

# Preliminary study of the mycorrhizal community according to the age of the Afares oak (*Quercus afares*) of the Akfadou forest, Algeria



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## Abstract

Afares oak (*Quercus afares* Pomel), a Numidian species endemic to the humid mountains of North Africa, is found in the central and eastern Algeria and the northwestern Tunisia. The aim of this study was to describe the mycorrhizal community in trees of this species at different age, in its natural environment. The study was conducted in the Akfadou forest (36°41'57" N, 4°33'25" E), characterized by a fresh wet temperate climate type and less clayey soil structure. During December 2016, sampling was performed from 16 trees with size diameter of two age categories: eight trees  $\leq 10$  years old and eight  $\geq 60$  years. Roots were sampled at four cardinal points at a distance of 1.50 m from the root collar. Endomycorrhizal structures at both ages were non-existent. A specific richness of 47 ectomycorrhizal morphotypes was observed, with 18 being common to all trees. Analysis of the external mycelium showed four different types of mycorrhiza with an exploration function. The Dices similarity index indicated similarity of the ectomycorrhizal communities in trees of both age groups, especially in the composition of the short-distance exploration type community.

**Key words:** Afares oak, Akfadou forest, ectomycorrhiza, exploration type mycorrhiza, tree age.

**Abbreviations:** ECM, ectomycorrhizae; OTMR, old tree morphotype richness; TTMR, total tree morphotype richness; YTMR, young tree morphotype richness..

## Introduction

Ectomycorrhizal (ECM) symbiosis is known to be a major player in the functioning of forest ecosystems (Bâ et al. 2011). Numerous species of the family Pinaceae and Fagaceae have ECM symbiosis (Smith, Read 2008). Without ECM, a healthy woodland community cannot be maintained (Rai, Varma 2010). Therefore, good knowledge on the ECM community is important for conservation and restoration of plant species in their habitats, especially in areas that are degraded due to overgrazing, climate change, stand conversion and other impacts. Knowledge of the ECM community in a particular geographical location could contribute to increasing the rate of tree adaptation and survival after afforestation.

*Quercus afares* Pomel is a relict tree species, representative of the restricted endemism of the Algerian and Tunisian littoral. *Q. afares* is considered to be a stabilized hybrid between *Quercus suber* and *Quercus canariensis* (Mir et al. 2006). It is a rather rare and threatened species with restricted distribution in the supramediterranean belt mountains, which is characterised by mean annual

temperature 8 to 13 °C and annual precipitation  $\geq 600$  mm (Quezel, Médail 2003). Many plant species are threatened with extinction worldwide, mainly because of land use changes, livestock grazing and unsustainable exploitation (Oldfield, Eastwood 2007). In the Akfadou forest in Algeria, especially in *Q. afares* habitat, intense regression of the plant cover under abusive and recurrent anthropogenic action has occurred (Quézel, Barbero 1990; Quézel 1991; Meddour 2010). It has been reported that the Afares oak area has regressed by 26.6% (Djema, Messaoudene 2009), compared to the area given by Boudy (1955) and Messaoudene (1989), and sometimes the degradation has been irreversible.

Afares oak has been the subject of several studies concerning botanical, phenological, phytogeographical, phytosociological, physiological and adaptive aspects (Boudy 1952; Maire 1952; Quezel, Bonin 1980; Messaoudene 1989; Acherar et al. 1991; Quezel, Médail 2003; Mhamdi et al. 2017). However, the symbiotic aspect, especially the mycorrhizal aspect, seems to have never been approached and, to our knowledge, functional aspects of the ectomycorrhizal community of the Afares oak are

unknown.

Therefore, the aim this study was to describe the mycorrhizal community of the Afares oak of Kabylia (Algeria) and to compare the morphotypic richness of mycorrhiza for this oak species between young trees and old trees in its natural environment.

## Materials and methods

### *Description of study site*

The study was carried out in pure Afares oak forest of the Bouchouled canton of the Akfadou forest, in Tizi-Ouzou (Algeria). It is located in the central part of the Tellian Atlas, 36°41'57" N and 4°33'25" E, 100 km east of Algiers. It belongs to the phytogeographical Djurdjura sub-sector of Kabylia (Quezel, Médail 2003). It is a rugged mountainous area, consisting of a succession of ridges, extensions of the coastal range and Djurdjura Mountain, which meet at the North-East end of the massif. At an altitude of 1200 m, the bioclimate is wet temperate to fresh wet of the supramediterranean belt.

### *Sampling of mycorrhizas*

The study was conducted in a pure stand of Afares oak, 100 m near a fire trench and 50 m from a forest road. Sampling was carried out in December 2016 when trees were dormant and bare of foliage. Several authors report that soil enzyme activities can remain high in winter (Dormaar et al. 1984; McClaugherty, Linkins 1990) and that the turnover and metabolic activity of fine roots and ECMs of deciduous trees do not slow down during the "resting season" (Buée et al. 2005).

In vegetated ecosystems, succession of some fungal guilds occurs in tandem with plant succession (Deacon et al. 1983). These observations allows the classification of "early-stage" and "late-stage" ECM taxa. In order to distinguish between early and late stage ECMs and to assess the degree of similarity between mushroom communities of young and old trees of Afares oak, we described the ectomycorrhizal communities of 16 trees over a radius of 20 m chosen according to a subjective sampling based on tree diameter (age). Pressler increment borer coring was carried out to determine the age of individual trees by tree-ring research, whereby thin cylinders of wood are retrieved using a hollow drill bit that is bored through the trunk cambial tissues and into the heartwood (Grissino-Mayer 2003). In order to avoid any adverse effects of coring on the state of health (Tsen et al. 2016) of individuals of the heritage species, samples were obtained from eight young and eight old trees. Age of these large trees was between 60 and 111 years (trees Nos. 1, 2, 9, 12, 13, 14, 15, 16). Diameter of the young trees was  $6 \pm 2$  cm, which corresponded to an age less than or equal to 10 years (trees Nos. 3, 4, 5, 6, 7, 8, 10, 11).

The ECM fungal community was evaluated in the

rootzone of trees in these two age categories. Four samples of roots and soil cores were collected for each individual tree, according to the cardinal points at 1 to 1.5 m from the trunk under the crown of the tree, at a depth of 10 to 30 cm. The soil temperature during sample collection was 7.6 °C and soil pH was 4.5.

### *Laboratory analysis*

A total of 64 soil and root samples were transported on the day of collection to the laboratory "Production, improvement and protection of plants and foodstuffs" at Mouloud Mammeri University in Tizi-Ouzou (Algeria). They were placed in labelled plastic bags and stored at 4 °C up to 15 days. A 20-cm sample of the long roots of each tree was pre-treated according to the method of Phillips and Hayman (1970) in order to detect the presence of endomycorrhizal structures. One part of the root sample was gently rinsed and then observed under a stereomicroscope. The study of morphological and anatomical characteristics of the three components of the vegetative ectomycorrhizal fungi is often referred to as "morphotyping" and it widely used to study ECM communities as an end in itself to characterize fungal diversity (Horton, Bruns 2001). The observed morphotypes were preserved using a fixative [3% formalin, 5% propionic acid and 92% ethyl alcohol (70%)]. Freehand anatomical sections were made and stained with lactophenol blue to complete the morphological description with anatomical features (emanating hyphae, ectomycorrhizal mantle: inner mantle, outer mantle OM), structure of rhizomorphs, shape of cystidia and Hartig's net according to the criteria of Agerer and Rambold (2004–2020), observed with a Zeiss microscope. Several authors consider that all anatomical features that include hyphae can be applied to characterize ECM (Agerer 1991a; Agerer, Rambold 2004–2020), but only four anatomical complexes are informative for recognition of fungal relationships: structure of outer mantle layers as seen in plane view, structure of rhizomorphs, shape of cystidia and features of emanating hyphae (Agerer 2006).

Individualized morphotypes are related to an exploration type according to the presence or absence of extramatrical, rhizomorphic hyphae and their mode of organization according to Agerer (2001): (i) contact (smooth structures mantle and only a few emanating hyphae, presence only of extramatrical hyphae), (ii) short distance (presence of a bulky hyphae without rhizomorphs), (iii) medium distance (presence of simple rhizomorphs with little or no differentiation), and (iv) long distance (few rhizomorphs, presence of well-differentiated rhizomorphs with central hyphae (vessel-like) of larger diameter allowing greater transport efficiency of water and nutrients). Agerer (2006) proposed that the last three groups correspond to different nutrient acquisition and translocation strategies.

The DEEMY database (Description EctoMycorrhiza, [www.deemy.de](http://www.deemy.de)) was used to determine of some morphotypes. Two ECM types were determined by

correspondence with a sporocarp. The rest of that we could not determine received only a reference number.

### Statistical analysis

Comparative analysis of the communities of the two age categories by morphotypic ectomycorrhizal richness was performed. Total tree morphotype richness (TTMR) was determined, which is the number of morphotypes observed in all trees of the two age categories. We also determined the morphotypic richness of young trees (YTMR) and the morphotypic richness of old trees (OTMR).

The similarity/dissimilarity between the ECM communities was obtained by calculating the Jaccard index of similarity:

$$J = C / (C + S1 + S2),$$

and Dice similarity coefficient:

$$DSC = 2C / (2C + S1 + S2),$$

where *C* is the number of common morphotypes between two samples, *S1* is the number of unique morphotypes for sample 1, and *S2* is the number of unique morphotypes for sample 2.

The statistical analysis was performed using computer software BioStat™ 2009 (AnalystSoft Inc., Vancouver, Canada), StatBox.6 and Microsoft Excel 2013.

## Results

Endomycorrhizal structures were not present in root samples from pure stands of Akfadou Afares oak trees in both age categories. Using the criteria established by Agerer and Rambold (2004–2020), root morpho-anatomical analysis allowed to determine 47 different ectomycorrhizal morphotypes. Nine morphotypes were determined to the fungal genus and four to the fungal species level (Table 1).

Morphotyping revealed the presence of morphotypes of the Basidiomycota branch, ECM formed by *Amanita muscaria* (L.:Fr.) Lam. and *Thelephora terrestris* (Pers.). Other ECMs were formed by the genera *Hydnum*, *Russula*, *Lactarius*, *Ramaria* and *Quercirhiza*.

HBc Ascomycota were well represented in the ECM community of Afares oak with, among others, species from genus *Tuber* of the discomycetes. ECMs formed by Dothideomycetes species *Cenococcum geophilum* Fr. as well as Agaricomycetes *Pisolithus tinctorius* (Mich.: Pers.) Coker were found.

Analysis of anatomical sections carried out for the different morphotypes highlighted the presence of a thick to medium-thick fungal mantle. Penetration of the hyphae forming the Hartig network usually did not exceed one to two layers of elongated cortical cells (Fig. 1).

From the 47 morphotypes, 18 were common, two were rare and 27 were very rare (Fig. 2). ECMs formed by *Pisolithus tinctorius*, *Cenococcum geophilum* and *Quercirhiza* sp. were rather commonly found.

Mean morphotypic richness of the ECM community per individual tree of *Q. afares* was TTMR = 20.18 ± 1.68. Similar morphotypic richness in the two age categories of Afares oak was observed: YTMR = 20.12 for young trees and OTMR = 20.25 for old trees (Table 2). The comparative analysis of the different ECM morphotypes at the level of the 16 characteristics revealed the presence of 18 common ECMs morphotypes, accounting for 38% of the community. Only 12 ECMs were specific to young trees and represented only 26% of the community. These can then be considered as early stage-specific ECMs of *Q. afares*. Seventeen ECMs were observed only in adult trees, accounting for 36% of the community; these can be considered to be late stage-specific ECMs.

**Table 1.** Morphological and anatomical characteristics (Agerer, Rambold 2004–2020) of common and determined ECMs anatomomorphotypes observed on *Quercus afares*. \*Correspondence with the sporocarp *in situ*. C, contact; LD, long distance; MD, medium distance; P, plectenchymatous; PP, pseudo-parenchymatous; SD, short distance

ECM No.	Macroscopic description	Inner mantle	Outer mantle	Emanating hyphae	Cystidium types	Rhizomorphs	Exploration type
1	Ramified, greyish	P	P	Brown, frequent	–	White	MD
2	Unbranched, constricted, brownish	PP	P	Frequent	–	Lacking	SD
3	Pinnate straight, brown	P	P	Lacking	–	Lacking	C
4	Branched, straight, greenish	PP	P	Brown, frequent	–	Whitish	MD
5	Monopodial-pinnate, sinuous, brown	PP	PP	Frequent	-	Lacking	SD
6	Unbranched, sinuous, yellowish	P	P	Whitish brown, frequent	Gloeocystidia	Lacking	C

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Table 1. continued

ECM No.	Macroscopic description	Inner mantle	Outer mantle	Emanating hyphae	Cystidium types	Rhizomorphs	Exploration type
7	Pyramidal, inflated, sinuous, dark	P	P	–	Lacking	Lacking	C
8 <i>Cenococcum geophylum</i>	Black, unbranched, grainy	P	P	Dark, long, hirsute	Lacking	Lacking	SD
9 <i>Pisolithus tinctorius</i>	Pyramidal to irregular branched, sinuous, brown	P	P	Infrequent	Lacking	Infrequent with vessel-like hyphae	LD
10	Irregularly branched, beaded, grey	P	P	Frequent	Lacking	Frequent cotoneous	MD
11	Unbranched, brownish	PP	PP	Infrequent	–	Lacking	SD
12 <i>Quercirhiza</i> sp.1	Monopodial irregular branched, black	P	PP, angular cells	Brown, hirsute, straight	Awl-shaped, bristle-like	Lacking	SD
13	Unbranched to branched straight end, yellowish	P	P	Frequent as patches	Lacking	Frequent, cotoneous	MD
14	Pyramidal, sinuous apex roundish, dark gray	PP	PP	Infrequent	–	Frequent	MD
15	Unbranched, bent, orange	PP	PP	Frequent, white	–	Lacking	SD
16	Unbranched, straight	PP	PP	Frequent	–	Lacking	SD
17	Unbranched, ramified	PP	PP	Frequent	Lacking	Lacking	SD
18	Pyramidal, bent, black shiny	P	P	Frequent	Lacking	Frequent with sclerote	MD
19 <i>Quercirhiza</i> sp.2	Unbranched, tubercle, dark brown	PP	PP, angular cell	Frequent, dark	Lacking	Lacking	SD
20 <i>Ramaria</i> sp.	Branched, thin; bent, yellowish	P	P	Infrequent	Lacking	Frequent, white	MD
25 <i>Russula</i> sp.1	Monopodial, straight, yellowish	PP	PP	Infrequent, yellowish	Lacking	Lacking	C
29 <i>Amanita muscaria</i>	Monopodial, bent, yellowish, silvery	P	P	Infrequent, white	Lacking	Frequent, white with clamp	MD
30 <i>Lactarius</i> sp.1	Pyramidal, smooth, greenish	PP, angular cells	P	Infrequent	–	Lacking	C
31 <i>Lactarius</i> sp.2	Unbranched, bent, smooth, orange to yellowish	PP, angular cells	P	Lacking	Flask-shaped with apical knob	Lacking	C
35 <i>Thelephora terrestris</i> *	Monopodial, straight, yellowish	P	P	Lacking	Lacking	Infrequent vessel-like hyphae	MD
37 <i>Hydnum</i> sp.*	Pyramidal, bent, grainy, white	P	P	–	–	Frequent white	MD
39 <i>Tuber</i> sp.	Unbranched, straight, grainy, orange	P	PP	Frequent, yellowish	Awl-shaped; bristle-like	Lacking	SD
42 <i>Russula</i> sp.2	Monopodial-pyramidal, orange	P	–	Frequent	Flask-shaped + apical knob	Infrequent	MD

According to the first two axes of the factorial correspondence analysis (total inertia of 86%), a set of common ECMs (C) in both young trees (Y) and old trees (O) was distinguished. The highest contributions of 3.40 to axis 1 (Fig. 3) were for the ECMs common to all trees on the positive side of the axis 1 (C). The two groups formed on the negative side of axis 1 distinguished the ECMs absent in young trees (Y): T3, T4, T5, T6, T7, T8, T11 and those absent in old trees (O): T1, T2, T9, T10, T12, T13, T14, T15, T16.

The Jaccard's index of similarity ( $J = 0.33$ ), and Dice similarity coefficient ( $DSC = 0.62$ ) indicated similarity of 45 to 62% for the ECM between the age categories of the trees (Table 3). This indicated similar ectomycorrhizal communities between the two age categories of the Afares oak population of the Akfadou forest.

The emanating hyphae of morphotypes were classified into the four types of exploration described by Agerer (2001). Short roots revealed the presence of the different exploration types in all the trees, but there were greater numbers of the medium and short-distance types. Long-distance and contact type exploration were less represented (Fig. 4).

The distribution of exploration types at the level of the ECM community of the short roots of the Afares oak showed 20 morphotypes (43%) for medium-distance exploration, 13 ECMs (28%) for the short-distance type, 10 ECMs (21%) for the contact type and four ECMs (8%) for the long-distance type. However, in the community common to both age groups, the proportion of exploration groups was reversed with eight short-distance types ECMs (44%), and six medium-distance types (33 %; Fig. 5).

The Jaccard's index of similarity ( $J = 0.61$ ), and Dice similarity coefficient ( $DSC = 0.76$ ) showed similarity of the short-distance type between the tree age groups. However, the contact, medium-distance and long-distance types showed heterogeneity of the ECM community between the two stages (Table 3).

## Discussion

The observation of the fungal structures in the cells of short and long roots of *Q. afares* confirmed the absence of arbuscules, vesicles and pelotons, as already reported by several authors for *Quercus* species (Smith, Read 2008; Garbaye 2013).

The presence of morphotypes of Basidiomycota and Ascomycota is common among forest species. Among the Basidiomycota, the ECMs formed by the *Russula* genus were reported by Beenken (2004) in *Quercus*, ECMs formed by the *Lactarius* genus were described by Luppi and Gautero (1967) in *Quercus petraea* and Palfner (1998) in *Quercus robur*. The ECMs formed by *Amanita muscaria* (L.:Fr.), and *Laccaria*, *Hydnum* and *Ramaria* genus were reported by Agerer and Rambold (2004–2020) in *Quercus*.

Ascomycota are well represented in the ECM community of Afares oak with, among others, the discomycetes genus *Tuber* already observed in *Q. robur* (Luppi, Gautero 1967) and in *Q. suber* and *Quercus ilex* (Lancellotti, Franceschini 2013). We noted the presence of the ECM formed by loculoascomycetes *Cenococcum geophilum*, previously found in *Q. ilex* (Agueda et al. 2001), *Q. suber* (Lancellotti, Franceschini 2013; Kadi-Bennane 2016) and in *Quercus cerris*, *Quercus frainetto*, and *Q. robur* in Romania (Fodor et al. 2011). The ECM formed by *Pisolithus tinctorius* was observed in *Q. ilex* (Agueda et al. 2001). This ECM was abundantly observed in *Q. suber* at all age stages (Kadi-Bennane 2016).

It appears that Afares oak has fungal partners common to species of the Fagaceae family and to the same genus. A dominance of Russulaceae, Cortinariaceae, Thelephoraceae, and Inocybaceae occurs in *Quercus*, *Fagus* and *Castanea* (Ortega, Lorite 2007). The ectomycorrhizal community is similar for species of Fagaceae and the genus *Quercus* (Garbaye 2013; Reis et al. 2017). Moreover, several authors reported that *Quercus* species have a similar community of ectomycorrhizal partners, which consists of Basidiomycota like Russulaceae (Russulales), Thelephoraceae (Thelephorales), *Boletus* (Boletales), Cortinariaceae, Inocybaceae and Amanitaceae (for the majority of Agaricales) (Ortega, Lorite 2007; Garbaye 2013).

Elongation of cortical cells was observed in anatomical sections, reflecting the efficiency of the symbiosis. This form of cortical cells in contact with the Hartig network offers a greater intensity of exchange between the two partners (Burgess et al. 1994). This anatomical characteristic confirms symbiotic activity in this vegetative rest period. Indeed, it was reported that the metabolic activity of fine roots and ECMs of deciduous trees does not slow down during the 'resting season' (Buée et al. 2005).

Our study allowed to distinguish 47 ECMs. This morphotype richness was similar to the species richness of ECMs observed previously by in oak forests – 43 species (Sharma 2017). Of the ECMs, 18 anatomo-morphotypes were common, two were rare and 27 were very rare. Higher proportion of infrequent morphotypes or even present only once, occurs in the majority of ectomycorrhizal communities (Garbaye 2013; Kadi-Bennane 2016). It is assumed that, from a functional point of view, the dominant fungal partners that account for most of the fungal biomass would be the most relevant in nutrient uptake and the least expensive in terms of nutrient uptake energy balance (Walker et al. 1999). It has been considered that the typical fungal partners participating in the beginning of succession, like *Cenococcum*, *Inocybe*, *Laccaria*, *Pisolithus*, and *Tuber*, tend to be persistent in disturbed habitats (Krpata et al. 2008).

The ECMs formed by *Pisolithus tinctorius*, *Cenococcum geophilum* and *Quercirhiza* sp.1 are among the common



**Fig. 1.** Examples of ectomycorrhizae observed on roots of *Quercus afares* in natural forest of Akfadou (Algeria). A, ECM *Lactarius* sp.1: contact exploration type, simple with orange color ( $\times 20$ ). B, ECM *Lactarius* sp.1: cross section, thick and compact pseudoparenchymatous mantle. C, ECM *Pisolithus tinctorius* long-distance exploration type ( $\times 40$ ). D, ECM *P. tinctorius*: cross section, with plectenchymatous  
*continued on page 243*

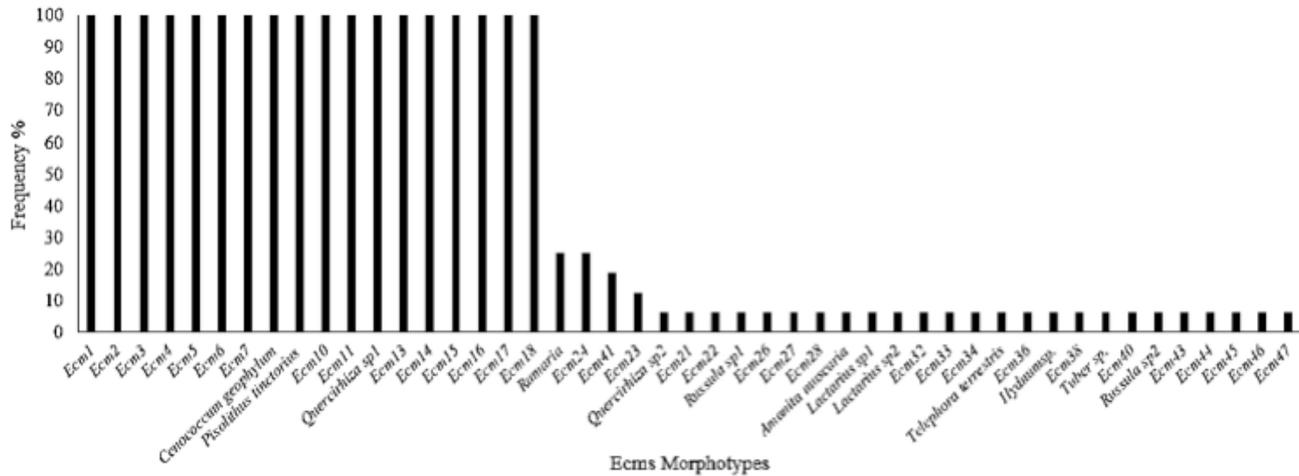


Fig. 2. Relative frequency of ectomycorrhizal morphotypes of *Quercus afares* in the Akfadou forest (Tizi-Ouzou, Algeria).

anatomy-morphotypes. They are known for their efficiency in the translocation of water and phosphorus (Reis et al. 2017). *Pisolithus tinctorius* was reported as a typical early-stage ECM fungus, which is well adapted to low pH and high temperatures of anthracite and hard coal spoils (Marx 1975). This common and widespread multi-host species is known from many highly disturbed sites (Marx 1977). Furthermore, the presence of *Quercirhiza* sp. morphotypes is a high stress indicator in *Quercus* (Kovacs et al. 2000) and *Cenococcum geophyllum* presence is a soil dehydration indicator (Garbaye 2013).

According to species richness, the  $\alpha$  diversity of fungal partners per root system of a tree is on average about eight to 20 types (Allen et al. 2003). For Afares oak, the mean morphotypic richness was  $20.18 \pm 1.68$  (YTMR = 20.12 in young trees and OTMR = 20.25 in old trees). The similarity of ECM communities and the presence of common

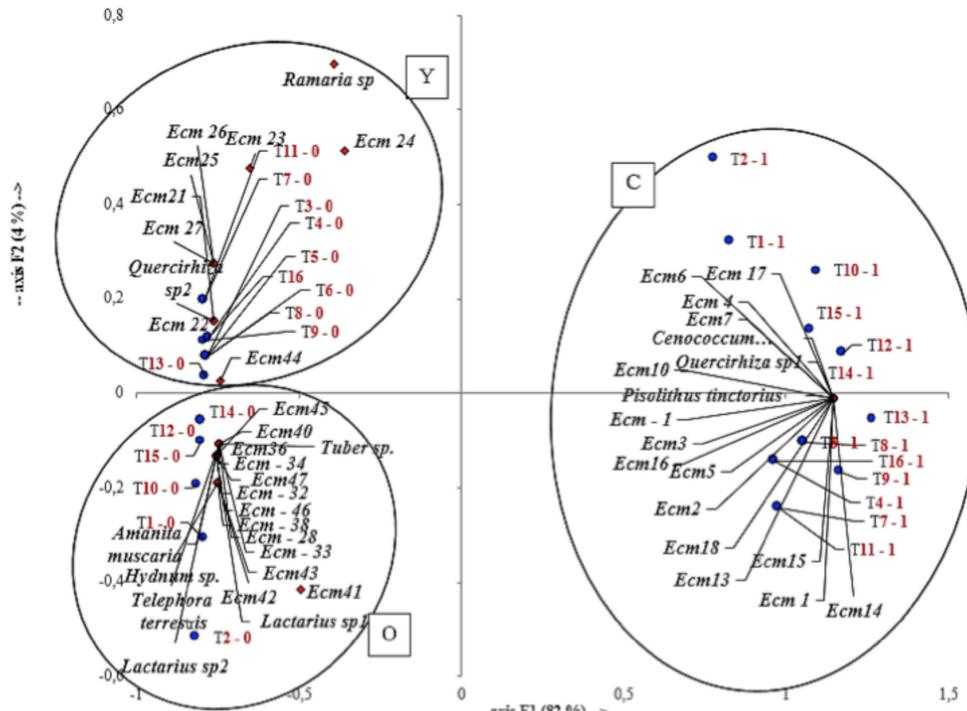
morphotypes can be explained by the maintenance of mycorrhizae from the early or pioneer stage (Guinberteau et al. 1990). In natural environments, late-stage ECM fungi colonize the roots of young plants via the rhizomorphs of emanating hyphae (Fleming 1983), referred to as the “wood-wide-web” (Helgason et al. 1998). They persist for a very long time in the state of “background noise” and rarely completely disappear. Ectomycorrhizal partners might occupy the periphery of the expanding root system even of old trees (Arnold 1991). For Afares oak of the Akfadou forest, our observations allowed to describe a similar ectomycorrhizal community between the two age categories of the population. In addition, there was an abundance of the medium-distance with short-distance exploration morphotypes. In forest ecosystems, “late stage fungi” can be found on roots closest to the trunk of the tree and “early stage fungi” on roots farthest from the base of the

Table 2. Richness and similarity indices of the ectomycorrhizal community in two age groups of *Quercus afares* of Akfadou forest (Tizi-Ouzou, Algeria)

Age group	Specific morphotype richness	Total morphotype richness per category	Morphotype richness per tree	Common ECMs	Total morphotype richness	Jaccard index	Dices index
Young trees	12	30	20.12	18	47	0.33	0.55
Old trees	17	35	20.25				

Fig. 1. continued

mantle and rhizomorphs ( $\times 400$ ). E, ECM 42: monopodial and yellowish brown color ( $\times 40$ ). F, ECM 42: cross section, plectenchymatous mantle with presence of tannin cells ( $\times 400$ ). G, ECM *Quercirhiza* sp.1: monopodial and dark color with emanating hyphae ( $\times 20$ ). H, *Quercirhiza* sp.1: longitudinal section, pseudoparenchymatous mantle and para-epidermal Hartig network. I, ECM *Thelephora terrestris* ( $\times 40$ ). J, ECM *T. terrestris*: longitudinal section, compact plectenchymatous mantle with rhizomorph ( $\times 400$ ). K, ECM 1: ramified and greyish, medium-distance type. L, ECM 1: cross section, thick plectenchymatous mantle with compact outer mantle. M, ECM 25: monopodial-pyramidal, orange ( $\times 20$ ). N, ECM 25: cross section, pseudoparenchymatous mantle and periepidermal Hartig network on one layer cell ( $\times 400$ ). O, ECM 31: monopodial, straight, yellowish contact type ( $\times 40$ ). P, ECM 31: longitudinal section, contact exploration type, pseudoparenchymatous mantle with angular cells ( $\times 400$ ). Q, ECM *Cenococcum geophyllum*: with rather thick emanating hyphae ( $\times 40$ ). R, *C. geophyllum*: cross section, with star-like tightly glued hyphae, cenococcoid mantle ( $\times 400$ ).



**Fig. 3.** Projection of individual trees and ECM types on the first two axes of the factorial correspondence analysis (86% inertia) of Afares oak *Quercus afares* of Akfadou forest (Algeria).

tree (Ford et al. 1980; Wolfe et al. 2009). Hyphal connections can also maintain physiological continuity between a tree and seedlings (Simard et al. 1997). Neighboring tree species identity shaped the ECMs community structure of the host, and the effects were specific to host–neighbor combination (Hubert, Gehring 2008); tree species may serve as reservoirs of ECM inoculation to one another. The theory of systematic change during successions has been reviewed and conservation in large part of the early successional species was reported, and that most types of mycorrhizae can dominate at any stage of ecosystem development (Dickie et al. 2013). The shifts the vegetation composition and function, including photosynthesis efficiency and nutrient requirements, are still considered strong determinants of ECM fungi successional traits (Buscot 2015).

The medium and long-distance types with emanating hyphae are more efficient for transport, while the short-distance types are more efficient in absorption (Lilleskov et al. 2011). In addition, it should be kept in mind that medium and long exploration types require large amounts of carbon

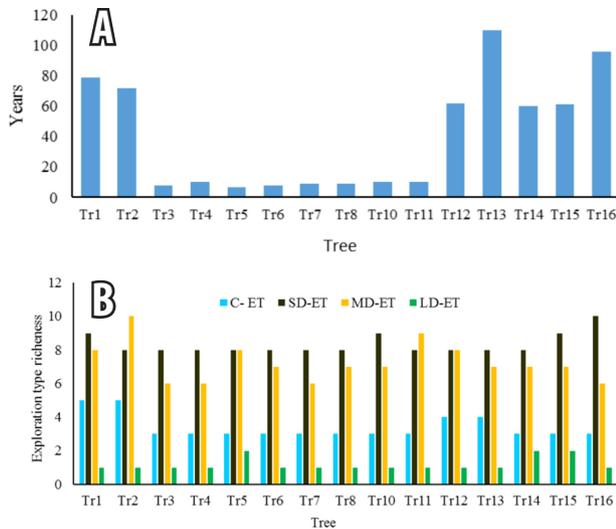
to establish an extensive mycelial exploration network (Baier et al. 2006). In conditions of reduced C supply from trees, this carbon is not available during drought and vegetative rest periods, which favours species that produce little external mycelium, such as the contact and short-distance types (Lilleskov et al. 2011). The richness of the medium-distance type ECMs can be explained by the poor forest soils in Mediterranean regions, with low amounts of available nitrogen and phosphorus. This creates a need for Afares oak to ally with fungal partners, despite their costly energy needs and the state of vegetative rest at the time of sampling.

Jaccard's index of similarity and Dice similarity coefficient indicated similarity of the communities in types of exploration for the two age groups. However, the short distance type most represented among the common community. Detailed analysis allowed to show differences in the ECM communities, in particular for the contact, medium distance and long distance types among the tree age categories.

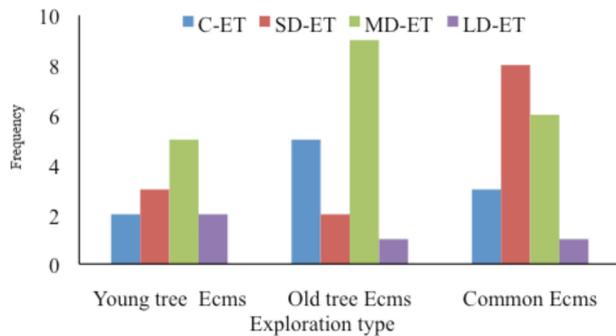
The similarity of the afares oak tree communities of the

**Table 3.** Comparative distribution of exploration type and similarity index by age category of Akfadou forest *Quercus afares*

Exploration type	Young tree ECMs	Old tree ECMs	Common ECMs	Jaccard index	Dices index
Contact	2	5	3	0.30	0.46
Short distance	3	2	8	0.61	0.76
Medium distance	5	9	6	0.30	0.46
Long distance	2	1	1	0.25	0.40



**Fig. 4.** Individual variability of age (A) and richness of exploration type (B) in *Quercus afares* of Akfadou forest (Algeria). C-ET, contact exploration type; SD-ET, short distance exploration type; MD-ET, medium distance exploration type; LD-ET, long distance exploration type.



**Fig. 5.** Distribution of exploration types in the ectomycorrhizal community of *Quercus afares* according to age categories in the Akfadou forest (Tizi-Ouzou, Algeria). C-ET, contact exploration type; SD-ET, short distance exploration type; MD-ET, medium distance exploration type; LD-ET, long distance exploration type.

two ages can be explained by the social relations and the “source – sink” mode (Simard et al. 2003). These transfer processes imply that nutrient, carbon and water deficiencies for a single species can be overcome by transfer strategies between individuals of the same species and between species of the same plant community (Van der Heijden et al. 1998). The networking by fungi can be made not only by long distance exploration types but also by short distance types when the roots of the trees spatially overlap (Horton, Bruns 2001). The ECM can interconnect roots of the same or different species (Simard, Durall 2004).

## Conclusions

*Quercus afares* of the Akfadou forest (Tizi-Ouzou, Algeria) has common mycorrhizal status of species of the Fagaceae family and more precisely those of the same genus.

Endomycorrhizal structures were absent, and the typical anatomo-morphotypes were mainly associated with sub-branching of Ascomycota and Basidiomycota with the presence of ectomycorrhizae formed by *Cenococcum geophilum*, *Pisolithus tinctorius* and *Quercirhiza* spp. Our study found similarity in the composition of the ectomycorrhizal community between the two age categories during the vegetative rest of Afares oak. Functional analysis of the morphotypes showed the short and medium-distance exploration types were more common in the ectomycorrhizal community of Afares oak, regardless of the age category.

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