EFFECTS OF ENVIRONMENTAL FACTORS ON THE RICHNESS AND EXPLORATION TYPE OF ECTOMYCORRHIZAS OF CORK OAK FOREST IN ALGERIA

Kadi-Bennane S., Adjoud-Sadadou D., Harchaoui–Bournine C., Mezaour N.
Laboratoire de Production, Amélioration et Protection des Végétaux, Faculté des sciences biologiques et sciences agronomiques, Université Mouloud MAMMERI de Tizi-Ouzou, UN1501, Algérie

Abstract

In order to study the effect of soil factors (physical and chemical) and seasons on the exploratory functional distribution of EcM communities of Quercus suber L., an inventory was conducted in the cork oak forest of Tazebt located in the upper limit of the sub-humid temperate-Mediterranean level of Tizi-Ouzou (Algeria) and located at 36 °46'28 "N, 4 °37'60" E. The samples were concerned with roots collected at the four cardinal points at 1.50m distance from the collar of thirty trees. Sampling is carried out during the summer of the year 2011 to spring 2012. The morphological and anatomical study - according to Agerer (1987-2008) - allowed us to identify 58 ectomycorrhizal morphotypes (EcMs) divided into four groups: 18 of contact exploration type (C-ET), 15 of short-distance exploration type (SD-ET), 13 of medium distance exploration type (MD-ET), and finally 12 of a long-distance exploration type (LD-ET). A symbiotic activity is noted for the seasons of the year including winter and summer. We observed a small variation in richness during the year. The most important richness is observed in winter and functional strategy in favour of the C and SD exploration groups. Our study allows us to highlight the direct effect of mineralization and pH on the richness of the LD exploration type.

Key words: ectomycorrhiza, exploration type, richness, soil, cork oak, Algeria

INTRODUCTION

In forest ecosystems, most tree roots are associated with fungi that form structures called ectomycorrhizas (EcMs) and involved in the exchange of water and nutrients (Smith and Read 2008). Symbiosis facilitates their acquisition and puts them at the disposal of the plant more efficiently. According to Bauhus and Messier (1999) and Querejeta et al. (2003), the ability of plant species to overpass the limitation of the resources of the soil may be partly due to the degree of colonization of the root system by EcM communities. The EcM communities are influenced by many abiotic factors (Brown 1995). A great number of studies have shown that the soil characteristics (Gehring et al., 1998; Dickie et al., 2002), water availability (Shi et al. 2002), temperature (Domisch et al., 2002), pH and the concentrations of nitrogen (Lilleskov et al., 2011; Averill et al., 2014), or other factors such as the quality of the gas component (Godbold and Berntson, 1997; Parrent et al., 2006) have significant effects on the assemblies.

The key role of ectomycorrhizas in biogeochemical cycles and maintaining soil structure has proved to be true (Baxter and Dighton, 2001; Jonsson et al., 2001; Rillig and Mummey 2006). In addition, several authors have determined the significant role played by ectomycorrhizal fungi involved in exchanges of water and in the mineral nutrition of trees (Mousain et al. 1997; Smith and Read, 2008 and Franklin et al., 2014). Moreover, Suz et al. (2014) argue that the Mediterranean oaks form such structures with numerous fungal species, thus forming EcM communities. These authors suggest that there may be large differences in the acquisition of nutrients between two species through their functional links with ECMS. On the other hand, Danielson, and Visser (1989) and Suz et al. (2014) have noted the importance of the soil factor (soil specificity) in the development of mycorrhizal fungi.

The Ectomycorrhizal community of Quercus suber L. aroused the enthusiasm of researchers because of the socio-economic perspective represented by this species. However, the aspect we are focusing on in this study is very few or no research on this issue: the effect of soil characters on functional exploratory distribution of the EcM communities of Quercus suber L.
MATERIALS AND METHOD

Sampling

Our study was conducted in the cork oak forest of Taksebt (36° 45'28"N 4° 38'49"E), located in the upper limit of the thermo-Mediterranean level in the wet temperate bio-climate of Tizi-Ouzou (Algeria). Through a subjective sampling; ten plots of 400 m² were installed, two of which were burned in the summer of the year 2011, and eight were observed for one year. For each plot, environmental variables were measured, such as altitude (Alt), the slope (St) and the depth of the horizon A0. The collection of root samples concerned 24 trees (3 trees/plot) aged 10 to 80 years, at a rate of one sample per season (Summer, Autumn, Winter and Spring). The roots collected at the four cardinal points, at a distance of 1.50 m and under tree crown of the trees, were placed in plastic bags, labeled and stored at 4°C. The roots were then gently rinsed with tap water and then observed under a stereoscope.

Ectomycorrhizas description

Based on the morpho-anatomical characteristics established by Agerer (1987-2008), the morphotypes are described, individualized and fixed in a conservative solution FPA. The ectomycorrhiza is characterized by the presence of three components: the fungal coat or tissue surrounding the root, the Hartig network of filaments developing between the cortical cells, and the extracellular matrix mycelium, which provides most of the connections between the fungus and soil (Smith and Read, 2008). The study of morphological and anatomical characteristics of these three components of the vegetative ectomycorrhizal fungi is often referred to as "morphotyping" (Horton and Bruns, 2001). Morphotyping is widely used to study EcM communities as an end in itself to characterize fungal diversity (Agerer 1987-2008). Anatomical cuts are made in order to complete the description of morphotypes.

The characterized morphotypes are distinguished into functional exploration groups as described by Agerer (2001): i: contact ET (C-ET, only a smooth mantle or emanating hyphae only exceptionally present), ii: short distance ET (SD-ET, a remarkable envelope of hyphae present without rhizomorphs), iii: medium distance ET (MD-ET, presence of simple rhizomorphs), iv: long distance ET (LD-ET, presence of well-differentiated rhizomorphs). The last three groups were proposed by Agerer (2006) as corresponding to different acquisition strategies and nutrient translocation. Morphotypes have received a reference number

Physico-chemical analysis

The soil collected around the tree and the rhizosphere soil are mixed and dried to be submitted to the physical and chemical analysis, in the laboratory of soil science department at the ENSA, El-Harrach (Algiers): texture, pH (water and KCl), electrical conductivity (EC), organic carbon (Co), total nitrogen (Nt) with Kjeldahl’s method, Phosphorus (Po) with the Olsen method, the comparable Potassium (K) with the amended Anna method.

Statistical analysis

In order to highlight the different relationships that lie between the distribution of types of ECMs exploration and ecological factors, in addition to descriptive statistics, the results were subjected to a principal component analysis (PCA) and a matrix correlation by means of the Statbox6.40 software.

RESULTS AND DISCUSSION

The observation of the soil profile of the forest of Taksebt, combined with the results of physicochemical analysis of the soil (Table 1), has allowed us to classify the latter according to the references of FAO (1998) in cambisol. The various soil plots are frankly to very strongly acidic with average values of respectively 5.68 ± 0.16 and 4.71 ± 0.25 for pH water and KCl pH; it is worth mentioning a slight difference between actual pH (active) of the soil and the pH of reserve (theoretical). This acidity is induced by very low rates of lime content in the soil, which falls within the ecological requirements of the cork oak known as calcifuge, a plant that is not suited to calcareous soil. A significantly negative correlation (R=-0.64, P = 5%) between water pH and concentration of soil phosphorus corroborate with
the results attained by Stout and Overstreet (1950), explaining that the mobility of phosphate ions is maximum between pH = 5.5 and pH = 7. Because of low pH values of the soil, many negative phenomena in plant growth are to be deplored, such as the decrease in nitrification and phosphorus deficiency (Landon, 1991; Strullu, 1991).

The Kjeldahl nitrogen concentrations obtained (mean 0.08 ± 0.03) can be described as poor to mediocre, based on the classification suggested by Calvet and Villemin (1986), for all plots except plot 3, which shows an average concentration. Moreover, according to the interpretation of Dabin (1970), soil fertility from pH and total nitrogen content turns out to be poor.

The percentage in CO that shows with an average of 3.26 ± 3.39 for the whole station attests to the high variability of this parameter in the different plots. The lowest concentration is deplorable at the level of plot 4 followed by plots 1, 5, 6, 7 and 8. As for plots 2 and 3, they have shown average grades. Given these values and soil texture, we distinguish very poor soils (P4, 5, 8, 6, 7 and 8), moderately filled soils (P1), and soils well supplied with organic matter (P2 and 3).

The level of carbon with respect to the total nitrogen ratio (C/N) is calculated to assess the rate of decomposition of organic matter and the potential supplying nitrogen from the soil, as well as the biological activity thereof. It is one of the main indicators of fertility and physicochemical soil quality. The C/N ratio, with an average of 43.71 ± 54.45, indicates a high variability of soil fertility of the different plots under study. In effect, based on the C /N, shows a slow ground mineralization with a strong reduction for plots 2 and 3, especially because of the acidity soils and anaerobic conditions.

Available phosphorus assay has revealed a mean value of 0.79 ± 0.45meq / 100g, which attests to the low Po soil of the cork oak forest under study. It is true that in forest soils, it is not uncommon to notice such low doses combined with the measured acid pH rate. In this respect, Smith and Read (2008) explain that an area of phosphorus depletion at the root system is a phenomenon induced by the very high absorption rate of phosphate by the growing plants.

The analysis of the concentrations of Potassium that appears with mean values of 0.79 ± 0.18 attests to the presence of this element in a relatively acceptable dose (Table 1).

<table>
<thead>
<tr>
<th>Plots</th>
<th>pH H₂O</th>
<th>pH Kcl</th>
<th>CE/µ/mg</th>
<th>Po (mg/kg)</th>
<th>CO %</th>
<th>C/N</th>
<th>Nt</th>
<th>K meq\100g</th>
<th>TEXT</th>
<th>A0</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5.91</td>
<td>5.02</td>
<td>34.8</td>
<td>0.42</td>
<td>2.41</td>
<td>34.3</td>
<td>0.07</td>
<td>0.71</td>
<td>L</td>
<td>9</td>
</tr>
<tr>
<td>P2</td>
<td>5.6</td>
<td>4.3</td>
<td>143.38</td>
<td>0.96</td>
<td>9.59</td>
<td>182.7</td>
<td>0.05</td>
<td>1</td>
<td>L</td>
<td>9.5</td>
</tr>
<tr>
<td>P3</td>
<td>5.51</td>
<td>4.77</td>
<td>46.94</td>
<td>0.96</td>
<td>8.49</td>
<td>52.7</td>
<td>0.16</td>
<td>0.86</td>
<td>L</td>
<td>4</td>
</tr>
<tr>
<td>P4</td>
<td>5.89</td>
<td>5.04</td>
<td>53.42</td>
<td>0.66</td>
<td>0.92</td>
<td>11.5</td>
<td>0.08</td>
<td>0.84</td>
<td>LA</td>
<td>4</td>
</tr>
<tr>
<td>P5</td>
<td>5.67</td>
<td>4.8</td>
<td>63.48</td>
<td>0.4</td>
<td>1.05</td>
<td>12.4</td>
<td>0.08</td>
<td>0.81</td>
<td>LA</td>
<td>3</td>
</tr>
<tr>
<td>P6</td>
<td>5.54</td>
<td>4.39</td>
<td>126.08</td>
<td>0.6</td>
<td>1.66</td>
<td>31.6</td>
<td>0.05</td>
<td>0.39</td>
<td>L</td>
<td>9</td>
</tr>
<tr>
<td>P7</td>
<td>5.51</td>
<td>4.6</td>
<td>89.96</td>
<td>1.84</td>
<td>0.43</td>
<td>6.1</td>
<td>0.07</td>
<td>0.94</td>
<td>SLA</td>
<td>6.5</td>
</tr>
<tr>
<td>P8</td>
<td>5.8</td>
<td>4.72</td>
<td>61.74</td>
<td>0.46</td>
<td>1.54</td>
<td>18.3</td>
<td>0.08</td>
<td>0.76</td>
<td>SLA</td>
<td>9.5</td>
</tr>
</tbody>
</table>

Morphotypes Richness

The specific richness in ECMs in the cork oak of the forest of Taksebt reveals a total of 58 morphotypes which represent a relatively large community if we consider the results of the inventory established by Azul et al. (2009), Adouane and Bediar (2011), and Lancellotti and Franceschini (2013), who recorded for the same species some respective specific richness of 74, 20 in ECMs and 57 morphotypes. The specific richness of ectomycorrhiza fungi exceeds in most cases a hundred taxa in a small area of land (Izzo et al., 2004). However, it is worth noting that this symbiotic community has morphotypes which
sporadically or rarely appear as already reported by Dahlberg (2001), Garbaye (2013) and Lancellotti and Franceschini (2013).

However, species richness by individual does not exceed a mean value of \(23.79 \pm 3.61\), and species richness in ECMs of the contact type (NC-ET), short distance (NSD-ET), Medium distance (NMD-ET) and long distance (NLD-ET) are encountered with respectively 31\%, 26\%, 21\% and 22\% (NC-ET = 18, NSD-ET = 15 NMD-ET= 13 and NLD-ET 12). Our results confirm those of several authors who have noticed the greatest species richness allotted to the C-ET and SD-ET, such as Hobbie and Agerer (2010), Rudawska et al. (2011), Shahin (2012), and Tedersoo et al. (2012). It could be explained by the potential activity of the battery laccase peroxidase, cellulase and hydrolase relatively higher in the type of exploratory contacts, which allows the transfer of nutrients directly from rotten leaves or dead wood (Courty and al., 2010; Rineau and Garbaye, 2009; Lilleskov et al., 2011; Shahin, 2012 and Garbaye, 2013) in slow mineralization conditions. The group of the SD type meanwhile is effective by its adaptation to growth and establish multiple contacts with the organic matter in surface organic horizons (Baier et al., 2006); efficiency in terms of lower carbon cost is also an important character in the selection of a fungal partner by the host plant.

The analysis of richness distributions for all four seasons attests to the activity of symbiosis whatever the weather conditions. A richness on low density in extra-matrix mycelium type (60\% and 62\%) is determined for the summer season and autumn. A slight regression of these exploration types (C-ET=57\% and SD-ET= 58\%) for the MD-ET and LD -ET is recorded for the winter and spring. The ectomycorrhizal community seems rich, dynamic and potentially active even in winter and summer, but a maximum richness of EcMs during is noted in spring (N= 51, R=0.51, P=5\%). This richness could be explained by the Sclerophyllous biology of the cork oak.

Considering the criteria of functional classification according to exploration types give them different abilities of C storage, of sampling and nutrient translocation (Courty et al., 2010; Hobbie and Agerer, 2010) and that the C-ET and SD-ET are the types of which the exchange energy equation is the least onerous for the cork oak, one can quite understand the evolution of the exploration types through the seasons. Indeed, this trend reveals a strategy of least energy expenditure throughout the year with a richer partnership in C-ET in autumn. So, it’s interesting to report the same wealth for both periods autumn and spring with NT = 49 EcMS. In spring, a frank back of this group as well as greater richness in EcMSDs (R = 0.57, P = 5\%) and EcMMDs are to be reported. MD-ET and LD-ET are widely sought in winter EcM (NMD-ET=9, R= 0.44, P =5\%) and (NLD-ET=12, R = 0.50 P =5\%) has been observed during the winter period, and this is probably related to their efficiency in the mobilization of organic nitrogen (Fig. 1).
C-ET: Mycorrhize contact exploration type; SD-ET: mycorrhize short distance exploration type; MD-ET: mycorrhize Medium distance exploration type; LD-ET mycorrhize long distance exploration type

Fig. 1. Distribution of exploration groups through the seasons

In order to transcribe the effect of different soil variables on the distribution of different functional groups of the EcMs, we have categorized the soils of the plots in various fertility classes. From the histogram analysis (Fig. 2) on the basis of a fertility gradient in available phosphorus, species richness in EcMS of the contact and short-distance groups are little influenced by the variation thereof, as evidenced by the respective Pearson correlations ($R = 0.09$ and $R = -0.13$, $P = 5\%$). Conversely, a negative sensibility is observed for MD-ET and LD-ET, thus having larger species richness for the average-fertility phosphorus class. Indeed, Rousseau et al. (1994) have reported a variation of the $P$ absorption capacity depending on the functional groups. If one considers, on the one part, that the fungi that produce an abundant network of extra-matrix hyphae better absorb phosphorus (Colpaert et al., 1999; Van Tichelen and Colpaert, 2000), and on the other part, that the interaction between fungi and minerals is stimulated only in conditions of low phosphorus concentrations only (Wallander and Hagerberg, 2004), we can quite understand the strategy of the cork oak in diversifying these fungal partners of medium and long-distance type in low phosphorus soils (Fig. 2a).
C-ET: Mycorrhize contact exploration type; SD-ET: mycorrhize short distance exploration type; MD-ET: mycorrhize Medium distance exploration type; LD-ET: mycorrhize long distance exploration type; Cl:Class.

**Fig. 2.** Breakdown of different functional groups of exploration through the fertility classes established for: a: Phosphorus, b: Nitrogen, c: C/N, d: Potassium.

Le Duc et al. (2013) have questioned the certainty of a relationship of distributions of ECMs communities with the availability of nutritious elements, especially organic nitrogen, in the soil. Several authors (Baxter and Dighton 2001; Lilleskov et al., 2011; Horton et al., 2013; and Suz et al., 2014) have reported the significant effect of this availability, with decrease in production of mycelium by the EcMs. Lilleskov et al. (2011) state that there is some sensibility (negative response) of MD-ET and LD-ET to the organic nitrogen content of the soil. Although, the variation of total nitrogen concentrations in the plots under scrutiny is small, it does not seem too overtly and positively influences any group other than the LD-ET one. Indeed, Agerer et al. (2012) explain that the abundance of nitrogen in the soil depends on the depth exploration of the fungal partner of the host plant. The potential enzyme activity induced by the average and long-distance type of EcMs (Finlay, 2008) relatively higher localized in the extra matrix phase with rapid growth has a large capacity of decomposition and mobilization of organic nitrogen (Agerer, 2001; Hobbie and Agerer, 2010). In the cork oak, this is true only for the LD -ET for which there is a gradual evolution of species richness depending on soil fertility in nitrogen although the correlation is not significant (R = 0.32 P = 5 %). Conversely, the richness of the C-ET and SD-ET evolve regressively according plots fertility gradient. The MD-ET (Fig. 2b) does not seem to be solicited and this is probably due to its slow development, a limited capacity to assimilate organic nitrogen (Agerer 2001; Hobbie and Agerer, 2010) and its poor capacity to thrive in terms of availability of inorganic N (Suz et al., 2014).
Recent studies (Rineau et al., 2012; Bodeker et al., 2014; Phillips et al., 2014; Lindahl and Tunlid, 2015) suggest that EcM fungi act as decomposers of organic matter in the soil, a widespread capacity in these EcMs with a selective N uptake (Lindahl et al., 2007; Clemmensen et al., 2013; Shah, 2015). The distribution of EcMs within the mineralization classes (C/N) shows some species richness in medium mineralization soils with a distribution that follows a regular norm, with sometimes a mismatch at the right for the C-ET and SD-ET, and sometimes at the left for the LD-ET. Correlations between NLD-ET and nitrogen (R = -0.57 P = 5%), pH KCL (R = -0.538 P = 5%) and CE (R = -0.55 P = 5%) attest to the negative effect of the slow mineralization of OM on the richness of the LD-ET (Fig. 1c).

Despite the lack of correlation between the different exploratory types, the distribution of functional groups following the potassium element shows distinctly the preference the cork oak in its strategy to associate with these partners of C-ET (R = -0.40 P = 5%) and SD-ET (R = -0.32 P = 5%) in the conditions of low potassium concentration (Fig. 2d).

The CAP (Fig. 3) divided factors and individuals into two sets in factorial terms, with 47% of total variance. A set with rich individuals in LD-ET with low nitrogen levels in a context of significant steep slopes, higher altitudes.

![Fig. 3. CAP exploratory groups of trees and ecological factors](image)

The second with less richness in LD-ET that are rich in exploratory types with low density in extra-matrix mycelium (C and SD), a soil with high nitrogen concentrations, a depth of a considerable A0 horizon, and slow mineralization.
CONCLUSION

Our study shows that the acidity conditions of the environment involve richness fungal partner of the cork oak in its natural environment. We have been able to describe the functional strategy adopted by the cork oak and to assert that *Quercus suber* L. appeals to fungal partners mostly of the contact and short-distance types, because of the slow mineralization of organic matter.

An activity and a significant richness are observed during all seasons with the dominance of the exploration types with low extra-matrix hyphae. However, a light regression of C-ET and SD-ET for the MD-ET and LD-ET is raised for the winter and spring. There does not seem to be any significant relationship between soil nutrients and species richness of the EcMs community.

The distribution of exploration types in the EcM communities is uncertain, particularly in connection with the physicochemical characteristics of soil horizons and seasons, except for the LD group, which seems to be negatively affected by slow mineralization. In all, it would be interesting to complement this work by molecular identification of these EcMs communities in order to provide inoculum strains for large-scale reforestation projects in the area under study.

ACKNOWLEDGEMENTS

The authors thank the Pedology Laboratory team of ENSA (El-Harrach, Algiers), the members of forest conservation of the riding of Azazga (Tizi Ouzou) especially Mr. Mebarki for their help and Mr. Hami for the English translation.

REFERENCES


Wallander H., Hagerberg D. 2004. Do ectomycorrhizal fungi have a significant role in weathering of minerals in forest soil? Symbiosis 37: 249-257