Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter?

Franck Richard1,2, Marc-André Selosse2 & Monique Gardes1

1UMR 5174 Evolution et Diversité Biologique, Université Toulouse III Paul Sabatier, Toulouse, France; and 2Centre d’Ecologie Fonctionnelle et Évolutive (CNRS, UMR 5175), Equipe Interactions Biotiques, Montpellier, France

**Correspondence:** Franck Richard, Centre d’Ecologie Fonctionnelle et Évolutive (CNRS, UMR 5175), Equipe Interactions Biotiques, 1919 Route de Mende, 34293 Montpellier cedex 5, France. Tel.: +33 4 67 61 32 62; fax: +33 4 67 41 21 38; e-mail: franck.richard@cefe.cnrs.fr

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**Abstract**

Positive plant–plant interaction is a widespread phenomenon, especially in harsh environments, which can contribute to secondary successions. Here, we investigated whether *Arbutus unedo* positively influences *Quercus ilex* establishment in shrub communities by abiotic and/or biotic interactions in a Mediterranean forest ecosystem, where we previously showed that *A. unedo* and *Q. ilex* share numerous species of mycorrhizal fungi. In a first field experiment, patterns of *Q. ilex* survivorship were documented. During the summer following germination, a majority of seedlings survived in *A. unedo* chaparral (AU), whereas most of them died in previous succession stages dominated by *Erica arborea* (EA). These results showed that survival of the *Q. ilex* seedling is succession stage dependent, probably due to the differential effects of the summer drought. In a second experiment, *Q. ilex* seedlings were used as bait plants to investigate the mycorrhizal inoculum in EA and AU. Morphotyping and molecular typing revealed 2.5 times higher colonization in AU than in EA, with more diverse fungi. Our results demonstrate that *A. unedo* facilitates mycorrhization of *Q. ilex* by hosting compatible ectomycorrhizal symbionts and positively influences seedling survival by buffering abiotic conditions. A comprehensive understanding of facilitation should thus include investigations of the soil biological patterns.

**Introduction**

Understanding the mechanisms that drive species’ and communities’ replacement over time, a process called succession (Begon *et al*., 2006), is among the most important topics of plant ecology. Soil microbiota have often been involved for example via the recruitment of specific soil pathogens that reduce the competitive abilities of plants and allow their displacement by others (Wardle *et al*., 2004; Kardol *et al*., 2006). The importance of facilitation, as a key process that strongly influences the species distribution and dynamics of plant communities, has received considerable attention, especially recently (Bruno *et al*., 2003; Maestre & Cortina, 2004). In Mediterranean-type ecosystems, where summer drought drastically limits plant establishment, most models of facilitation suggest that late-successional tree species transiently benefit ‘safe sites’ where microclimatic conditions are buffered by early-successional shrubs (Gómez-Aparicio *et al*., 2004, 2005; Kennedy & Sousa, 2006; Sanchez-Gomez *et al*., 2006).

Most plant communities are dominated by species that obligately associate with mycorrhizal fungi (Smith & Read, 2009). For this reason, the availability of compatible fungal inoculum in soil has been suggested to locally influence the ability of tree seedlings to establish in Mediterranean-type ecosystems (Horton *et al*., 1999; Azcon-Aguilar *et al*., 2003). Recently, experimental investigations from a wide variety of natural habitats have demonstrated that interspecific hyphal links may facilitate seedling establishment and contribute to plant community dynamics (Dickie *et al*., 2004; Nara, 2006; Selosse *et al*., 2006). The general idea is that mycorrhizal fungi mediate plant species coexistence by integrating the associated plant species into common mycelial networks. In this scenario, nutrient acquisition and the growth of...
seedlings should improve in the vicinity of plants due to an increased availability of compatible fungal symbionts already established at the cost of these plants (Simard et al., 2002; Dickie et al., 2004; Bever & Schultz, 2005). Despite recent attention, microorganism-mediated plant–plant interactions are rarely included in modern facilitation theories, because their extent is still insufficiently documented and the ecophysiological responses to increased mycorrhization are poorly understood at the plant community level.

Mediterranean forests have recently experienced a dramatic increase in fire frequency (Le Houérou, 1987; Scarascia-Mugnozza et al., 2000), resulting in a progressive replacement of oak forests by shrub-dominated communities that offer outstanding models of ecological successions (Fig. 1). *Quercus ilex* L. (the holm oak), an evergreen sclerophyllous oak species covering > 7.5 Mha, characterizes the vegetation of the western Mediterranean basin (Quézel & Médail, 2003). In this area, postfire secondary succession on siliceous soils starts with pioneer dwarf shrubs, followed, within a few decades, by several species of midsuccessional shrubs (e.g. *Erica arborea* L., *Phillyrea latifolia* L.). The resulting shrub-dominated community (a chaparral locally called 'macchia') resists holm oak invasion for several decades, but it is slowly colonized by *Arbutus unedo* L. (the strawberry tree; Fig. 1) that outcompetes *E. arborea* due to its high potential to reproduce asexually. *Quercus ilex* naturally invades *A. unedo*-dominated macchia (AU), but not *E. arborea*-dominated macchias (EA; Allier & Lacoste, 1981; Mesléard & Lepart, 1991; Gamisans, 1999). At the forest stage (QI), *Q. ilex* trees dominate an understory composed of conspecific seedlings, of *P. latifolia* and *A. unedo* (Panaïotis et al., 1997; Gamisans, 1999). At the landscape level, variations of the succession rate from site to site result in a patchy mosaic of different succession stages.

In a previous study conducted in an old *Q. ilex* forest from Corsica, we showed that a large number of ectomycorrhizal fungal species are shared among canopy trees, oak seedlings and *A. unedo* mature shrubs (Richard et al., 2005). The two-host fungal taxa represented 13% of the taxonomic diversity, but colonized 70% of all mycorrhizal sampled. This pattern led us to investigate the role of ectomycorrhizal fungi in the establishment of holm oak in the context of a secondary succession. While *A. unedo* forms mycorrhizae with the same fungi as ectomycorrhizal plants, *E. arborea* forms mycorrhizae with a restricted group of Ascomycetes that are predominantly absent from ectomycorrhizal tree species such as *Q. ilex* (Fig. 1; Villarreal-Ruiz et al., 2004; Vrålstad, 2004). In the present study, we tested whether the fungal diversity supported by *A. unedo* facilitates ectomycorrhizal colonization of *Q. ilex* seedling in AU, and whether the observed differences in *Q. ilex* establishment patterns between the two macchia types could result from the availability of ectomycorrhizal fungi.

To address this, pregerminated acorns of *Q. ilex* were planted under (1) EA and (2) AU. In Experiment 1, we documented the patterns of seedling emergence and survival in the two situations. Based on the results, we set up a second experiment comparing *Q. ilex* seedlings from AU and EA to (1) investigate the patterns of ectomycorrhizal root colonization, (2) document the species richness and diversity of ectomycorrhizal fungi and (3) test for differences in growth and foliar nutrients. The ectomycorrhizal communities growing on seedlings were compared with the ectomycorrhizal community in an old *Q. ilex* forest from the research area. In addition, we examined the two macchia habitats for various microclimatic and edaphic parameters. Our objectives were to determine the potential for ectomycorrhizae–plant interactions to be a mechanism underlying a 'nurse plant effect.'

**Materials and methods**

**Study systems**

Research was conducted during 1999–2003 at the Fango Man and Biosphere Forest Reserve (42° 20' N; 8° 49' E) in Corsica Island, France. The climate is of Mediterranean type with a monthly temperature varying up to 29.9 °C (July) and the mean annual precipitation ranging from 730 to 1070 mm depending on elevation, with rainfall occurring principally during the spring and autumn months (for other site features, see Richard et al., 2004). The vegetation at low elevation is a mosaic of *Q. ilex* stands (QI) adjacent to AU and EA patches (Fig. 1; Panaïotis et al., 1998). Field trials were conducted at three sites, Monte Estremo, Perticato and Tetti, located in three adjacent valleys from 3.4 to 7.9 km apart. These study sites were selected to provide accessible and large areas of AU and EA. Very scattered individuals of...
E. arborea remaining from the previous successional stage.

Two to three (depending on the experiment) replicate plots of (1) AU and (2) EA communities were chosen at each site. Plots were at least 20 m in radius, and situated from 200 m to 2 km apart. The following rules applied: (1) each plot was devoid of Q. ilex, C. monspeliensis and C. salviifolius individuals; (2) EA plots were devoid of A. unedo mature shrubs; and reciprocally, (3) AU plots were devoid of E. arborea mature shrubs, although they contained dead E. arborea remaining from the previous successional stage.

Edaphic and microclimatic conditions

The canopy cover was determined at each of the three sites using a silicon pyranometer (LI-200SB, Li-cor, Lincoln, NE). For each plot, measurements were taken at the ground level under overcast conditions (from January 15 to 17, 2001) (1) metrically along three 10-m-long transects and (2) at the four corners of each outplanted seedling group. The data were compared with a reference location in the open. The average light flux value was used to calculate a leaf area index (LAI) as described by Mc Naughton & Jarvis (1983).

Field capacity and soil gravimetric water content were assessed on soil cores collected at the center of each plot and at three supplementary random locations per succession stage per site. After removing litter, samples were collected from 0 to 10 cm using a 9-cm-diameter soil corer, and transported to the laboratory for measurements. In addition, four additional soil samples (25 cm depth, 10 cm diameter) were collected in EA and AU at the Perticato site only, for analysis of physical and chemical characteristics.

Air-dried soils were sieved and the < 2-mm fraction was analyzed for pH, C to N ratio, total Kjeldahl phosphorus, Olsen phosphorus and exchangeable sodium, potassium, calcium, aluminum and magnesium.

Experiments

Holm oak acorns drop to the ground in autumn (November), and germinate soon afterwards (January), with the shoot emerging during April to mid-May. Acorns were collected yearly in November from various locations across the Fango forest, and stored at 4 °C in dry sand for pregermination until planting. In late January, acorns were examined for insects and fungal damages. Healthy pregerminated acorns were buried a 2-cm depth. For protection against mammals and birds, we used 50 cm × 50 cm × 30 cm tall protection cages with a 1-cm mesh wire netting and edges secured with steel spikes.

Experiment 1: dynamics of seedling survival in EA and AU

This first study was conducted at the Perticato site. On January 15, 2000, three plots were established per succession stage (AU and EA), each consisting of 49 pregerminated acorns buried under the protection cages described above. Seedling emergence, shoot survival and shoot height were recorded monthly in each plot until January 15, 2001.

Experiment 2: seedling performance and mycorrhization in EA and AU

This second experiment was conducted at Perticato, Tetti and Monte Estremo sites, from January 15, 2001 to January 15, 2002. A total of 36 healthy acorns were planted under each cage on two plots per succession stage at each site (i.e. 2 × 2 × 3 = 12 cages in all). Seedling emergence and survival as well as shoot height were recorded monthly. Seedlings were watered every two weeks from July 1 to August 31 to limit the effect of summer drought (Jimenez et al., 2007).

In order to examine the time course of ectomycorrhizal infection, two sets of 12 randomly selected seedlings (i.e. one per plot) were harvested from each plot on July 1, 2001 and on September 15, 2001 and examined for the presence of ectomycorrhizae on their root system. At the end of January 2002, the remaining seedlings were carefully uprooted in order to avoid fine root disruption. Root systems were washed, and short root tips were counted and classified as ectomycorrhizae if a fungal mantle was observed under a dissecting microscope. For each seedling, ectomycorrhizal infection was calculated as the percentage of short roots forming ectomycorrhizae. Seedlings were then oven dried at 50 °C for 72 h. The roots, stems and leaves were measured and weighed separately. Leaves of 30 seedlings per succession stage were analyzed for total nitrogen and phosphorus concentrations using the Autoanalyser II Technicon method (O’Neill & Webb, 1970).

Morphotyping and molecular identification of the fungal symbionts

Ectomycorrhizae from 30 seedlings (five individuals randomly located at each plot) were collected in each macchia type, AU and EA. All live root tips of each seedling were hand picked and sorted into morphotypes under a dissecting microscope by characteristics such as color, shape and distinct features of the mantle. No attempt was made to match morphotypes between seedlings.

Cenococcum geophilum Fr. mycorrhizae were considered characteristic enough to be identified by morphology. However, in order to confirm this identification, two randomly selected C. geophilum mycorrhizae from 20 plant individuals at the Perticato site (five Q. ilex seedlings, five Q. ilex saplings, five Q. ilex trees and five A. unedo mature shrubs) were screened by restriction fragment length polymorphism (RFLP) analysis, and sequenced (Richard et al., 2005).
Thus, they were counted separately and not picked. All other mycorrhizae were stored individually at −20°C in 700 μL cetylammomiumbromide lysis buffer (2% cetylammomiumbromide, 100 mM Tris-HCl, 20 mM EDTA and 1.4 M NaCl). A representative tip from each unique morphotype was subjected to DNA extraction, PCR amplification of the intergenic transcribed spacer (ITS) of the nuclear rRNA gene and subsequent ITS-RFLP analysis (using endonucleases CfoI, Hinfl, MboI and HaeIII) as in Richard et al. (2005). For taxonomic identification, ITS-RFLP types were compared with those from reference databases assemblies from ectomycorrhizal fruitbodies and roots collected at Perticato in a previous study [Richard et al. (2004, 2005); the latter studies showed that a perfect match between two ITS-RFLP types using four endonucleases well approximates for species identification at our study site]. Symbionts that were absent from databases were sequenced and tentatively identified by BLAST analysis as in Selosse et al. (2002).

Statistical analyses

Data analyses were performed using MINITAB 12.2 software (Minitab Inc., Paris, France). All statistics were considered significant at \(P = 0.05\). Differences in Q. ilex survival between EA and AU (Experiment 1) were tested using \( \chi^2 \) tests (Schwartz, 1993). Comparisons were performed (1) at the end of May (spring survival), (2) at the end of September (summer survival) and (3) at the end of the experiment (annual survival). Differences in the foliar nutrient concentration of 1-year-old seedlings were analyzed using \( t \)-tests, with succession stage (EA vs. AU) as the variation factor. Before running the \( t \)-tests, the variances were confirmed to be homogenous using Levene’s tests (\(P > 0.05\)). Differences in incident light and soil physical and chemical characteristics between EA and AU (Experiment 2) were tested using Mann–Whitney nonparametric tests with succession stage as the variation factor.

To compare the ectomycorrhizal richness between EA and AU, the relative abundance distribution of all ITS-RFLP types was plotted in both types of macchia. As the number of ribotyped ectomycorrhizae differed between EA and AU, data were rarefied (Simberloff, 1972) to the size of the smaller sample (i.e. EA) using BIODIVERSITY PRO 2 (http://biodiversity-pro.software.informer.com/) and the curves obtained were visually compared. The ectomycorrhizal diversity was estimated using Simpson’s index, because this estimator is less biased by differences in sample size (Mouillot & Lepretre, 1999). The similarity of the ectomycorrhizal composition among the three succession stages was compared using \( S_\alpha \), Sorensen’s coefficient (Krebs, 1999). Correlations between Q. ilex seedling survival, on the one hand, and LAI, ectomycorrhizal infection, \(C. geophilum\) relative abundance, ectomycorrhizal richness and diversity, on the other, were statistically tested on averaged data at the plot level using the Pearson correlation coefficient.

Before analysis, data distributions were tested for normality by the Anderson–Darling analysis. The effects of succession stage and site on the seedling height and biomass, root to shoot ratio, ectomycorrhizal colonization, \(C. geophilum\) relative abundance and number of ribotypes per seedling (ectomycorrhizal richness) were tested on averaged data at the plot level using two-way ANOVAs (general linear model procedure) with succession stage and site as the variation factors, followed by Bonferroni multiple comparisons. To test whether the non-normally distributed variables (i.e. number of short roots and ectomycorrhizal diversity) differed between sites and succession stages, we conducted Friedman’s nonparametric tests.

Results

Edaphic and microclimatic conditions in EA and AU

Averaged canopy cover was significantly lower at all sites under \(E. arborea\) than under \(A. unedo\), as shown by lower LAI values in EA than in AU (Table 1). At Perticato, both the soil field capacity and the water gravimetric content were significantly higher in AU than in EA (Table 1). At Perticato, there was no significant difference between AU and EA soils in the pH, C to N ratio, exchangeable cations and Kjeldahl and Olsen phosphorus (Supporting Information, Table S1).

Dynamics of seedling survival in EA and AU (Experiment 1)

Survival of Q. ilex seedlings after 1 year was significantly higher in AU than in EA (\(\chi^2 = 100.49; P < 0.001\); Table 2). At the end of the experiment, 91.8% of the planted pregerminated acorns were dead in EA. In contrast, the seedling survival rate was high in AU, and 1 year after acorn planting, 74.7% of Q. ilex seedlings were still alive (Table 2).

Table 1. Vegetation and soil physical and chemical features in EA and AU

<table>
<thead>
<tr>
<th>Variable</th>
<th>Succession stage</th>
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<tbody>
<tr>
<td></td>
<td>EA</td>
</tr>
<tr>
<td>LAI (m² m⁻²) (average on all sites)</td>
<td>5.44 (0.52) a</td>
</tr>
<tr>
<td>Field capacity (%) at Perticato</td>
<td>110.98 (5.54) a</td>
</tr>
<tr>
<td>Soil water gravimetric content</td>
<td>1.19 (0.06) a</td>
</tr>
<tr>
<td>(g water per g soil) at Perticato</td>
<td></td>
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</table>

*Perticato, Tetti and Monte Estremo.

Values are averaged data with SE within parentheses. Different small letters indicate significant differences between EA and AU at \(P < 0.05\) using the Mann–Whitney nonparametric test.
Variables that significantly differed between EA and AU at values are numbers of seasonally dead seedlings per plot (in %).

The survival of seedlings was thus dependent on both the succession stage and the season (Table 2), with (1) a moderate and similar mortality during the first 5 months after acorn planting (spring mortality, i.e., mostly before shoot emergence from soil) in all macchias and (2) a pulse of mortality during the annual drought from May to September in EA only (Table 2). The high survival of *Q. ilex* seedlings observed in AU, as well as the pulse of summer mortality in EA, were consistent from year to year (as found in our other experiments, not shown).

### Patterns of seedling development across sites in AU and EA (Experiment 2)

One year after acorn planting, the survival of watered *Q. ilex* seedlings (Experiment 2) averaged 51.4% and 63.4% in EA and AU, respectively. Survival in EA was thus more than six times better than that in Experiment 1, but the seedling survival rate was still significantly higher in AU than that in EA ($\chi^2 = 6.40; P < 0.05$). A total of 148 seedlings (64 and 84 seedlings from EA and AU, respectively) were used for biometric analyses, foliar nutrient measurements and ectomycorrhizal colonization investigations.

Seedings were heavier in EA than in AU ($P = 0.009$; Table 3). The root to shoot ratio, plant height and number of short roots did not differ between macchias ($P > 0.05$; Table 3). The total nitrogen and phosphorus contents in the leaves of 1-year-old *Q. ilex* seedlings did not differ significantly between AU and EA ($P > 0.05$; Table S1). In contrast, due to the lower foliar biomass in AU, the phosphorus concentration in *Q. ilex* leaves was significantly higher in AU than in EA ($P = 0.019$; Table S1), whereas, differences in the foliar nitrogen concentration were not significant between AU and EA (Table S1).

The plant mass, plant height and root to shoot ratio were significantly site dependent (Table 3). Bonferroni multiple comparisons indicated (1) heavier *Q. ilex* seedlings at Tetti than at Perticato ($P = 0.028$; Fig. 2a), (2) higher root to shoot ratios at Perticato as compared with Monte Estremo ($P = 0.020$; Fig. 2b) or Tetti ($P = 0.016$; Fig. 2b) and (3) taller seedlings (higher shoot height) at Perticato than at Tetti ($P = 0.036$; Fig. 2c).

### Patterns of ectomycorrhizal colonization, richness and diversity across sites in EA and AU (Experiment 2)

Most seedlings were either poorly or not colonized by ectomycorrhizal fungi until fall (data not shown). At the end of the experiment, a total of 7890 ectomycorrhizal tips were collected out of 37 661 short roots from the 148 1-year-old seedlings harvested.

Mycorrhization patterns differed significantly between the two succession stages (Table 3). Averaged ectomycorrhizal colonization per seedling, averaged at the plot level, was significantly higher in AU than in EA (22.9% in AU and 9.4% in EA, respectively; $P = 0.048$; Table 3 and Fig. 3a). Similarly, the mean ectomycorrhizal taxa richness was more than twice when grown in AU, as compared with EA (6.7 and 2.8 taxa per seedling in AU and in EA, respectively).
respectively; \( P = 0.004; \) Table 3 and Fig. 3b). There was no difference between EA and AU in either averaged \( C. \text{geophilum} \) relative abundance (\( P > 0.05; \) Fig. 3c) or in ectomycorrhizal diversity as estimated using Simpson's index (\( P > 0.05; \) Fig. 3d).

DNA extraction and ITS-RFLP typing were carried out on 153 ectomycorrhizal root tips, and succeeded for 105 of them (68.6\%). A total of 44 ITS-RFLP types were found on seedlings (Table S2). Using matches with RFLP patterns and BLAST analyses of fruitbodies from an adjacent oak forest at the Perticato site (Richard et al., 2004, 2005), 14 taxa were identified, including five species in Russulaceae, three species in Cortinariaceae, two species in Thelephoraceae and Boletaceae and one species in Clavulinaceae and Sebacinaeae (Table S2). In all macchias, the most abundant ectomycorrhizal species was \( C. \text{geophilum} \), accounting for 14.8\% and 70.2\% of the total number of ectomycorrhizae in EA and AU, respectively (Fig. 3c). In all, 17 types occurred in EA, 36 types occurred in AU (Fig. 4) and nine were common to both macchias (Fig. 4). Only four ITS-RFLP types were represented by more than five mycorrhizae (Fig. S1a), including \( C. \text{geophilum} \), a tomentelloid symbiont (present in EA at two of the three sites, on roots of nine different seedlings distributed in four plots), \( Russula \) persicina var. rubrata Romagn. and \( Hygrophorus \) eburneus var. carneipes Kühner. At the other extreme of the rank abundance curve, we found a majority of singletons (56.3\% and 57.1\% of the total number of taxa in EA and AU, respectively), and many taxa represented by either two or three mycorrhizae (31.3\% and 22.9\% of the total number of taxa in EA and AU, respectively; Fig. S1a). Based on rarefied data, ectomycorrhizal taxa richness (i.e. the expected numbers of ITS-RFLP types contained in a sample of 31 types of ectomycorrhizae) was higher in AU than in EA (20.38 and 14.27 species in AU and EA, respectively; Fig. S1b).
Correlations between temporal patterns of *Q. ilex* mortality and patterns of seedling development and mycorrhization (Experiment 2)

The spring, fall and annual mortalities of *Q. ilex* seedlings were not correlated to any of the tested variables (data not shown). On the other hand, there was a significant and negative correlation between summer mortality of *Q. ilex* seedlings in macchias and the intensity of canopy cover ($r = -0.809; P = 0.049$; Table 4). None of the tested mycorrhization parameters correlated significantly with patterns of summer mortality in macchias (Table 4).

Similarities of ectomycorrhizal fungal communities among AU, EA and QI

Overall, 64.7% of ITS-RFLP types found in EA were also present in QI (Fig. 4), including (1) six taxa on roots of old *Q. ilex* trees, (2) one taxon on roots of *A. unedo* mature shrubs and (3) four taxa on roots of both plant species. A similar pattern was observed in AU, with 18 ITS-RFLP types out of 36 (50%) also present in QI (Fig. 4), including (1) 14 taxa (38.9%) found exclusively on roots of old *Q. ilex*, (2) two taxa (8.3%) found exclusively on roots of *A. unedo* shrubs and (3) two taxa (8.3%) found on roots of both species. A low ectomycorrhizal taxa similarity was found between QI and the two different types of macchia (Fig. 4). In contrast, the similarity between EA and AU was twice that between each macchia and QI (Fig. 4).

**Fig. 4.** Distribution of ectomycorrhizal fungal diversity according to three successive vegetation stages along the secondary succession: EA, AU and QI [estimated by Richard et al. (2005) using a sample of 2294 ectomycorrhizae] and ectomycorrhizal taxa similarities among EA, AU and QI. Values inside the circles are percentages of the total number of RFLP types ($n$=number of RFLP types). Values outside the circles are similarity coefficients $S$, of Sorensen.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$r$ value</th>
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<tbody>
<tr>
<td>Ectomycorrhizal infection</td>
<td>-0.650</td>
</tr>
<tr>
<td><em>C. geophilum</em> abundance</td>
<td>-0.082</td>
</tr>
<tr>
<td>Ectomycorrhizal richness (number of ITS-RFLP types)</td>
<td>-0.505</td>
</tr>
<tr>
<td>Ectomycorrhizal diversity (Simpson’s index)</td>
<td>-0.003</td>
</tr>
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</table>

Significant correlation ($P < 0.05$) is shown in bold.

**Table 4.** Pearson’s $r$ correlation coefficients between mean spring and summer *Quercus ilex* mortality per plot and averaged leaf area index, ectomycorrhizal infection, *Cenoccocum geophilum* relative abundance and ectomycorrhizal richness and diversity (Experiment 2).
test for the impact of shade on oak survival in our system, the level of understory irradiance was significantly lower in AU than in EA (Table 1), and canopy cover positively and significantly correlated with Q. ilex summer survival (Table 4). Moreover, field capacity and gravimetric water were higher in AU than in EA (Table 1). These results are in accordance with previous field works by Mesléard & Lepart (1991), showing that A. unedo is associated with mesophilic microhabitats. Altogether, this suggests that A. unedo favors Q. ilex early recruitment by both providing buffered above-ground microclimatic conditions and dominating sites with increased soil water availability.

Potential explanations for the summer mortality in EA (Table 2) include: (1) severe water losses due to a high level of photosynthetically active radiation and (2) belowground competition for soil water by drought-tolerant shrubs. These species may interfere either directly (Schiller et al., 2002; Maestre et al., 2004) or even indirectly via their mycorrhizal associates, which may impact ectomycorrhizal fungal colonization negatively (Jones et al., 2003; Mc Hugh & Gehring, 2006). However, even when Q. ilex seedlings were watered (Experiment 2), summer survival was still significantly higher in AU than in EA. These data may suggest that the watering was not sufficient to negate the drought effects experienced by Q. ilex seedlings in EA. Alternatively, these results may indicate that soil water availability was not the only factor to drive the dynamics of seedling survival.

**Arbutus unedo facilitates ectomycorrhizal colonization of Q. ilex seedlings**

One year after acorn planting, ectomycorrhizal colonization of Q. ilex seedlings was 2.5 times higher when grown in AU than in EA (Table 3; Fig. 3). In addition, ectomycorrhizal taxa communities on 1-year-old seedlings grown in AU were richer than those from EA (35 vs. 16 RFLP types, respectively). This difference can be explained by the high potential for common mycelial networks with them than those from EA (35 vs. 16 RFLP types, respectively). This difference can be explained by the high potential for common mycelial networks with previous seedlings grown in AU during the relatively wet and warm autumn. Further experiments, including a 2-year-long monitoring in AU, are needed to estimate the importance of the ectomycorrhizal fungal linkages between A. unedo mature shrubs and Q. ilex seedlings and to evaluate whether an increased ectomycorrhizal infection represents a nurse effect.

Despite strong differences in ectomycorrhizal colonization on oak seedlings, the fungal communities in both succession stages were surprisingly equally diverse (Figs 3d and 2d). As on adult Q. ilex (Richard et al., 2005), C. geophilum, a drought-tolerant fungus, was the most

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Potential explanations for the summer mortality in EA (Table 2) include: (1) severe water losses due to a high level of photosynthetically active radiation and (2) belowground competition for soil water by drought-tolerant shrubs. These species may interfere either directly (Schiller et al., 2002; Maestre et al., 2004) or even indirectly via their mycorrhizal associates, which may impact ectomycorrhizal fungal colonization negatively (Jones et al., 2003; Mc Hugh & Gehring, 2006). However, even when Q. ilex seedlings were watered (Experiment 2), summer survival was still significantly higher in AU than in EA. These data may suggest that the watering was not sufficient to negate the drought effects experienced by Q. ilex seedlings in EA. Alternatively, these results may indicate that soil water availability was not the only factor to drive the dynamics of seedling survival.

**Arbutus unedo facilitates ectomycorrhizal colonization of Q. ilex seedlings**

One year after acorn planting, ectomycorrhizal colonization of Q. ilex seedlings was 2.5 times higher when grown in AU than in EA (Table 3; Fig. 3). In addition, ectomycorrhizal taxa communities on 1-year-old seedlings grown in AU were richer than those from EA (35 vs. 16 RFLP types, respectively). This difference can be explained by the high potential for common mycelial networks with A. unedo (Richard et al., 2005) in AU, in contrast with EA, which was devoid of ectomycorrhizal fungi. Our data do not indicate whether ectomycorrhizal abundance and the richness of ectomycorrhizae in AU had an influence on seedling survival in the first year. However, higher taxonomical diversity may entail higher functional diversity in the ectomycorrhizal community (Courty et al., 2005) and thus better soil exploitation during the relatively wet and warm autumn. Further experiments, including a 2-year-long monitoring in AU, are needed to estimate the importance of the ectomycorrhizal fungal linkages between A. unedo mature shrubs and Q. ilex seedlings and to evaluate whether an increased ectomycorrhizal infection represents a nurse effect.

Despite strong differences in ectomycorrhizal colonization on oak seedlings, the fungal communities in both succession stages were surprisingly equally diverse (Figs 3d and 2d). As on adult Q. ilex (Richard et al., 2005), C. geophilum, a drought-tolerant fungus, was the most colonization is poorly influenced by ectomycorrhizal Cistus occurring in EA, perhaps because of the higher specificity of Cistus ectomycorrhizal assemblages (Comandini et al., 2006).

Our results provide little support for the hypothesis that early ectomycorrhizal colonization facilitates Q. ilex survival during the first year following germination. We could not find any correlation between ectomycorrhizal colonization patterns, seedling development and nutrient content 1 year after the start of the experiment (Table 4). This is likely because no substantial ectomycorrhizal colonization by ectomycorrhizal fungi occurs before the end of the dry season (i.e. 8 months after planting, data not shown). However, oak seedlings from AU exhibited the highest phosphorous foliar concentration (Table S1). Interestingly, we detected no differences in edaphic factors that could explain these results (Table S1). These data raise the possibility that oak seedlings may benefit from ectomycorrhizal colonization in AU. At the end of this study, the net effect of enhanced mycorrhization in AU on Q. ilex seedlings is still unclear. In addition, the mycorrhizae in AU are likely to be part of mycelia that previously established on A. unedo roots and have already developed large extraradical mycelia; whether this represents a gain for the seedlings, connecting to a ‘prepaid’ network (Selosse et al., 2006), or a cost in the short term, as such fungi need more carbon, is unclear from our study. A positive effect may well explain that, after watering, seedlings in EA are heavier than these in AU (Fig. 2a). This indicates that an observable relationship does not necessarily translate into an immediate and easily quantifiable ecophysiological response at the individual level.

Interestingly, seedlings in AU had more fungi available to them than those from EA (35 vs. 16 RFLP types, respectively). This difference can be explained by the high potential for common mycelial networks with A. unedo (Richard et al., 2005) in AU, in contrast with EA, which was devoid of ectomycorrhizal fungi. Our data do not indicate whether ectomycorrhizal abundance and the richness of ectomycorrhizae in AU had an influence on seedling survival in the first year. However, higher taxonomical diversity may entail higher functional diversity in the ectomycorrhizal community (Courty et al., 2005) and thus better soil exploitation during the relatively wet and warm autumn. Further experiments, including a 2-year-long monitoring in AU, are needed to estimate the importance of the ectomycorrhizal fungal linkages between A. unedo mature shrubs and Q. ilex seedlings and to evaluate whether an increased ectomycorrhizal infection represents a nurse effect.

Despite strong differences in ectomycorrhizal colonization on oak seedlings, the fungal communities in both succession stages were surprisingly equally diverse (Figs 3d and 2d). As on adult Q. ilex (Richard et al., 2005), C. geophilum, a drought-tolerant fungus, was the most
abundant species, particularly in AU (Fig. 3c). To what extent it also protects seedlings from drought remains to be tested during the second year, after ectomycorrhizal colonization.

**Ectomycorrhizal communities across secondary succession**

Seedlings from both macchia types had many ectomycorrhizal species in common with the oak forest (Fig. 4), suggesting a high dispersal rate of spores or mycelium from the surrounding forested zones in this patchy vegetation. The dispersal agents probably included wind and animals, because mammal fecal pellets are important sources of ectomycorrhizal inoculum before establishment of ectomycorrhizal plants in primary succession (Pyare & Longland, 2000; Nara et al., 2003; Ashkannejhad & Horton, 2006). Alternatively, a dormant spore bank inherited from previous forest stages may also persist (Taylor & Bruns, 1999), for example for *C. geophilum* that forms resistant mycelial sclerotia. In contrast, the low level of ectomycorrhizal colonization in EA (Fig. 3) is probably explained by the lack of an ectomycorrhizal host and a patchy distribution of the ectomycorrhizal inoculum (Dickie & Reich, 2005).

Ectomycorrhizal tree species such as oaks often associate with ericoid-mycorrhizal fungi (Vrålstad, 2004) or Glomalean fungi (Egerton-Warburton & Allen, 2001), and some ericoid-mycorrhizal fungi may coexist in ericaceous and ectomycorrhizal plants (Bergero et al., 2000; Perotto et al., 2002). We did not investigate the presence of these fungi on *Q. ilex* seedlings at the end of the experiment. If present on roots during the first year, these fungi probably did not significantly contribute to oak performance, given the low survival rate observed in EA.

**Conclusion**

During the course of postdisturbance succession, *A. unedo* dominance is transient between pioneer stages, where most of the *Q. ilex* seedlings die during the first summer following germination due to summer drought, and oak-dominated stands, where acorns do not survive various postdispersal predators (Gomez, 2004). We show that during the first year, *A. unedo* facilitates (1) *Q. ilex* survival during the summer drought by creating buffered pedo-climatic conditions, and (2) later in the year, *Q. ilex* mycorrhization, likely by providing its own ectomycorrhizal fungi. Further experiments (e.g. artificial shading or reciprocal soil transfers) are required to investigate the temporal variations of the net effect of biotic interactions on the survival of *Q. ilex* seedlings along the succession.

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**References**


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Ectomycorrhizal fungal community in *Erica arborea* macchia (EA – white circles) and *Arbutus unedo* macchia (AU – black circles).

**Table S1.** Vegetation and soil physical and chemical characteristics in *E. arborea* macchia (EA) and *A. unedo* macchia (AU).

**Table S2.** ITS-RFLP types of ECM collected on the 148 sampled seedlings introduced in *E. arborea* macchia (EA) and in *A. unedo* macchia (AU) (Experiment 2).

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