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SHORT COMMUNICATION

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Degradation of mycorrhizal fungal communities associated with cork oak and understory vegetation by the anthropogenic factors

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Abstract

The cork oak (*Quercus suber* L.) forests are ecosystems of high environmental and socioeconomic value in the Mediterranean basin. However, in Algeria, the cork oak forests are highly threatened by repeated fires, overgrazing and the anthropogenic pressure that weaken the ecosystem of cork oak and affect its natural regeneration. This degradation results in an alteration of many ecological components of the cork oak, such as fungal communities in the soil. Thus, the aim of this work is to study the effects of cork oak stand degradation on the diversity of mycorrhizal communities associated with *Quercus suber* and some of its understory shrubs (*Cistus monspeliensis*, *Lavandula stoechas* and *Thymus vulgaris*) in the forest of Brabtia (northeastern Algeria).For this purpose, two sites were chosen: one degraded by the anthropogenic factors and the other non-degraded one. Moreover, it is suggested that some plants of the understory shrub vegetation of cork oak, such as the cistus, lavender and thyme, prove to be favourable to the juvenile growth of this tree.

The results obtained showed that the ectomycorrhizal (EcM) root colonization of cistus was higher compared to that of cork oak in both stations. The estimation of arbuscular mycorrhizal (AM) colonization showed significantly higher levels in the roots of cork oak, cistus, lavender and thyme in the degraded station compared with the non-degraded stations. In contrast, the rhizosphere soils of cork oak and cistus had low number of AM propagules and fungal spores, while under the roots of lavender and thyme, these two parameters were greatly improved with the abundance of the genera *Glomus* and *Gigaspora*. These results underline the need to take into account the plant–fungus interactions in the development of restoration strategies of the degraded soils and forest ecosystems.

Key words

Quercus suber, ectomycorrhizal colonization, cistus, degradation, fungal diversity, lavender, arbuscular mycorrhizal fungi, mycorrhizal potential

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Introduction

The cork oak (*Quercus suber* L.) is an emblematic component of Western Mediterranean forests (Acácio& Holmgren 2014). Native cork oak forests occupy 1.3 million ha in southern Europe (Portugal, Spain, France and Italy) and 0.9 million ha in North Africa (Morocco, Algeria and Tunisia) (Lancellotti and Franceschini 2013; Ksentini et al. 2020).

They play a major role in the landscape formation and are of great ecological and socio-economic value (soil protection, cork production, logging, grazing space, mushroom production, aromatic and medicinal plants, ecotourism, etc.) for Mediterranean populations (Eriksson et al. 2017; Ksentini et al. 2020). However, declining regeneration of cork oak was observed, notably in Algerian cork oak populations (Bouhraoua 2008). The cork oak health status degradation is an ancient problem, going back to the end of the 19th century (Bouhraoua 2008). However, it was not until these years that cork oak mortality became a matter of concern for the future of these ecosystems. In fact, cork oak decline was defined as a gradual process involving several factors acting sequentially or simultaneously and leading directly or indirectly to a gradual tree weakness (Ben Jamaa 2011), followed by a decrease in its defensive ability and the establishment of favourable conditions for infection by biotic agents (De Sousa et al. 2008).Cork oak decline has multiple impacts at aboveground and belowground levels, strongly affecting resilience and productivity of cork oak forests. In addition to symptoms directly affecting the cork oak itself, cork oak decline was shown to negatively impact key elements of ecosystem functioning, notably, the soil microbiome(Lancellotti and Franceschini 2013). Indeed, deterioration of the condition of cork oak forests has been worsened by the mentioned anthropogenic activities and climatic disturbances, combined with a lack or scarcity of natural cork oak regeneration (Maghnia et al. 2019).

Among soil microbiota, mycorrhizal fungi are considered as key components of the sustainable soil–plant system (Dickie et al. 2004). They are generally considered to be 'soil engineers' that are particularly involved in the productivity and stability of ecosystems and agrosystems. Ectomycorrhizal (EcM) symbiosis is a mutualistic relationship in which the fungal hyphae surround and grow between the cortical cells of specialised fine plant roots. EcM fungi play an important role in the regeneration of forests by facilitating nutrient and water uptake by their host plants (Smith and Read 2008). However, in contrast to arbuscular mycorrhizal fungi (AMF), EcM symbionts are not uniformly distributed within ecosystems in terms of presence/absence, abundance or community composition (Boudiaf et al. 2013).

Several studies reported the strong impact of cork oak degradation on soil microbiome, with the main focus being on the about the role and diversity of soil EcM fungal communities establishing a symbiosis with cork oaks (Ksentini et al. 2017; Boudiaf et al. 2013; Lancellotti and Franceschini 2013). However, *Q. suber* and, more generally, *Quercus* spp. establish biotic interactions with a broader range of soil microorganisms, from AMF (Dickie et al. 2001; Egerton-Warburton and Allen 2001; Toju et al. 2013a), ericoid mycorrhizal (ErM) fungi (Bergero et al. 2000) and root endophytic fungi (Toju et al. 2013a, b) to soil bacteria (Marongiu et al. 2006; Boudiaf et al. 2013).

The effects of some shrub species on cork oak recruitment and survival have also been observed. For example, *Cistus salviifolius* and *Cistus ladanifer* were shown to have a negative impact on seedling survival of cork oak, whereas *Erica arborea* was positively correlated (Acácio et al. 2007; Pérez-Devesa et al. 2008).

Therefore, the objectives of this study were to determine the impact of degradation by anthropogenic pressure on (i) the rate of mycorrhizal colonization of root-associated fungal community of *Quercus suber* and some of its understory vegetation (*Cistus monspeliensis*, *Thymus vulgaris*, *Lavandula stoechas*), (ii) the mycorrhizal potential of the soil and (iii) the total number of fungal spores in the soil.

Material and methods

Site characterisation

The study was carried out in the forest 'Brabtia' (36°52′09.27″ N, 8°20′16.58″ E) located in northeastern Algeria at an altitude of 30 m (Fig. 1).The climate is sub-humid with a hot and dry season between May and November, while the rest of the year, it is humid and rainy(Sarri et al. 2014). The average annual rainfall is 225 mm. The Brabtia forest is composed of various shrub species such as *Cistus salviifolius* L., *Phillyrea angustifolia* L., *Thymus vulgaris* L., *Pistacia*

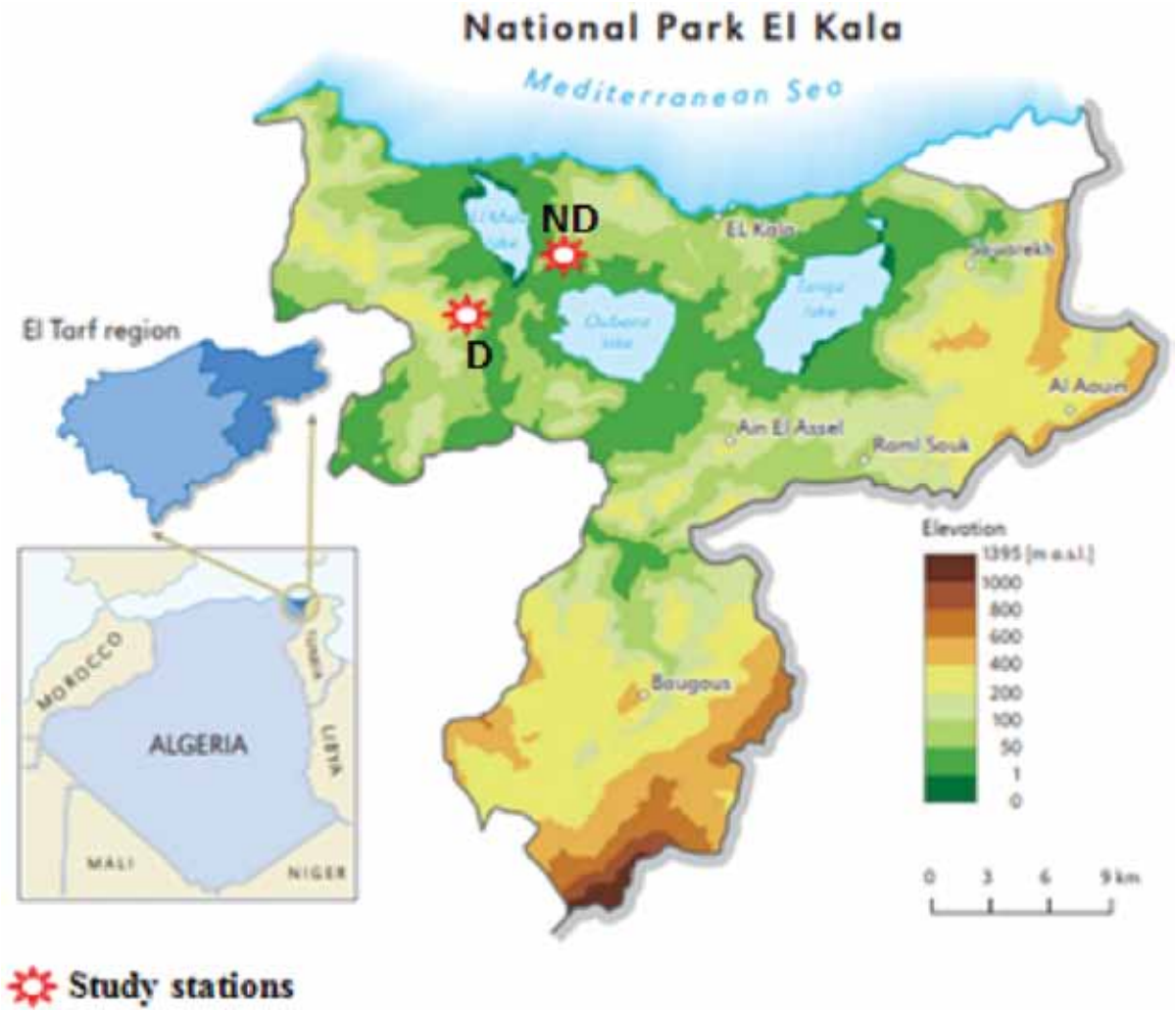

Figure 1. Location of the cork oak forest 'Brabtia' and the study stations (Diaf et al. 2019). ND – non-degraded station, D – degraded station

lentiscus L., *Cistus monspeliensis* L., *Lavendula stoechas* L. and *Erica arborea* L. Unfortunately, the plant cover is sparse and degraded due to multiple attacks of overgrazing, repeated fires and anthropogenic pressure, which strongly affecting the resilience and the productivity of cork oak forests (Ksentini et al. 2020).

Two stations within the Brabtia forest (Fig. 1), 17 km apart, have been chosen: one (ND) non-degraded and the other (D) degraded by various anthropogenic pressures (harvesting cork oak, cutting wood, grazing). The non-degraded station was chosen according to the following criteria: (i) less accessible to grazing livestock, (ii) lower frequency of fires and (iii) high density of cork oak trees and their understory shrub vegetation. The degraded station was chosen according to the following criteria: (i) decrease in the density of the cork oak and its understory shrubs vegetation to about 50 trees and (ii) higher repeated fires.

Soil and root sampling

At each station, soil samples of cork oak (CO), Cistus (CI), lavender (L) and thyme (T) were collected from the foot of six trees (2 kg/tree) at a depth of 20 cm. After a composite soil sample was collected in every station, very fine roots of cork oak, CI, L and T more likely to be mycorrhizal and more easily observable under a micro-

scope were collected at the same time as the soil. Soils were air-dried for further physicochemical analyses, assessment of the AM propagules in the soil, spore isolation and morphological identification.

Soil physicochemical analysis

Rhizosphere soil of cork oak (*Quercus suber*) was analysed for its physicochemical properties, that is, pH, texture, organic carbon (OC), nitrogen (N), phosphorus (P) and potassium (K). The analyses were made in the soil laboratory of FERTIAL (Annaba, Algeria).

Determination of EcM root colonization

EcM colonization assessment was made by counting the presence or absence of colonized root tips under a stereomicroscope according to the method of Brundrett et al. (1996). The percentage of root colonization was determined for each sample by examining three hundred 1cm root pieces and was calculated using the following formula:

$$\text{EcM colonization rate (\%)} = \\ = (\text{number of mycorrhizal root pieces/total number of observed root pieces}) \times 100.$$

Estimation of the mycorrhizal intensity (M%)

The sampling of the same roots, performed as described above, was cleared and stained for assessment of mycorrhizal colonization using the technique of Phillips and Hayman (1970), and the percent of AM colonization was quantitatively estimated following Trouvelot and Kough (1986). Samples were processed through the following stages: soil washing, cutting into segments of 1.5 cm, hot cleaning with KOH 10% (15 min), immersed into a solution of HCl 20% (10 min) and then staining with 0.03% trypan blue solution at 90°C. Root observations were done made in five replicates for 30 root fragments of 1 cm length, which were then placed between the slide and cover in a drop of glycerol. The annotation was made using the software MYCOCALC (http://www2.dijon.inra.fr/mychintec/. Mycocalc-prg/download.html).

Assessment of the most probable number of AM propagules

The mycorrhizal potential of the rhizosphere of four tested species (cork oak, lavender, thyme, and cistus) collected from both stations (degraded and non-degraded) was measured by the most probable number (MPN) of AM propagule (Porter1979), which consists in making a series of successive soil dilutions and determining the limiting dilution at which no AMF propagules can be detected. For this, the collected soil was mixed with sterilised soil to make five serial dilutions (1/10) and five replicates of 50 g per pot. One pre-germinated clover (*Trifolium repens* L.) seed was planted into each pot. The seedlings were transferred under controlled conditions to a greenhouse (average daily temperature 18–22°C with 60–70% relative humidity) and watered daily with distilled water. After 6 weeks, the entire root system per plant was stained according to the method of Philips and Hayman (1970) using lactic acid. The number of propagules per kilogram of soil was evaluated using Cochran's table (1950).

Recovery and counts of AMF spores

Spores were extracted for the rhizosphere of the four tested species (cork oak, lavender, thyme, and cistus) collected in winter, 201 from both stations (degraded and non-degraded),using the wet sieving method (Gerdemann and Nicholson 1963). In a 1 L beaker, 100 g of each composite sample of the soil was immersed with 0.5 L of tap water and stirred for 1 min with a spatula. After 10–30 seconds of decanting, the supernatant was passed through two superposed sieves with decreasing meshes 250 and 50 µm, respectively). This operation was repeated twice. The contents retained by the sieves were divided in two tubes and centrifuged for 4 min at 2000 rpm. The supernatant was discarded, and a viscosity gradient was created by adding 20 mL of sucrose solution at 50% in each centrifuge tube (Brundrett et al. 1996). The mixture was rapidly stirred, and the tube provided was centrifuged again for 1 min at 3000 rpm. Unlike the first centrifugation, the supernatant was poured onto the sieve of 50 µm; the resulting substrate was rinsed with distilled water to remove sucrose. The spores were then recovered with little distilled water in an Erlenmeyer flask and counted, and their density (spore number per 50 g dry soil) was determined.

Identification of AMF species

Apparently viable spores were observed in a stereomicroscope and separated into morphotypes according to their colour, size and shape. They were mounted on slides with polyvinyl lactoglycerol (PVLG) and Melzer's reagent for observation under a microscope and taxonomic identifi-

cation through spore morphology. Spores were extracted and separated into morphotypes by their colour, shape and size. Characteristics of spores, such as the number of spore layers, ornamentation of the outer layers, type of hyphal attachments and the wall layer reactions to Melzer's reagent, were also recorded. The identification of species was made according to the description of the species provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) following the classification of Redecker et al. (2013).

Data analysis

Data are expressed as the mean ± standard error of the mean. Statistically significant differences between the groups were evaluated by one-way of variance (ANOVA) followed by post hoc multiple comparisons with Tukey's honestly significant difference (HSD) test using IBM Statistical Package for the Social Sciences (SPSS) statistical software, ver.25.0 (SPSS, Inc., Chicago, IL, USA). Differences with $P < 0.05$ indicate statistical significance.

Results

Physicochemical characteristics of Brabtia soil

Degradation by anthropogenic pressure had a significant negative influence on most of the tested physicochemical parameters of the soil of *Quercus suber*, compared to those of the non-degraded station (control) (Tab.1). Phosphorus and organic matter showed the highest levels in the soil of non-degraded station in comparison to the degraded station.

Table 1. Soil physicochemical properties of *Quercus suber* rhizosphere soil in the two stations

Station	pH	OM (%)	N %	P (mg/kg)	K total
ND	5.95[a]	3.50[a]	0.31[a]	6.80[b]	21.17[b]
D	5.88[a]	2.00[b]	0.27[a]	4.60[a]	11.27[a]

Note: ND – non-degraded station; D – degraded station; OM – organic matter; N – nitrogen; P – phosphorus; K – potassium.

Effect of cork oak stand degradation on EcM root colonization

EcM root colonization was assessed in the roots of cork oak and cistus in the degraded and non-degraded stations (Fig. 2). A negative influence of anthropogenic pressure on the EcM root colonization of *Cistus monspeliensis* and *Quercus suber* was found because the total percentage of EcM colonization was significantly higher in the non-degraded station than in the degraded one ($F = 19.47$, $P \leq 0.000$). EcM colonization of the roots of CI in the non-degraded station and degraded station was high (57.70% and 43.65%, respectively; Fig. 2), compared to the roots of CO in both stations (37.36% and 30.42%, respectively).

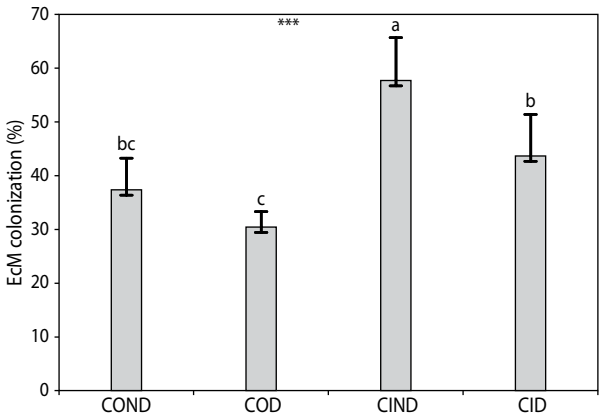

Figure 2. Rate of EcM colonization of cork oak (*Quercus suber* L.) and *Cistus* (*Cistus monspeliensis* L.). Different letters indicate significant differences between treatments according to Tukey's HSD test ($P < 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. COND: cork oak in non-degraded station, COD: cork oak in degraded station, CIND: *Cistus* in non-degraded station, CID: *Cistus* in degraded station, EcM: ectomycorrhizal, HSD: honestly significant difference

Mycorrhizal intensity (M%) of cork oak, lavender, cistus, and thyme roots

The estimation of mycorrhizal intensity revealed, on average, significantly higher rates ($F = 8.37$, $P \leq 0.000$) in cork oak, cistus, lavender and thyme roots in the less-degraded station compared to those in the degraded station (Fig. 3). The colonization of T and L roots taken from the less-degraded station reached rates of 1.01% and 0.60%, respectively. Whereas in the degraded station, the AM colonization was significantly decreased for the two species, compared to those calculated in the less-degraded station. CI and CO roots showed AM colonization rates of 0.50% and 0.31% in the non-degraded station compared to the degraded station, these intensities are 0.41% and 0.25%, respectively.

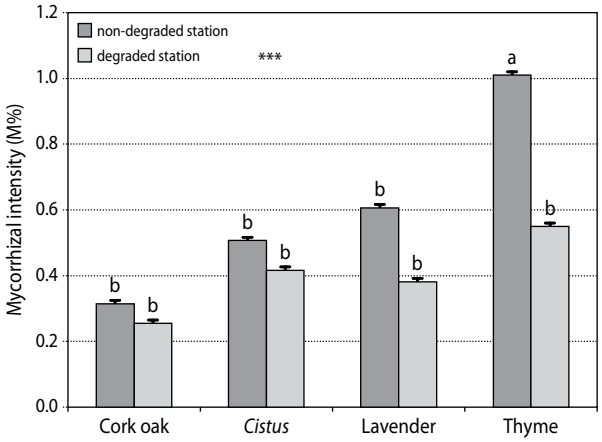

Figure 3. Mycorrhizal intensity (M%) of the root system of *Quercus suber*, *Cistus monspeliensis, Lavendula stoechas* and *Thymus vulgaris* in the two stations. Different letters indicate significant differences between treatments according to Tukey's HSD test ($P < 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

COND: cork oak in the non-degraded station; COD: cork oak in the degraded station; CIND: *Cistus* in the non-degraded station, CID: *Cistus* in the degraded station, LD: lavender in the degraded station; TND: thyme in the non-degraded station, TD: thyme in the degraded station, HSD: honestly significant difference

MPN of AMF propagules

The results obtained show that the number of AMF propagules in the four studied soils of both stations had been affected negatively by degradation due to anthropogenic pressure. The soil of cork oak roots had the lowest number of 133 propagules per kilogram of soil (Fig. 4). However, under the T and CI, the number of propagules (in the non-degraded and degraded stations) was higher (566 and 400, respectively). On the other hand, the number of propagules in the soil under L was 666 and 466 in both stations, respectively.

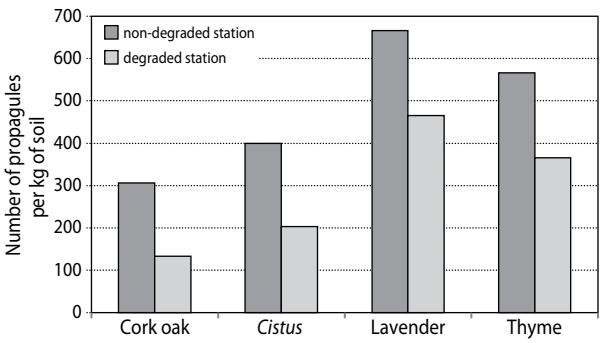

Figure 4. The number of AM propagules per kilogram of soil in the degraded and the non-degraded stations

AMF diversity and abundance of spores in the rhizosphere

Estimation of the number of arbuscular fungal spores in 50 g of soil taken from cork oak, cistus, lavender and thyme in the non-degraded station showed high values compared to the number in the soil taken from the degraded station. The soil of T was the richest in fungal frequency with the total number of AM spores being 125 (Fig. 5), followed by L with 89 spores, while

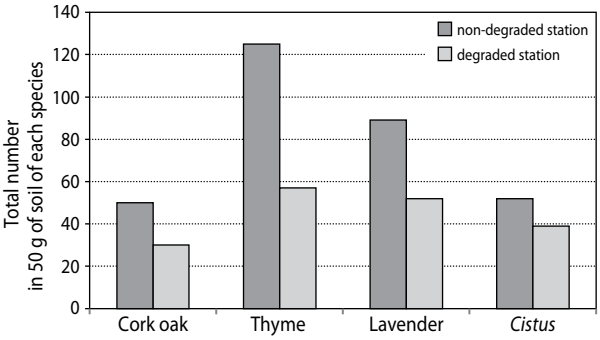

Figure 5. The total number of the fungal diversity of spores of the four rhizospheres in the degraded and the non-degraded stations

Table 2 . Frequency of morphotypes of spores in the four rhizospheres

Fractions	Spores collected from		
Quercus suber rhizosphere			
250 µm 50 µm	*Glomus* sp. 1 +++	*Glomus constrictum* +++	*Glomus* sp. 2 +++
Lavandula stoechas rhizosphere			
250 µm 50 µm	*Gigaspora* sp.1 +++	*Glomus* sp. 2 ++	*Glomus* sp. 3 +
Thymus vulgaris rhizosphere			
250 µm 50 µm	*Glomus* sp. 4 ++	*Glomus* sp. 3 +	*Gigaspora* sp. 1 ++
Cistus monspeliensis rhizosphere			
250 µm 50 µm	*Glomus mosseae* +++	*Glomus* sp. 3 ++	*Glomus* sp. 4 ++

Note: + present, ++ abundant, +++ highly abundant.

the lowest numbers of spores were recorded in the soil of CI and the CO with 52 and 50 spores, respectively. On the other hand, the degraded station showed lower numbers with 30, 39, 52 and 57 in the soil of CO, CI, L and T, respectively. The morphological criteria of the AMF spores isolated from the soil under study allowed us to find five spore species in thyme and lavender and three spore species in cork oak and cistus. The various genera and species identified in all rhizospheres belonged to the genera *Glomus* and *Gigaspora* with a total abundance (Tab. 2).

Discussion

Mediterranean forest ecosystems are severely threatened by the impact of humans and climatic conditions in numerous ways (Gauquelin et al. 2018). For this purpose, before restoring a degraded ecosystem, it was essential to know the effect of this degradation on the mycorrhizal communities associated with *Quercus suber* roots and some of its understory vegetation(cistus, lavender and thyme). This study clearly shows that the soil degraded by the anthropogenic pressure was strongly altered in its chemical characteristics, leading to a decrease in water pH, nutrients (N and P) and organic matter and decrease in its mycorrhizal communities rate of EcM colonization, rate of AM colonization and the number of spores associated with the roots. Numerous studies in the world have revealed variable results about the symbiotic relationship between plants and fungi, depending on many factors such as age of the host species, physicochemical properties of the soil, environmental characteristics and the geographical location (Zhao et al. 2007; Castillo et al. 2016).

In our study, the soil in both stations was characterized by low chemical fertility due to low levels of major assimilable nutrients (nitrogen and phosphorus). This degradation of our soil may be due to the combined factors of repeated fires, overgrazing, and anthropogenic pressure. Requena et al. (2001) also showed that loss and degradation of plant cover affect the physicochemical and biological properties of soils. Indeed, the influence of these factors on soil degradation mainly leads to deterioration of the ecological characteristics of the environment, whose first sign is degradation of the physical, chemical and biological properties of the soil, which makes the soil poor in mycorrhizae and fungal diversity (Zhao and Zhao 2007).

The results of EcM root colonization of cork oak and the cistus showed high EcM colonization in the non-degraded station, while in the degraded station, these rates were low. This may be due to the greater competition among these plants for water and nutrients in the soil, especially in more degraded soils. Our findings are consistent with the results obtained by Albuquerque-Martins et al. (2019) and de Vega et al. (2010) who have found that under different growing conditions, shrubby Cistaceae species showed a greater degree of EcM colonization. According to Shi et al. (2017), specific EcM fungi are capable of improving the juvenile growth of some forest species and mitigating the effects of transplant stress. The interaction between trees and EcM fungi is dependent on many factors, namely, tree species, environmental conditions and belowground interactions, among others (Opik et al. 2006). Even seasonal variations have an important role in EcM fungal dynamics in the soil. According to Voriskova et al. (2014), seasonal changes have a significant impact on fungal activity, biomass content and composition, as well as on the relative abundance of different fungal groups in temperate oak forests.

The presence of anthropogenic pressure had also negatively affected the rate of AM colonization and the number of spores. AMF are obligate biotrophic symbionts that require host plants to live (Smith and Read 2010), and changing the dominant plant species in the ecosystem could change the established balance in favor of certain fungal species. However, reduction in the diversity of plant communities that occurs in the degraded site should decrease the density of the AMF community. We suggest that the change in plant composition and dominance of ecosystem structure may be the primary forces driving the change in AMF structure. Therefore, selective forces towards more competitive and adaptable fungal species are likely active, contributing to the change in AMF community structure.

In Mediterranean ecosystems, the host tree can interact with a hundred different EcM fungal species. The functional complementarity of symbionts is key to the flexibility and resilience of forests to changing natural conditions (Azul et al. 2010; Courty et al. 2010). The majority of studies treated ectomycorrhizas because of

their economic importance, using mainly artificial inoculation of seedlings (Agueda et al. 2006).

The number of AM propagules found in the soil of cork oak and cistus was low in the degraded station compared with the non-degraded station, whereas under the rhizosphere of lavender and thyme, this number was higher. The low number of propagules could be due to the nature of the soil, which is strongly anthropogenic due to human pressure, and overgrazing, which causes degradation of native plant communities (population structure and species diversity), resulting in loss or decrease of mycorrhizal propagules in the soil (Asmelash et al. 2016). Soil degradation and vegetation cover perturbation lead to loss of nutrients, reduction of microbial diversity (Kennedy and Smith 1995) and, in particular, propagules of mycorrhizal fungi, which therefore the reduction of the potential of the inoculum for the formation of soil mycorrhizae (Azcon-Aguilar et al. 2003). Our results are also in agreement with the findings of Duponnois et al. (2001), who have shown that degradation of soil by anthropogenic activities decreased its diversity and the number of AM propagules.

Indeed, degraded areas still show low densities of native mycorrhizal propagules. AMF species also have very different life cycles, and the relative abundance of every species at each stage of the cycle (spores, hyphae, colonised roots) may change over time (Nehila et al. 2015).

The natural diversity of spores was low in all the rhizospheres studied, with the dominance of the genus *Glomus*. The low spore density could be due to the season of soil sampling, which was winter when all the climatic conditions are unfavourable, especially precipitation. The results of this study are also in agreement with the findings of Han et al. (2019), which showed that the fungal spore density was low in spring, increased in summer, reached its highest level in autumn and then decreased in winter. The AMF frequency is based on seasonal changes and host metabolic pathway (Yang et al. 2018). Li et al. (2020) also suggested that the seasonal dynamics of the soil AMF spore density differed depending upon the soil type and the host plant species.

Asmelash et al. (2016) showed that the degraded lands harbour low levels of AMF abundance and diversity. It was also reported that human disturbance decreased AMF spore density, root colonization and nutrient availability (Birhane et al. 2017). Smith (1987) also showed that the variation in the number of spores of AMF depend on the season, formation processes, and germination. Glomus species are widely distributed in various habitats of the world, and they dominate in degraded soils (Uhlmann et al. 2006). According to Schenck and Perez (1990), this AM species is ubiquitous. However, we noticed that the genus *Glomus* is represented by a large number of morphotypes that are distinguished by colour and shape, which probably suggests the presence of several species.

Conclusions

The degradation of forest sites by anthropogenic pressure has been found to have a negative impact on the physicochemical properties of soil in degraded soils compared to the non-degraded station. The changes in soil properties demonstrated a negative impact on the EM-root colonization levels of *Cistus monspeliensis* and *Q.suber*; however, *Q.suber* showed the lowest level of AM colonization.

The mycorrhizal potential of the soil under cork oak was the lowest in the degraded station compared to that assessed under lavender, cistus and thyme in both stations. The presence of fungal spores was also low in all rhizospheres studied, with the dominance of the genus *Glomus*. The knowledge of the richness of EcM and AMF is a priority to understand the processes which allow its adaptation in some environments and the setting of symbiosis with plants.

Thus, in the case of our degraded Mediterranean ecosystems, the mycorrhizal potential is very low to ensure the development of forest species the selection of native AM and EcM fungi and their multiplication in trap plants could be reintroduced in the degraded oak forests and thus improve the quality of seedlings of this last in order to use them in the ecological restoration of degraded ecosystems.

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