DNA from 2 g of each homogenized soil. Bacterial and fungal diversity were assessed by high-throughput sequencing of the amplified V3-V4 regions of the 16S rRNA gene (~ 460 bp) and ITS1-2 (~ 300 bp). PCR was carried out with primers S-D-Bact-0341-b-S-17/S-D-Bact0785-a-A-21 (Berni Canani et al., 2017) and BITS1fw/ B58S3- ITS2rev (Bokulich & Mills, 2013) using conditions reported in the original studies. For bacterial primers S-D-Bact-0341-b-S-17 (5'-CCTACG GGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GAC TACHVGGGTATCTAATCC-3'), PCR conditions were: 25 cycles of 95°C for 3 min, 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, 72°C for 5 min and held at 4°C. For fungal primers BITS1fw (5'-ACCTGCGGARGGATCA-3') and B58S3-ITS2rev (5'-GAGATCCRTTGYTRAAAGTT-3') PCR conditions were: 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s, and a final extension of 72°C for 5 min. PCR products were purified with the Agencourt AMPure beads (Beckman Coulter, Milan, IT) and quantified using an AF2200 Plate Reader (Eppendorf, Milan, IT). Equimolar pools were obtained and sequencing was carried out on an Illumina MiSeq platform, yielding to 2× 250 bp, paired-end reads.

Sequence data analysis

Demultiplexed fastq files were processed using the DADA2 package (version 1.16.0 pipeline) (Callahan et al., 2016) in R software (4.0.4) (Team, 2016). DADA2 provides better taxonomic resolution than other methods because it retains unique sequences and calculates sequencing error rates rather than clustering to 97% similarity (Hugerth & Andersson, 2017). The resulting taxonomic units are referred to as amplicon sequence variants (ASVs) rather than operational taxonomic units (OTUs). For bacterial sequences, forward and reverse reads were trimmed to 250 bp, primer sequences were removed from all reads, and filter parameters used were the following: maxN = 0, maxEE for both reads = 2, truncQ = 2 (MaxEE corresponds to the maximum expected errors, TruncQ represents the parameter that truncates reads on the first occurrence of a quality score less than or equal to two, and MaxN is the maximum number of 'N' bases accepted). Error rates were estimated by learnErrors using nearly 4 million reads. Sequences were dereplicated using derepFastq with default parameters and exact sequence variants were resolved using the dada algorithm. The RemoveBimeraDenovo function was then used to remove chimeric sequences. For fungal sequences, the pipeline was pre-empted by a preliminary step of trimming adapter sequences and low-quality ends (< Q20) using Cutadapt software (Martin, 2011). For both the bacterial and fungal datasets, reads with more than three errors in the forward reads and five errors in the reverse reads were removed. Taxonomy was then assigned using assignTaxonomy based on the SILVA (v132) and UNITE (v7) databases for

bacterial and fungal communities, respectively (Quast et al., 2013; Nilsson et al., 2019). *Chloroplast* and *Streptophyta* contaminants and singleton ASVs were removed, and relative abundances of the other taxa were recalculated.

Statistical analysis and data visualization

Plotting was performed using PRIMER 7 software (Primer-E Ltd, Plymouth; UK). Alpha diversity metrics were calculated. Heatmaps were created to assess variation in community composition at lowest taxonomic levels. Heatplots were used clustering variables according to the results of an index of association similarity matrix and samples according to Bray-Curtis dissimilarity. The 50 most abundant taxa in the fungal and bacterial communities are shown in the heatplots. A resemblance matrix calculated based on Bray-Curtis dissimilarity was used to perform non-metric multidimensional scaling (nMDS) to assess variation in species composition under the different investigated canopies for the bacterial and fungal communities. The significance of changes in composition of the two communities analysed were tested by PERMANOVA (999 permutations), using the shrub species as fixed factor. The significance of variation in the alpha diversity metrics of the two communities was assessed along with the soil and litter chemical characteristics using the ANOVA test, and the means were pairwise separated using the *post-hoc* Tukey test. The level of significant differences was assessed at $P < 0.05$. All statistical analyses were performed using STATISTICA 13.3 software.

Furthermore, we analysed functional group variation for the fungal community, identifying putative fungal functional groups as well as their trophic modes using FUNGuild (Nguyen et al., 2016). The core microbiome was identified by constructing Venn diagrams for 7 sets (i.e., 6 canopies + the grass) for bacterial and fungal community using R software and the VennDiagram package (Chen & Boutros, 2011). Co-occurrence networks incorporating communities containing bacteria and fungi were based on single ASVs and generated to assess co-occurrence or potential interactions between species. To reveal microbiome complexity and potential interrelationships among microbial community members, network analyses were carried out for communities at the seven different sampled canopies. Recent studies have shown that network inference techniques are useful for deciphering microbial relationships from co-occurrence patterns (Barberán et al., 2012). For the purpose of focusing on the most commonly occurring ASVs and decreasing the effects of rare ones, only the most abundant 50 ASVs were analysed for each bacteria and fungi. The pairwise correlations between the ASVs were calculated using the Spearman correlation in R (version 3.3.2 and Hmisc package 4.0–1). Based on the statistical analysis, only strong and significant (Spearman's $r > 0.6$ or $r < -0.6$ and $p < 0.05$) correlations were considered. The network visualization was made using Gephi (version 0.9.2, Bastian & Jacomy, 2009). Each edge represents a robust and significant correlation and each node represents an ASV. A set of integrative metrics were calculated and compared to describe the network topology. For example, the average number of neighbours explains the complex pairwise connections and the average path length describes node distribution. Number of modules, defined as clusters of nodes that form coherent structural subsystems of interacting units, and modularity were measured according to the Louvain algorithm within Gephi.

Results

Litter and soil chemistry

Leaf litter chemistry varied largely among shrub species and the grass *Ampelodesmos* (Table S4 & Table 1). *Ampelodesmos* had the highest cellulose content and C/N and lignin/N ratios, while it had the lowest N content and relatively low lignin content. On the other hand, the highest lignin content was found in *Rosmarinus* litter, which also had high N content and the lowest cellulose content. *Pistacia* had relatively high contents of all proximate parameters except N content, which was relatively low. *Juniperus* had very low N content, and thus the highest C/N and lignin/N ratios. *Euphorbia* leaf litter, on the other hand, had the highest N content and the lowest C/N and lignin/N ratios. Finally, *Myrtus* leaf litter recorded the lowest lignin content among all other canopies and thus a very low lignin/N ratio.

Table 1

Chemical traits of the studies shrubs and the dominant perennial plant in the grassland (*Ampelodesmos mauritanicus*).

		<i>Euphorbia</i>	<i>Juniperus</i>	<i>Myrtus</i>	<i>Olea</i>	<i>Pistacia</i>	<i>Rosmarinus</i>	<i>Ampelodesmos</i>
Elemental and proximate parameters	Cellulose (%)	14.2b	13.0c	15.8bc	10.6d	17.9b	8.6d	27.3a
	Lignin (%)	13.2d	23.8b	10.6e	16.3c	23.9b	41.4a	18.1c
	N (%)	2.1a	0.7d	1.2c	1.7b	1.1c	1.9a	0.5d
	C / N	20.5d	69.3a	41.1b	31.2c	45.4b	22.8d	77.6a
	Lignin / N	6.3d	33.0a	8.7d	16.3c	21.7b	21.6b	34.5a
¹³ C-CPMAS NMR-derived parameters	Carboxylic C	9.8a	4.4c	6.2b	5.6c	5.3c	6.9b	4.8c
	– 161–190 ppm							
	O-substituted aromatic C	4.4d	4.8cd	5.7c	3.6e	9.3a	7.4b	4.0d
	– 141–160 ppm							
	di-O-alkyl C – 91–110 ppm	10.8bc	9.7cd	11.4b	8.9d	12.6b	11.1a	14.9a
	O-alkyl C – 61–90 ppm	44.8b	38.4c	42.4b	39.1c	34.7c	37.5c	55.7a
	Methoxyl C – 46–60 ppm	8.1a	6.2b	6.2b	8.3a	4.5c	5.6bc	4.6c
	Alkyl C – 0–45 ppm	17.4d	27.5a	19.2c	24.9b	21.5bc	19.9c	6.7e
Values are mean of five replicates. Different letters within each column indicate significant difference (Duncan test, P < 0.05).								

The ¹³C-CPMAS NMR data showed that *Euphorbia* leaf litter had the highest content of carboxylic C, followed by *Rosmarinus* and *Myrtus*, while the others, including *Ampelodesmos*, had significantly low values. The O-substituted aromatic C fraction was highest in *Pistacia* and lowest in *Ampelodesmos* and *Euphorbia*. Moreover, *Pistacia* and *Rosmarinus* showed the highest H-C substituted aromatic C content while the lowest was found in *Euphorbia*. However, *Ampelodesmos* and *Rosmarinus* had the highest di-O-alkyl C and O-alkyl C fractions, while the lowest methoxyl C and alkyl C fractions were recorded for *Ampelodesmos* alone. In addition, *Euphorbia* and *Olea* had the highest methoxyl C fraction, and *Juniperus* was the one that contained the highest alkyl C fraction content instead.

Soil chemical parameters showed significant variation among canopies (Table S5 & Table 2). Clustering of the canopies, by D1 Euclidean distance, based on chemical parameters resulted in 5 clusters for the six studied shrubs and the grass, *Ampelodesmos*, separating *Euphorbia*, *Myrtus*, *Juniperus*, Grassland-*Rosmarinus* and *Pistacia-Olea* (Fig. S2). In details, *Euphorbia* and *Myrtus* showed the highest soil pH compared to grassland and other canopies. In addition, *Euphorbia* soil proved to enclose the highest total limestone by a maximum of 4.11% and soil potassium by a maximum of 1.27 g/kg. Electrical conductivity, chlorides and sodium were higher under *Olea* and *Myrtus*, while significantly lower values were

measured under the rest of the canopies, with an exception for sodium under *Juniperus*. The organic C and total N contents were higher under *Myrtus*, *Pistacia* and *Olea* than under the rest of the canopies. In addition, the highest phosphorus (P) and Mg contents were found in the soils under *Euphorbia* and *Myrtus* canopies. The distribution of Ca, Cu, Zn and Mn was different among the canopies. Fe content was found to be significantly higher only in the grassland and under *Rosmarinus* compared to other canopies, while the lowest value was found under *Euphorbia* canopy.

Table 2
Chemical analysis of the soils collected under the canopy of plant species in the studied shrubland as well as the grassland.

Parameters	Grassland	<i>Juniperus</i>	<i>Rosmarinus</i>	<i>Pistacia</i>	<i>Olea</i>	<i>Myrtus</i>	<i>Euphorbia</i>
pH	5.62c	6.00c	5.82c	6.32b	6.34b	6.74a	6.93a
Water content (%)	5.82c	11.19b	6.31c	9.13b	9.42b	10.46b	16.99a
Total limestone (%)	0.87b	1.75b	1.39b	0.82b	0.67b	1.11b	4.11a
Electrical conductivity (mS/cm)	0.31b	0.26b	0.21b	0.34b	0.42a	0.47a	0.31b
Chlorides Cl (g/Kg)	0.21a	0.15bc	0.13bc	0.18b	0.278a	0.30a	0.087c
Sodium Na ₂ O (g/Kg)	0.25b	0.40a	0.19c	0.31b	0.43a	0.43a	0.26b
Organic Carbon (%)	12.48b	11.90b	11.53b	16.6a	16.27a	16.17a	9.80b
Total Nitrogen (%)	0.50b	0.47b	0.42b	0.77a	0.85a	0.99a	0.70a
P (mg/Kg)	20.47b	21.73b	12.55c	20.6b	20.88b	35.20a	39.89a
K (g/kg)	0.35c	0.65b	0.44bc	0.51b	0.53b	0.55b	1.27a
Mg (g/Kg)	0.57b	0.91a	0.46b	0.89ab	0.97a	1.20a	1.32a
Ca (g/Kg)	3.76b	3.91b	2.63b	9.01a	8.65a	10.92a	8.40a
Cu (mg/Kg)	1.37b	1.62b	1.43b	2.04a	2.52a	1.87a	2.05a
Zn (mg/Kg)	8.08c	3.96d	5.53d	17.95b	18.68ab	22.59a	4.69d
Mn (mg/Kg)	26.66b	30.84ab	25.48b	43.98a	39.82a	27.77b	13.29c
Fe (mg/Kg)	56.78a	29.80b	44.57a	23.25b	30.00b	23.23b	5.21c
Different letters within each parameter indicate significant differences (Duncan test, P < 0.05).							

Microbial diversity

Significant variation in the two indices (Shannon diversity and species richness) was found for bacterial and fungal diversity. Compared with the grassland, bacterial species richness was significantly higher in the soil under the canopy of *Euphorbia* and significantly lower under *Rosmarinus*, while slight, although not significant, variations were observed for the other canopies (Fig. 2a). The Shannon index of bacteria was significantly higher in the soil under the canopy of *Juniperus* and *Euphorbia* compared to the canopy of *Rosmarinus* and the grassland. On the other hand, no significant variation was found in fungal species richness, while the *Olea* showed the highest Shannon diversity index of the soil fungi, which showed a statistical significance compared to *Myrtus* and grassland soils (Fig. 2b).

Bacterial community composition

At the phylum level, considerable significant variation was found among shrubs in the bacterial community (Fig. 3a, Table S1). All shrubs harboured mainly *Actinobacteria* under their canopies, ranging from 30.1% in *Rosmarinus* to 40.3% in *Olea*, except for *Pistacia*, which harboured equally *Actinobacteria* and *Proteobacteria*, with a proportion of 25.4% each. On the other hand, the lowest abundance of *Proteobacteria* was found in the grassland soil at 15.7% compared to 20.9% – 25.4% among the other canopies. *Acidobacteria*, on the other hand, was most abundant in the grassland soil at 18.5% followed by *Pistacia* at 15.8% and less than 10% among the other canopies. In addition, the highest levels of *Verrucomicrobia* were found in *Myrtus* canopy (13.2%), followed by *Pistacia* (8.7%) and the remaining canopies with less than 5%. On the contrary, *Planctomycetes* was higher in *Rosmarinus* (20.2%) compared to the other canopies (< 13%).

Moreover, the Venn diagram (Fig. 4a) confirmed that the canopies of *Euphorbia*, *Juniperus*, and *Pistacia* are the ones that include the highest number of unique ASVs, where they attained 47, 34, and 31 ASVs, respectively. While the lowest number of unique ASVs was found under the canopy of *Rosmarinus* and *Olea*. However, 219 ASVs were determined as the core bacterial microbiota of the studied microbiomes.

At the lowest taxonomic level (Fig. 5), the clustering of the canopies, based on the 50 most frequent ASVs, is highly distinct using the Bray-Curtis similarity index across different shrubs of the grassland ($P = 0.001$; Table S2), where it revealed five different clusters for the six canopies and the grass. *Rosmarinus* soil is clearly separated from the other samples by a difference of 40% (cluster A). Cluster B includes *Pistacia* soil, separated from the rest by a difference of 35%, while grassland soil is separated from the rest by a difference of 25% (Cluster C). All the other canopies have more than 80% similarities. The main driving ASVs of the clustering were 1) *Acidobacteria* subgroup_6, which was more abundant under *Pistacia* soil compared to the other canopies, 2) *Rubrobacter*, which was highly abundant under the canopy of *Rosmarinus*, grassland and *Olea*, while very rare under the canopy of *Pistacia*, 3) *Candidatus Udaeobacter*, which was abundant only under *Myrtus* canopy, followed by grassland and *Pistacia*, 4) *Isosphaeraceae*, which was abundant under *Rosmarinus*, less abundant under other canopies and almost absent under *Pistacia* canopy, and 5) WD2101_soil_group ASV belonging to *Planctomycetes*, which was highly abundant under *Rosmarinus* and less abundant under the rest of the canopies.

The nMDS analysis of the microbial community distribution according to the chemical parameters (Fig. S3a) showed a strong positive correlation between Fe and the distribution of grassland by its bacteria. However, *Rosmarinus* and *Olea* were positively correlated with Cu, Na, total N, Mg, and Cl; and negatively correlated with OC, total limestone, P, Mn, Ca, and Zn; with an opposite pattern for the bacterial distribution of *Pistacia* and *Myrtus*, which was positively correlated with OC, P, and total limestone and negatively correlated with total N, Na, and Mg. Finally, *Juniperus* and *Euphorbia* showed a high negative correlation with Fe, which was the main reason for their bacterial community distribution

Fungal community composition

The fungal community showed a clear variation among the shrub canopies and also in comparison with the grassland (Fig. 3b, Table S1). Specifically, all the soils studied were dominated by the phylum *Ascomycota*, with abundance ranging from 60.5% in the grassland soil to 80.3% in *Myrtus* and *Rosmarinus*. However, the highest proportion of the phylum *Basidiomycota* was found in the grassland soil (25.1%), followed by *Juniperus*, *Euphorbia* and *Myrtus* with ~ 16%, while in the other canopies their abundance did not exceed 10%. On the other hand, the phylum *Chytridiomycota* was found with an abundance of 5.7% under the canopy of *Olea*, and less than 2% in the rest of the canopies, with the exception of *Myrtus*, in which it was completely absent. In addition, *Pistacia* showed exclusivity in harbouring the *Mortierellomycota*

phylum (15.4%), followed by *Euphorbia* (10.9%), while it was almost absent under the *Rosmarinus* canopy. Finally, the phylum *Zoopagomycota* was present in only 2.4% of the *Pistacia* soil, and the phylum *Rozellomycota* was found only in *Juniperus*, *Euphorbia* and *Pistacia* at a very low abundance.

Moreover, the Venn diagram showed that all the canopies have enclosed a high amount of exclusive ASVs compared to the bacterial community (Fig. 4b). The highest number of unique ASVs was found under the grassland (i.e., 46) and the lowest was found under *Euphorbia* canopy (21) and *Myrtus* (22). However, the core microbiota, responsible for the most frequent ASVs, was represented by 115 ASVs.

At the lowest taxonomic level (Fig. 6), clustering of the canopies, based on the 50 most abundant ASVs, using the Bray-Curtis similarity index showed clear representation of variation among the fungal community across different shrubs and grassland ($P = 0.001$; Table S2). This clustering resulted in 7 separated clusters. The first cluster (A) representing *Myrtus*, which is separated from the rest by 70% of dissimilarity, followed by cluster B where *Juniperus* is separated from the rest by almost 65% of dissimilarity; while the rest of the canopies are separated by more than 50% of dissimilarity. The heatmap shows that the main ASVs responsible for the observed clustering differ from one canopy to another, comprising a group of genera instead of a specific one (Fig. 6). For example, the group that includes the genera *Penicillium*, *Gibberella*, *Helicoma*, and *Pilidium* is responsible for separating *Myrtus* from the rest because they are more abundant under its canopy. In addition, the genera *Didymella*, *Sarocladium*, *Phaeosphaeria*, *Xylariales* and *Russoella*, all belonging to the phylum *Ascomycota*, distinguish the *Rosmarinus* canopy from the rest. In addition, the overall diversity of *Juniperus* was associated with a group of ASVs that included *Stemphylium*, *Agaricomycetes*, *Mortierella* and other *Ascomycota* genera. While the grassland was characterized mainly by *Claviceps* genus belonging to the *Ascomycota*.

As revealed by FUNGuild analysis (Fig. S1), the distribution patterns of dominant ecological guild functions were more similar between *Pistacia*, *Rosmarinus* and the grassland. However, each plant canopy was found to host one or more guilds exclusively or more frequently compared to the others. For example, algal parasites are present only under the canopy of *Euphorbia* and *Pistacia*; however, bryophyte parasites are more abundant under the canopy of *Euphorbia*, while they are almost absent in the canopy of *Rosmarinus* and the grassland. In addition, ectomycorrhizal fungi were present under all canopies with low abundance except in grasslands where they were almost absent.

As for the distribution of fungal community, *Pistacia* had the highest positive correlation with Ca (Fig. S3b). However, Fe was responsible for the distribution of grassland and *Rosmarinus* with the highest positive correlation observed, while the large negative correlation between Fe and the fungal community of *Myrtus* was responsible for their distribution. In addition, P, Zn and OC were negatively correlated with grassland, *Rosmarinus* and *Olea* and positively correlated with *Myrtus*. Finally, the distribution of the fungal community of *Juniperus* was due to its strong negative correlation with Ca.

Microbial co-occurrence

We constructed two co-occurrence networks dividing samples from the grassland and the shrubland areas (Fig. 7) and calculated seven topological parameters to assess the interactions between the ASVs in the two networks (Table S3). The microbial network of the grassland area contained 91 nodes and 1946 edges, while the network of the shrubland area contained 97 nodes and 1159 edges. The proportions of positive ASVs correlations in the microbial networks of the grassland and shrubland areas were 81.9% and 99.7%, respectively. Network centralization, network heterogeneity, and characteristic path length values were significantly higher in the shrubland than in the grassland area. Compared with the shrubland, the network density, and clustering coefficient values were much higher in the grassland. Microbial assemblages in the two groups exhibited modular structures, and the modularity was notably higher in the shrubland (Fig. 7b1) than in the grassland area (Fig. 7a1). Nodes with high degrees, high closeness centrality, and low betweenness centrality were considered keystone taxa. In the grassland, 37.4% of the top 13 keystone taxa were *Ascomycota*, 18.7%

were *Actinobacteria*, and 14.3% were *Proteobacteria*. In the shrubland, *Ascomycota*, *Actinobacteria* and *Proteobacteria* represented 39.2%, 17.5% and 13.4% of the top 13 keystone taxa, respectively.

Discussion

Grassland matrix

Our proximate and ^{13}C NMR analysis show that litter of *Ampelodesmos*, the dominant perennial grass in the monitored grassland, contained the highest levels of cellulose, di-O-alkyl-C, and O-alkyl-C compounds, while the levels of lignin, N, carboxyl-C, O-substituted aromatic C, methoxyl-C, and alkyl-C were the lowest. This litter type decomposes relatively quickly on the ground (Bonanomi et al., 2019), but is also highly flammable and most of it remains in the plant tussock, a condition that promotes the occurrence of fire events in this grassland (Incerti et al., 2013). Previous studies have shown the significant and diverse effects of plant litter on soil physio-chemical properties and also on microbial communities (Schneider et al., 2012; Ping et al., 2019). Since litter input could affect soil ecological processes, including soil C and N cycling via litter decomposition, we observed that soil under *Ampelodesmos* had correspondingly lower levels of organic carbon, total nitrogen, and P, while soil Fe content was highest. This is probably due to the occurrence of summer fires, which allow low accumulation of litter and OC in the soil profile. Moreover, we generally found low bacterial and fungal taxonomic richness and diversity in the grassland soil. These results could be explained by the low chemical diversity of the litter combined with low structural heterogeneity, resulting in low trophic specialisation that does not allow the coexistence of different saprotrophic communities.

In addition, we found high abundance of *Acidobacteria* and *Actinobacteria* at the phylum level, whereas the abundance of *Proteobacteria* was low compared to the soil under shrub canopies. *Proteobacteria* are generally considered to be more copiotrophic, while *Acidobacteria* are considered oligotrophic in soil (Leff et al., 2015). Fierer et al., (2007) described *Acidobacteria* as oligotrophs that prefer poor soils with lower carbon availability. Therefore, their high proportion in grassland could be explained by the low C and N contents in the soil. In addition, the *Verrucomicrobia* phylum has been shown to utilise a variety of carbon compounds and may be closely linked to carbon cycling (Fierer et al., 2013), making it more adaptable to oligotrophic environments in soils (Noll et al., 2005). Indeed, our results are in agreement with those of Qiu et al., (2020), who found that the proportion of *Verrucomicrobia* was significantly lower in grasslands than in shrublands at different soil depths.

On the other hand, we found the highest abundance of *Basidiomycota* and the lowest of *Ascomycota* compared to the soil under shrub canopies. This is surprising considering the low lignin content of *Ampelodesmos* litter. The low abundance of *Ascomycota* in grasslands compared to shrub canopies suggests strong litter selection, as they are considered important decomposers in the early and intermediate stages of litter succession (Boanomi et al., 2019). Therefore, abundance of *Ascomycota* is likely promoted by more complex plant litter (Purahong et al., 2016). In detail, the genus *Trechispora*, which belongs to the phylum *Basidiomycota*, was found only in grassland soils. This saprophytic fungus is involved in wood decomposition processes, and its occurrence in grasslands rather than under shrub canopies is consistent with the results of Kirker et al., (2017), who found higher abundance of wood decomposition fungi for the exposed areas compared to the unexposed areas, and many of these species are well-studied wood decay fungi or at least the genera, with the exception of *Trechispora* spp, which is more abundant on decaying wood debris on the forest floor than on solid wood. Finally, we recored a high abundance of the genus *Claviceps* in the grassland soil, whereas it was almost absent under all shrub canopies. This result suggests a rather specific association between *Ampelodesmos* and *Claviceps*, which are known for the ergot disease infecting ~ 200 species of wild and cultivated grasses (Boestfleisch et al., 2015).

Are shrubs signatures specific?

We hypothesized that the diversity, richness, and composition of the microbial community would be altered by each shrub canopy, possibly due to the higher amount and/or diversity of litter trapped under the shrub canopy, which would enter the soil C and N cycles (Hooper et al., 2000). Our ^{13}C NMR analysis shows high diversity within the litter characteristics of the shrubs studied compared to the grassland. We found that the litter of the evergreen, sclerophyllous *Rosmarinus* had a high content of lignin, N and di-O-alkyl C, while it had the lowest cellulose content. On the other hand, *Pistacia* had the highest content of O-substituted aromatic C while it had the lowest N content. Moreover, *Olea* had the highest value of methoxyl-C, while *Myrtus* litter had the lowest lignin content. *Euphorbia*, a deciduous species that sheds its leaves in summer to avoid drought period, had high N content associated with high carboxyl-C and methoxyl-C content. As a result, *Euphorbia* accumulated little OC in the soil compared to other shrubs such as *Pistacia* and *Olea*. Previous studies suggest that litter decomposition in soil can alter microbial biomass, composition and community structure by increasing substrate variability and diversity of chemical compounds, and that this can vary depending on litter quality (Meier & Bowman, 2008; Chapman et al., 2013). Accordingly, the evergreens *Pistacia*, *Olea* and *Myrtus* have demonstrated high C levels and low N levels in their soils, while the coniferous *Juniperus* and *Rosmarinus* enclose low C and N levels. Our results, at microbiota scale, showed that bacterial diversity was significantly higher under *Juniperus* and *Euphorbia* canopies than under *Rosmarinus*, while fungal diversity was significantly higher under *Olea* than under *Myrtus*. Collins et al., (2020) found that SE did not assign a "global signature" but was associated with increased, decreased, or no change in alpha microbial diversity when compared to soils from nearby herbaceous plant communities. Our data, instead, indicate that the microbiota signature among coexisting shrub species is highly specific.

The increase in *Proteobacteria* in shrub soils is consistent with the findings of Wallenstein et al., (2007), who found an increase in *Proteobacteria* in Arctic shrub soils, suggesting that these bacteria thrive in C- and nutrient-rich soils that develop under shrub canopies and exhibit copiotrophic properties. Previous work has shown that SE increases oxygenation and nutrient content in surface soil (Bragazza et al., 2015), suggesting that SE may influence the distribution of bacterial life strategies in the soil, i.e. enrichment of copiotrophic and depletion of oligotrophic bacteria. Moreover, all shrubs harbored a significant amount of *Actinobacteria* under their canopy. In particular, the *Streptomyces* genus was more abundant under the *Myrtus* canopy compared to the other canopies and the grassland. In this context, Qiao et al., (2017) studied microbial communities in nutrient-rich soils and found that *Actinobacteria* were more abundant than other microbes. It is now widely accepted that the establishment of bacterial communities in soils is not random but is controlled by specific compositional rules (Reinhold-Hurek et al., 2015), including plant species (Edwards et al., 2015). Interestingly, we found high abundance of free-living N-fixing bacteria, including the genera *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Neorhizobium*, under the canopies of *Euphorbia* and *Pistacia*, while it was lower under the canopy of *Rosmarinus*. Surprisingly, the abundance of these free-living N-fixing bacteria was positively correlated with soil pH, phosphorus content and cations, while it was negatively correlated with soil Fe content, which could partially explain their distribution. Our results also showed that *Rosmarinus* had the highest Fe content, which assigns for it an intermediate-like level to grassland, while *Euphorbia* had a very low soil Fe content. Fe plays a fundamental role in all isozymes of nitrogenase, the ubiquitous enzyme involved in biological N fixation (Raymond, 2003). This contradicts the negative correlation between Fe and the abundance of N-fixing bacteria under *Rosmarinus* and grassland soils, which contain high amounts of mineral Fe. In this regard, it is possible that Fe is immobilized in the grassland matrix and thus become unavailable to microbes, as well as to plants.

The abundance of fungal phyla showed a marked variation among shrub canopies. Our findings are in line with earlier studies showing that the *Ascomycota*, which are early colonisers of litter and the major decomposers, are litter type specific (Štursová et al., 2020) and thus highly abundant under shrub canopies. The phylum *Basidiomycota* is generally better equipped for lignin degradation (Lundell et al., 2010); our results confirm that the highest amount of lignin was found in the litter of *Rosmarinus*, so the abundance of *Basidiomycota* is positively correlated with the lignin content in the litter of shrub canopies. Moreover, *Mortierellomycota* are known to be saprobic and ubiquitous, and several studies show that they have the ability to solubilize phosphorus and are associated with increasing yields and establishing symbioses

with plants (Grządziel et al., 2019). In our study, *Mortierellomycota* showed significant accumulation mainly in soils under *Pistacia*, followed by *Euphorbia*, indicating the good quality of these soils due to the quality of their falling decomposed litter. In detail, our results showed that *Myrtus* was characterised by a group of fungi composed mainly of *Penicillium* (soil-inhabiting *Penicillium*), which are among the common producers of secondary metabolites in soil (Zhelifonova et al., 2010) and have an important function in the decomposition of organic matter (Frisvad & Samson, 2004). Park et al., (2020) found that most *Penicillium* species from soil are highly selective and unique to each plant. In our study, all shrubs do not form symbiosis with ectomycorrhiza, except *Juniperus* (Mejstrik & Cudlin, 1983), therefore, their presence under shrub canopies is in the form of free-living spores. The fact of the presence of these mycorrhizal fungi confirms the formation of islands of fertility under the shrub canopies, indicating that the soil is ready for vegetation succession. Furthermore, *Euphorbia* was characterised by the presence of a significant amount of weak saprophytes, including *Aspergillus*, *Alternaria* and *Cladosporium*, while *Rosmarinus* exclusively hosted the genus *Didymella*, which are opportunistic parasitic microorganisms that often exploit special conditions to colonise on plants and occasionally cause severe damage (Blancard, 2012). *Euphorbia* also exclusively harbours the genus *Aureobasidium*, a typical phyllospheric endophyte that is mainly found in fresh litter and rapidly disappears upon decomposition (Bonanomi et al., 2019). According to previous culture-based studies conducted on different tree species, the persistence of *Aureobasidium* in decomposed litter is unusual due to its limited competitive ability (Voříšková & Baldrian, 2013). Our finding, that different functional groups of litter stimulated different fungal taxa, suggests that fungi have a preference for certain litter types, probably because the ability to degrade specific organic compounds varies among taxa (Schneider et al., 2012; van der Wal et al., 2013).

Our results suggest that the composition of the fungal microbiota converges in part by the types of litter functional group enclosed by each shrub canopy (Reinhart & Callaway, 2006). This could be due to differences in chemical composition between litter types (Fanin et al., 2014; Schneider et al., 2012), with plant functional groups often playing an important role in explaining differences in chemical traits (Diaz et al., 2004). In contrast to our results for fungal communities, we did not find strong shifts in the community composition of bacteria. That the effects were particularly pronounced for fungal communities may not be surprising, considering that fungi play a key role in the degradation of more recalcitrant organic compounds (van der Wal et al., 2013). It could be that fungi are more specialised to certain litter types, while bacteria use simpler carbon compounds from litter and fungal degradation products and are therefore less responsive to different litter types. Our results are consistent with previous work showing that the addition of organic amendments to agricultural soils can influence fungal biomass and community composition in soils (Clocchiatti et al., 2020; Reardon & Wuest, 2016) and that leaf litter is associated with a unique microbiome enhancement (Asplund et al., 2018; Lin et al., 2019).

Co-occurrence network of microbial community

Microbial co-occurrence patterns have been analysed to assess the rules of community assembly and interaction networks in highly complex systems (Gotelli & McCabe, 2002). Compared to that in shrubland, the microbial co-occurrence network in grassland was more complex, which might have been caused by the temporal and spatial continuity of connections between microbes, in contrast to shrubland, where connections might have been disrupted by the process of shrub encroachment. Previous studies have also suggested that positive correlations between nodes in co-occurrence networks of microbial communities in desert soil could be the result of functional interdependencies among microbial taxa under different environmental conditions (Neilson et al., 2017). In our study, the proportion of positive correlations was higher in the shrubland than the grassland, suggesting a high level of interdependence between microbial taxa in the shrubland. Topological parameters provide important information to understand microbial community structure (Newman, 2006; Ren et al., 2017). Higher values of network centralization and heterogeneity were observed in the shrubland than in grassland, indicate that there are many closely connected microbial modules (subnetworks/subcommunities in the overall network) in the shrubland.

Interactions between species are more frequent and intense in one module than in the rest of the community (Newman, 2006). Therefore, a small disturbance in the main module of a microbial network can have a large effect on the entire network of microbial communities (Liu et al., 2020). Moreover, higher mean degree and high closeness centrality and lower betweenness centrality could be used together to identify bacterial keystone taxa (Banerjee et al., 2018), which were mainly classified as *Ascomycota*, *Actinobacteria* and *Proteobacteria* in both networks. These keystone taxa could have a significant impact on microbial community structure and function (Banerjee et al., 2018). Removal of these highly connected taxa in a network would cause the collapse of the ecosystem structure and function (Saavedra et al., 2011).

Conclusion

Our study discovered that, under the same climate and rock type, coexisting shrubs generate specific signature of bacterial and fungal microbiota in the soil. We found that shrubs not only differentiate from the *Ampelodesmos* grassland matrix, but also between shrubs belonging to different functional groups, i.e. deciduous, evergreen coniferous and evergreen sclerophyllus. Differences in litter chemistry probably play a key role in controlling soil chemistry and, in cascade, in shaping the soil microbiota. The observation that shrub signatures are specific is not trivial, and highlights the limitation and imprecision of the common approach, which often generalises the effect of shrubs compared to open vegetation habitats. Moreover, the presence of specific microbiota under shrub canopies is likely the result of a progressive plant-soil feedback that probably took decades to generate the observed signature. This, indeed, opens the door for further studies that would investigate the functional consequences and direction of such feedback for species coexistence as well as for litter decomposition in the context of home field advantage framework.

Abbreviations

SE

Shrub encroachment; CO₂:Carbon dioxide; N:Nitrogen; P:Phosphorus; C:Carbon; USA:United states of America; CPMAS:Cross-polarization magic angle spinning; NMR:Nuclear magnetic resonance; Bruker AV:Bruker Avance; MAS probe:Magic Angle Spinning Probe; EC:Electrical conductivity; Cl:chloride content; DNA:Deoxyribonucleic acid; rRNA:Ribosomal ribonucleic acid; ITS:Internal transcribed spacer; PCR:Polymerase Chain Reaction; DADA:Divisive Amplicon Denoising Algorithm; ASV:Amplicon sequence variants; OUT:Operational taxonomic units; PRIMER:Plymouth Routines In Multivariate Ecological Research; nMDS:non-metric multidimensional scaling; PERMANOVA:permutational multivariate analysis of variance; ANOVA:Analysis of variance; Mg:Magnesium; Ca:Calcium; Cu:Copper; Zn:Zinc; Mn:Manganese; Fe:Iron; Na:Sodium; OC:Organic carbon;

Declarations

Ethical Approval and Consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

Reads of the sequence data have been deposited in the NCBI Sequence Read Archive (SRA) under the bioproject Palinuro microbiome with accession no. PRJNA744707.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MI, GB, and MZ have realized the soil collection in the field along with the monitoring of the shrubs within the grassland. MI, FDF, and GS have extracted the DNA and performed the molecular analysis. AMA and TF have done the chemical analysis of the litter and the soil. MI, SF, and GB have analysed data and written the manuscript. All authors read and approved the final manuscript.

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References

1. Asplund J, Kauserud H, Bokhorst S, Lie MH, Ohlson M, Nybakken L (2018) Fungal communities influence decomposition rates of plant litter from two dominant tree species. *Fungal Ecology* 32:1–8
2. Association of Official Analytical Chemists International (AOAC) (1990) *Official Methods of Analysis*, 15th edn (edited by K. Helrich). Arlington, VA: Association of Official Analytical Chemists Inc. 1990; 1028–1039
3. Banerjee S, Schlaeppi K, van der Heijden MGA (2018) Keystone taxa as drivers of microbiome structure and functioning. *Nat Rev Microbiol* 16:567–576
4. Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6:343–351
5. Bastian M, Jacomy M. Gephi: an open-source software for exploring and manipulating networks International AAAI Conference on Weblogs and Social Media: San Jose, CA, USA. 2009

6. Berni Canani R, De Filippis F, Nocerino R, Laiola M, Paparo L, Calignano A, De Caro C, Coretti L, Chiariotti L, Gilbert JA (2017) Specific signatures of the gut microbiota and increased levels of butyrate in children treated with fermented cow's milk containing heat-killed *Lactobacillus paracasei* CBA L74. *Appl Environ Microbiol* 83:10
7. Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol* 157:465–473
8. Blacard D (2012) *Tomato Diseases: Identification, Biology and Control*. Manson Publishing, London
9. Boestfleisch C, Drotleff AM, Ternes W, Nehring S, Pazoutova S, Papenbrock J (2015) The invasive ergot *Claviceps purpurea* var. *spartinae* recently established in the European Wadden Sea on common cord grass is genetically homogeneous and the sclerotia contain high amounts of ergot alkaloids. *Eur J Plant Pathol* 141:445–461
10. Bokulich NA, Mills DA (2013) Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-highthroughput profiling of fungal communities. *Appl Environ Microbiol* 79:2519–2526
11. Bonanomi G, De Filippis F, Cesarano G, La Stora A, Zotti M, Mazzoleni S, Incerti G (2019) Linking bacterial and eukaryotic microbiota to litter chemistry: combining next generation sequencing with ¹³C CPMAS NMR spectroscopy. *Soil Biol Biochem* 129:110–121
12. Bonanomi G, Incerti G, Giannino F, Mingo A, Lanzotti V, Mazzoleni S (2013) Litter quality assessed by solid state ¹³C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. *Soil Biol Biochem* 56:40–48
13. Bragazza L, Bardgett RD, Mitchell EAD, Buttler A (2015) Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytol* 205:1175–1182
14. Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK (2005) An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *Bioscience* 55:243–254
15. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583
16. Callaway RM (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht
17. Chapman SK, Newman GS, Hart SC, Schweitzer JA, Koch GW (2013) Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition. *PLoS ONE* 8:e62671
18. Chen H, Boutros PC. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics*. 2011;12
19. Clocchiatti A, Hannula SE, van den Berg M, Korthals G, de Boer W (2020) The hidden potential of saprotrophic fungi in arable soil: Patterns of short-term stimulation by organic amendments. *Appl Soil Ecol* 147:103434
20. Collins CG, Spasojevic MJ, Alados CL, Aronson EL, Benavides JC et al (2020) Belowground impacts of alpine woody encroachment are determined by plant traits, local climate, and soil conditions. *Glob Change Biol* 26:7112–7127
21. Czekala W, Malińska K, Cáceres R, Janczak D, Dach J, Lewicki A (2016) Co-composting of poultry manure mixtures amended with biochar – the effect of biochar on temperature and C-CO₂ emission *Bioresour. Technol* 200:921–927
22. De Marco A, Meola A, Maisto G, Giordano M, De Santo AV (2011) Non-additive effects of litter mixtures on decomposition of leaf litters in a Mediterranean maquis. *Plant Soil* 344:305–317
23. Dean WRJ, Milton SJ, Jeltsch F (1999) Large trees, fertile islands, and birds in arid savanna. *J Arid Environ* 41:61–78
24. Diaz S, Hodgson JG, Thompson K, Cabido M, Cornelissen J, Jalili A, Montserrat-Martí G, Grime JP, Zarrinkamar F, Asri Y, Band SR, Basconcelo S, Castro-Díez P, Funes G, Hamzehee B, Khoshnevi M, Pérez-Harguindeguy N, Pérez-Rontomé MC, Shirvany A, ... Zak MR. The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*. 2004;15: 295–304
25. Ding L, Shang Y, Zhang W, Zhang Y, Li S, Wei X, Zhang Y, Song X, Chen X, Liu J, Yang F, Yang X, Zou C, Wang P (2020) Disentangling the effects of driving forces on soil bacterial and fungal communities under shrub encroachment on the Guizhou Plateau of China. *Sci Total Environ* 709:136–207

26. D'Odorico P, Fuentes JD, Pockman WT, Collins SL, He Y, Medeiros JS, DeWekker S, Litvak ME (2010) Positive feedback between microclimate and shrub encroachment in the northern Chihuahuan desert. *Ecosphere* 1:art17
27. Dong WY, Zhang XY, Dai XQ, Fu XL, Yang FT, Liu XY, Sun XM, Wen XF, Schaeffer S (2014) Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Appl Soil Ecol* 84:140–147
28. Du BM, Ji HW, Peng C, Liu XJ, Liu CJ (2016) Altitudinal patterns of leaf stoichiometry and nutrient resorption in *Quercus variabilis* in the Baotianman Mountains, China. *Plant Soil* 413:193–202
29. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences, USA*. 2015;112: 911–920
30. Eldridge DJ, Beecham G, Grace JB (2015) Do shrubs reduce the adverse effects of grazing on soil properties? *Ecohydrology* 8:1503–1513
31. Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JF, Whitford WG (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. *Ecol Lett* 14:709–722
32. Facelli JM, Brock DJ (2000) Patch dynamics in arid lands: localized effects of *Acacia papyrocarpa* on soils and vegetation of open woodlands of south Australia. *Ecography* 23:479–491
33. Fanin N, Hättenschwiler S, Fromin N (2014) Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. *Plant Soil* 379:79–91
34. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88:1354–1364
35. Fierer N, Ladau J, Clemente JC, Leff JW, Owens SM, Pollard KS, Knight R, Gilbert JA, McCulley RL (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science* 342:621–624
36. Frisvad JC, Samson RA. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins R.A. Samson, J.C. Frisvad (Eds.), *Studies in Mycology* 49: *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. 2004;pp. 1-174
37. Gessner MO (2005) "Proximate lignin and cellulose. In: Practical A, Guide, eds MAS, Graca F, Bärlocher, Gessner MO " in *Methods to Study Litter Decomposition*. Springer, Dordrecht, pp 115–120
38. Gherardi LA, Sala OE, Yahdjian L (2013) Preference for different inorganic nitrogen forms among plant functional types and species of the Patagonian steppe. *Oecologia* 173:1075–1081
39. Gotelli NJ, McCabe DJ (2002) Species Co-occurrence: A Meta-Analysis of J. M. Diamond's Assembly Rules Model. *Ecology* 83:2091–2096
40. Grządziel J, Furtak K, Gałazka A (2019) Community-level physiological profiles of microorganisms from different types of soil that are characteristic to Poland – a long-term microplot experiment. *Sustainability* 11:56
41. Guido A, Salengue E, Dresseno A (2017) Effect of shrub encroachment on vegetation communities in Brazilian forest-grassland mosaics. *Perspectives in Ecology Conservation* 15:52–55
42. Hooper DU, Bignell DE, Brown VK, Brussard L, Dangerfield JM, Wall DH, Wardle DA, Coleman DC, Giller KE, Lavelle P, Van der Putten WH, De Ruiter PC, Rusek J, Silver WL, Tiedje JM, Wolters V (2000) Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. *Bioscience* 50:1049–1061
43. Hu G, Liu H, Yin Y, Song Z (2015) The role of legumes in plant community succession of degraded grasslands in Northern China. *Land Degrad Dev* 27:366–372
44. Huang C, Archer SR, McClaran MP, Marsh SE (2018) Shrub encroachment into grasslands: end of an era? *PeerJ* 6:e5474

45. Hugerth LW, Andersson AF (2017) Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. *Front Microbiol* 8:1–22
46. Idbella M, Bonanomi G, De Filippis F et al (2021) Contrasting effects of *Rhizophagus irregularis* versus bacterial and fungal seed endophytes on *Trifolium repens* plant-soil feedback. *Mycorrhiza*. 2021;31: 103–115
47. Incerti G et al (2013) Fire occurrence and tussock size modulate facilitation by *Ampelodesmos mauritanicus*. – *Acta Oecol* 49:116–124
48. Kirker GT, Bishell AB, Jusino MA, Palmer JM, Hickey WJ, Lindner DL (2017) Amplicon-Based Sequencing of Soil Fungi from Wood Preservative Test Sites. *Front Microbiol* 8:1997
49. Kochy M, Wilson SD (2001) Nitrogen deposition and forest expansion in the northern Great Plains. *J Ecol* 89:807–817
50. Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol Biochem* 34:139–162
51. Kulmatiski A, Beard KH (2013) Woody plant encroachment facilitated by increased precipitation intensity. *Nat Clim Chang* 3:833–837
52. Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL et al. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc. Natl Acad. Sci.* 2015;112: 10967–10972
53. Lin D, Pang M, Fanin N, Wang H, Qian S, Zhao L, Yang Y, Mi X, Ma K (2019) Fungi participate in driving home-field advantage of litter decomposition in a subtropical forest. *Plant Soil* 434:467–480
54. Liu Y, Ren Z, Qu X, Zhang M, Yu Y, Zhang Y, Peng W (2020) Microbial community structure and functional properties in permanently and seasonally flooded areas in Poyang Lake. *Sci Rep* 10:4819
55. Lundell TK, Mäkelä MR, Hildén K (2010) Lignin-modifying enzymes in filamentous basidiomycetes-ecological, functional and phylogenetic review. *J Basic Microbiol* 50:5–20
56. Martin M. Cutadapt removes adaptor sequences from high-throughput sequencing reads *EMBnetjournal*. 2011;17: 10–12
57. McKinley DC, Blair JM (2008) Woody plant encroachment by *Juniperus virginiana* in a mesic native grassland promotes rapid carbon and nitrogen accrual. *Ecosystems* 11:454–468
58. Meier CL, Bowman WD. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences*. 2008;105: 19780–19785
59. Mejstrik VK, Cudlin P (1983) Mycorrhiza in some plant desert species in Algeria. *Plant Soil* 71:363–366
60. Meldrum W, Forbes J (1928) The volumetric determination of chloride. *J Chem Educ* 5:205
61. Neilson JW et al (2017) Significant Impacts of Increasing Aridity on the Arid Soil Microbiome. *MSystems* 2:e00195–e00116
62. Newman MEJ. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. USA*. 2006;103: 8577–8582
63. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community data sets by ecological guild. *Fung Ecol* 20:241–248
64. Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res* 47:D259–D264
65. Noll M, Matthies D, Frenzel P, Derakshani M, Liesack W (2005) Succession of bacterial community structure and diversity in a paddy soil oxygen gradient. *Environ Microbiol* 7:382–395
66. Park MS, Lee JW, Kim SH, Park JH, You YH, Lim YW (2020) Penicillium from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea. *Mycobiology* 48:431–442
67. Peters J, Combs S, Hoskins B, Jarman J, Kovar J, Watson M, Wolf N (2003) Recommended methods of manure analysis. University of Wisconsin Cooperative Extension Publishing, Madison

68. Ping Y, Pan X, Li W et al (2019) The soil bacterial and fungal diversity were determined by the stoichiometric ratios of litter inputs: evidence from a constructed wetland. *Sci Rep* 9:13813
69. Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F (2016) Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Mol Ecol* 25:4059–4074
70. Qiao J, Yu X, Liang X, Liu Y, Borriss R, Liu Y (2017) Addition of plant-growth-promoting *Bacillus subtilis* PTS-394 on tomato rhizosphere has no durable impact on composition of root microbiome. *BMC Microbiol* 17:131
71. Qiu J, Cao J, Lan G, Liang Y, Wang H, Li Q (2020) The Influence of Land Use Patterns on Soil Bacterial Community Structure in the Karst Graben Basin of Yunnan Province, China. *Forests* 11:51
72. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–D596
73. Ratajczak Z, Nippert JB, Collins SL (2012) Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology* 93:697–703
74. Raymond J (2003) The natural history of nitrogen fixation. *Mol Biol Evol* 21:541–554
75. Reardon CL, Wuest SB (2016) Soil amendments yield persisting effects on the microbial communities –A 7-year study. *Appl Soil Ecol* 101:107–116
76. Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytol* 170:445–457
77. Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial activity. *Annu Rev Phytopathol* 53:403–424
78. Ren Z et al (2017) Taxonomic and Functional Differences between Microbial Communities in Qinghai Lake and Its Input Streams. *Front Microbiol* 8:2319
79. Reynolds JF, Smith MS, Lambin EF, Turner IBL, Mortimore M, Batterbury SPJ, Downing TE, Dowlatabadi H, Fernández RJ, Herrick JE, Huber-Sannwald E, Jiang H, Leemans R, Lynam T, Maestre FT, Ayarza M, Walker B (2007) Global desertification: building a science for dryland development. *Science* 316:847–851
80. Reynolds JF, Virginia RA, Kemp PR (1999) Impact of drought on desert shrubs: effects of seasonality and degree of resource island development. *Ecol Monogr* 69:69–106
81. Saavedra S, Stouffer DB, Uzzi B, Bascompte J (2011) Strong Contributors to Network Persistence are the Most Vulnerable to Extinction. *Nature* 478:233–235
82. Sankaran M, Ratnam J, Hanan NP (2004) Tree-grass coexistence in savannas revisited-insights from an examination of assumptions and mechanisms invoked in existing models. *Ecol Lett* 7:480–490
83. Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrell WM, Virginia RA et al (1990) Biological feedbacks in global desertification. *Science* 247:1043–1048
84. Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K (2012) Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME J* 6:1749–1762
85. Silva MEF, de Lemos LT, Nunes OC, Cunha-Queda AC (2014) Influence of the composition of the initial mixtures on the chemical composition, physicochemical properties and humic-like substances content of composts. *Waste management* 34:21–27
86. Stinca A, Battista Chirico G, Incerti G, Bonanomi G (2015) Regime shift by an exotic nitrogen-fixing shrub mediates plant facilitation in primary succession. *PLoS One* 10:e0123128
87. Štursová M, Šnajdr J, Koukol O, Tláškal V, Cajthaml T, Baldrian P (2020) Long-term decomposition of litter in the montane forest and the definition of fungal traits in the successional space. *Fungal Ecology* 46:100913
88. Team RC. R: A language and environment for statistical computing. *R Found. Stat. Comput.* 2016

89. Van Auken OW (2000) Shrub invasions of North American semiarid grasslands. *Annu Rev Ecol Syst* 31:197–215
90. van der Wal A, Geydan TD, Kuyper TW, de Boer W (2013) A thready affair: Linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiol Rev* 37:477–494
91. Voříšková J, Baldrian P (2013) Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME J* 7:477–486
92. Wallenstein MD, McMahon S, Schimel J (2007) Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. *FEMS Microb Ecol* 59:428–435
93. Wigley BJ, Bond WJ, Hoffman T (2010) Thicket expansion in a South African savanna under divergent land use: local vs. global drivers? *Glob Change Biol* 16:964–976
94. Xiang X, Gibbons SM, Li H, Shen H, Chu H (2019) Proximate grassland and shrub-encroached sites show dramatic restructuring of soil bacterial communities. *PeerJ* 7:e7304
95. Xiang XJ, Shi Y, Yang J, Kong JJ, Lin XG, Zhang HY, Zeng J, Chu HY (2014) Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. *Sci Rep* 4:3829
96. Yannarell AC, Menning SE, Beck AM (2014) Influence of Shrub Encroachment on the Soil Microbial Community Composition of Remnant Hill Prairies. *Microb Ecol* 67:897–906
97. Zhelifonova VP, Antipova TV, Kozlovsky AG (2010) Secondary metabolites in taxonomy of the *Penicillium* fungi. *Microbiology* 79:277–286

Figures

Figure 1

Sampling site of Capo Palinuro located in Southern Italy in Campania region (black circle on the map) on the grassland matrix, at the top of the figure, dominated by *Ampelodesmos mauritanicus* in which the studied shrubs have encroached; followed by the six encroached shrubs studied at the shrubland.

Figure 2

Box plots showing the variation in the Shannon diversity and species richness indices for bacterial (A) and fungal (B) communities under each plant canopy across the shrubland of Capo Palinuro. Different letters indicate significant ($P < 0.05$) differences in the indices under different plant canopies. The lower and upper bounds of the boxplots show the first and third quartiles (the 25th and 75th percentiles); the middle line shows the median, whiskers above and below the boxplot indicate inter-quartile range.

Figure 3

The relative abundance of various bacterial (A) and fungal (B) phyla under the canopy of each plant across the shrubland and grassland.

(a) - Bacteria

(b) - Fungi

Figure 4

Venn diagram for bacterial (A) and fungal (B) communities show the numbers of ASVs (97% sequence identity) that were shared or not shared by the studied plant individuals depending on overlaps.

Figure 5

Heatmap showing relative abundance of the 50 most frequent Amplicon Sequence Variants (ASVs) in the bacterial community under the canopy of each plant across the shrubland and grassland. The hierarchical clustering of variables is based on Whittaker's association index.

Figure 6

Heatmap showing relative abundance of the 50 most frequent Amplicon Sequence Variants (ASVs) in the fungal community under the canopy of each plant across the shrubland and grassland. The hierarchical clustering of variables is based on Whittaker's association index.

Figure 7

Correlation base network between bacterial and fungal families within the grassland (A) and the shrubland (B) communities. Nodes with black borders correspond to fungal amplicon sequence variants (ASVs) and nodes with no black borders corresponding to bacterial ASVs. Only the top 50 frequent ASVs, for each kingdom, reported. The nodes are coloured by phylum level. Modularity networks of grassland (A1) and shrubland (B1) communities. Edges connecting nodes show either positive (light grey) or negative (red) co-occurrence relationships. Edges length represents correlation strength. The connection stands for significant correlations ($P\text{-value} < 0.05$).

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