

# Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*

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## Summary

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- We analysed the ectomycorrhizal (ECM) fungal diversity in a Mediterranean old-growth *Quercus ilex* forest stand from Corsica (France), where *Arbutus unedo* was the only other ECM host.
- On a 6400 m<sup>2</sup> stand, we investigated whether oak age and host species shaped below-ground ECM diversity. Ectomycorrhizas were collected under *Q. ilex* individuals of various ages (1 yr seedlings; 3–10 yr saplings; old trees) and *A. unedo*. They were typed by ITS–RFLP analysis and identified by match to RFLP patterns of fruitbodies, or by sequencing.
- A diversity of 140 taxa was found among 558 ectomycorrhizas, with many rare taxa. *Cenococcum geophilum* dominated (35% of ECMs), as well as Russulaceae, Cortinariaceae and Thelephoraceae. Fungal species richness was comparable above and below ground, but the two levels exhibited < 20% overlap in fungal species composition.
- *Quercus ilex* age did not strongly shape ECM diversity. The two ECM hosts, *A. unedo* and *Q. ilex*, tended to share few ECM species (< 15% of the ECM diversity). Implications for oak forest dynamics are discussed.

**Key words:** *Arbutus unedo*, ectomycorrhizas, ITS–RFLP, Mediterranean forests, molecular typing, old trees, *Quercus ilex*, seedlings.

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## Introduction

*Quercus ilex* L. (Holm oak) is a characteristic evergreen oak species in the Mediterranean basin (Quézel, 1985; Scarascia-Mugnozza *et al.*, 2000). Despite heavy anthropic pressure, old-growth forests still exist in the island of Corsica (Quézel & Médail, 2003). In such stands, overstorey oaks coexist with understorey chaparral shrubs such as *Arbutus unedo* L. (strawberry tree) and *Erica arborea* L. (tree heath). In general, oak trees do not exceed 200 yr of age (Panaiotis *et al.*, 1997). In old-growth forests, mortality leads to tree falls that create numerous small-scale canopy gaps.

A wide variety of ectomycorrhizal (ECM) fungi are symbionts of many tree species in temperate climatic zones. More than 5000 species from the Ascomycetes and Basidiomycetes

form ectomycorrhizas on secondary tree roots (Trappe, 1962; Smith & Read, 1997). The majority of ECM species have large host spectra (Molina *et al.*, 1992). This allows a diffuse interaction, i.e. the sharing of common fungal associates by plant individuals of identical or different species. Ectomycorrhizas are critical for nutrition of both partners, and plant protection against soil parasites and toxic compounds. The mycorrhizal network can also reduce carbon costs of ectomycorrhiza formation for some plants, as the extraradical mycelium is already established and sustained by other plants (Högberg *et al.*, 1999).

The fungi that form ectomycorrhizas with trees also form arbutoid mycorrhizas on the roots of ericaceous plants from the *Arbutoidea* suborder (e.g. *Arctostaphylos* and *Arbutus* spp.; Molina & Trappe, 1982). In addition to the fungal sheath and

hyphal intercellular growth (Hartig net) that are typical of ECM, hyphae penetrate the cell wall and produce intracellular coils in living cells (Smith & Read, 1997). These fungi may mediate interactions between arbutoid plants and ECM trees. For instance, in Californian chaparral *Arctostaphylos glandulosa* Eastw. may allow the establishment of *Pseudotsuga menziesii* (Mirb.) Franco seedlings (Horton *et al.*, 1999) by acting as a symbiont reservoir that may contribute to successional transition to forest stages. In the Mediterranean basin *Q. ilex* naturally establishes in *A. unedo*-dominated chaparral (Gamisans, 1999). However, sharing of fungal symbionts between *Q. ilex* and *A. unedo* has hitherto not been explored. To our knowledge, studies of *A. unedo* symbionts have mainly focused on mycorrhizal ultrastructure (Fusconi & Bonfante-Fasolo, 1984; Giovannetti & Lioi, 1990; Münzenberger *et al.*, 1992).

Studies of ECM communities are based either on identification of mycorrhizas (the so-called below-ground view), or on monitoring of fruitbody production (above-ground view). Identification of mycorrhizas can be conducted according to root-tip morphotype or using molecular tools, such as restriction fragment length polymorphism (RFLP) or sequencing of the internal transcribed spacer (ITS) region, an efficient way to dissect ECM communities (Gardes & Bruns, 1996; Horton & Bruns, 2001; Tedersoo *et al.*, 2003). Fruitbody surveys reveal the presence of ECM taxa in a fast and inexpensive way (Vogt *et al.*, 1992; Richard *et al.*, 2004). However these studies, mainly carried out on fleshy macromycetes, often underestimate the presence of numerous resupinate taxa (e.g. Thelephoraceae or Sebacinaceae), hypogeous fungi, and taxa lacking an apparent sexual stage (e.g. *Cenococcum geophilum* Fr.) (Horton & Bruns, 2001).

Little is known about the below-ground community of ECM fungi in broadleaved forests. For instance, most descriptions of ECM communities in *Q. ilex* forests have been based on fruitbody surveys (Signorello, 1996; Laganà *et al.*, 1999; Richard *et al.*, 2004). The problems with the use of fruitbody sampling are obvious to anyone who has collected fungi for many years. First, fruiting may vary tremendously from year to year. Second, sampling must be intensive because fruit bodies of many species are short-lived. Thus, in addition to the analysis of fruitbody patterns, there is a need to explore the ECM community in the soil from either ectomycorrhizas or mycelia. A study conducted recently by De Román & De Miguel (2002) has revealed the presence of numerous species of Thelephoraceae in managed *Q. ilex* stands. However, further research using molecular tools is necessary to document the below-ground diversity in *Q. ilex* forests.

In a previous study (Richard *et al.*, 2004), we analysed the temporal and spatial patterns of fruitbody production in an old Holm oak forest in Corsica during three consecutive fruiting seasons. Fleshy epigeous macromycetes were surveyed in a permanent plot (160 × 40 m) from September 1999 to March 2002. Here we sampled ectomycorrhizas from *Q. ilex* and arbutoid mycorrhizas from *A. unedo* shrubs at the same

research site in March 2001. On *Q. ilex* we collected ectomycorrhizas from seedlings, young saplings and old trees. Our objectives were to: (i) document the below-ground ECM richness in an old-growth Mediterranean forest; (ii) investigate two factors potentially shaping this richness, i.e. host age and host species; and (iii) relate the structure of the ECM community, as determined by mycorrhizas, to that obtained from fruitbody surveys. To identify the fungal symbionts on roots, we compared RFLP types from mycorrhizas to those from fruitbodies of known species collected from the same site. Dominant fungal associates of *A. unedo* were also sequenced to investigate in more detail the composition of the below-ground community. We relied on this typically Mediterranean plant species to ascertain the relative importance of fungal groups not sampled during our fruitbody survey, such as resupinate or hypogeous fungi.

## Materials and Methods

### Study site

The research transect was the same as that studied in our previous paper (Richard *et al.*, 2004). It was located in the Fango valley (42°20' N; 8°49' E) in Corsica, on slightly acidic soils. The vegetation at the research site is an old *Q. ilex* forest that consists mainly of old *Q. ilex* trees ( $\approx 460$  stems ha<sup>-1</sup>), 2- to 10-yr-old saplings, and 1-yr-old seedlings (Panaiotis *et al.*, 1997). A dense 7 m high oligospecific chaparral develops under oak canopies, made up of *Phillyrea latifolia* L., *Erica arborea* and *A. unedo* (Panaiotis *et al.*, 1997). Two small individuals of *Cistus monspelliensis* L. were also present at this site. A detailed description of the research site is provided by Richard *et al.* (2004).

### Sampling of mycorrhizas

To investigate the structure of ECM diversity, we sampled four plant categories: old *A. unedo* shrubs (*A.un.*) and three *Q. ilex* categories representing the age sequence: 1-yr-old seedlings (*Q.il.1*); 2- to 10-yr-old saplings (*Q.il.2*); and 170-yr-old senescent trees (*Q.il.3*) (Table 1). In each category, 30 individuals scattered over the whole study site (from 30 different 100 m<sup>2</sup> plots) were sampled (Fig. 1). Plant age was determined either by counting shoot ring number (for *Q.il.1* and *Q.il.2*) or using data from Panaiotis *et al.* (1997) (for *Q.il.3*). Host species was ascertained by tracking roots to the shoot (for *Q.il.1* and *Q.il.2*) or using cambium colour and root architecture which differ between the two host species (for *A.un.* and *Q.il.3*).

Seedlings (*Q.il.1*) and saplings (*Q.il.2*) were carefully removed from the soil in order to keep the root system intact and avoid fine root disruption. ECM fungal diversity was evaluated in these two plant categories by exhaustive hand-picking of ECM after pulling up. Soil cores were collected for old *A. unedo* shrubs (*A.un.*) and *Q. ilex* senescent trees (*Q.il.3*): for each individual, four 10 × 10 cm soil samples

**Table 1** Sampling design and colonization by the dominant *Cenococcum geophilum* for the four host categories investigated

| Sampling features                     | <i>Quercus ilex</i> |               |               | <i>Arbutus unedo</i><br>Old shrubs |
|---------------------------------------|---------------------|---------------|---------------|------------------------------------|
|                                       | Seedlings           | Saplings      | Old trees     |                                    |
| Code name                             | <i>Q.il.1</i>       | <i>Q.il.2</i> | <i>Q.il.3</i> | <i>A.un.</i>                       |
| Number of plants                      | 30                  | 30            | 30            | 30                                 |
| Number of mycorrhizas                 | 190                 | 304           | 900           | 900                                |
| Number of mycorrhizas with:           |                     |               |               |                                    |
| <i>C. geophilum</i> morphotype        | 107 (56.3%)         | 229 (75.3%)   | 221 (24.5%)   | 261 (29.0%)                        |
| Other morphotypes                     | 83                  | 75            | 679           | 639                                |
| Colonization by <i>C. geophilum</i> : |                     |               |               |                                    |
| Relative abundance per plant (%)*     | 64.3 ± 31.0 a       | 73.5 ± 30.4 a | 29.2 ± 24.6 b | 29.1 ± 14.3 b                      |
| Relative frequency among plants (%)   | 100                 | 90            | 100           | 100                                |

\*Mean number ± SD, values followed by different letters differ significantly according to ANOVA ( $P < 0.05$ ).

were taken at the four cardinal points, from the humus organic horizon to a depth of 20 cm. Roots were sieved from the soil cores, carefully washed, and samples from a given plant were pooled. For each individual plant, five root samples were randomly selected, and 30 ECMs from 30 distinct aggregates. They were then hand-picked and stored at  $-20^{\circ}\text{C}$  in 700  $\mu\text{l}$  CTAB lysis buffer (2% cetyltrimethylammoniumbromide, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl). Picking was performed to maximize the number of morphotypes recovered on each plant, in order to obtain the most complete description of the community.

*Cenococcum geophilum* mycorrhizas were considered characteristic enough to be identified by morphology (Agerer, 1987–93). However, in order to ascertain this identification, two randomly selected *C. geophilum* mycorrhizas from five different individuals per plant category were screened by RFLP analysis, and three were sequenced. All other ECM morphotypes were submitted to molecular analysis according to the following sampling design: (i) exhaustive typing for *Q.il.1* and *Q.il.2* because of the low number of non-*C. geophilum* ECM recovered; (ii) five mycorrhizas per plant for *A.un.*; (iii) seven ECM per plant for *Q.il.3*.

### Polymerase chain reaction

DNA was extracted as described by Gardes & Bruns (1993). Extracted DNA was resuspended in 30  $\mu\text{l}$  TE buffer (1 mM Tris-HCl, 0.1 M EDTA pH 8.0) and diluted for PCR with sterile distilled water at a ratio of 1 : 300 (v/v). The internal transcribed spacer (ITS) region of the rDNA was amplified by PCR using the primer pair ITS1-F/ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) in a PTC 200 DNA thermocycler (MJ Research, Inc., Waltham, MA, USA). A fraction of 12.5  $\mu\text{l}$  of the diluted DNA extract was added to 12.5  $\mu\text{l}$  of the PCR mix (final concentrations: 2 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 1 mM of each primer, 1 $\times$  buffer for *Taq* DNA polymerase, 1 U *Taq* DNA polymerase) and amplified using the same temperature profile as Gardes & Bruns (1996). Negative controls without

DNA were used to detect DNA contaminations of the reagents in every PCR.

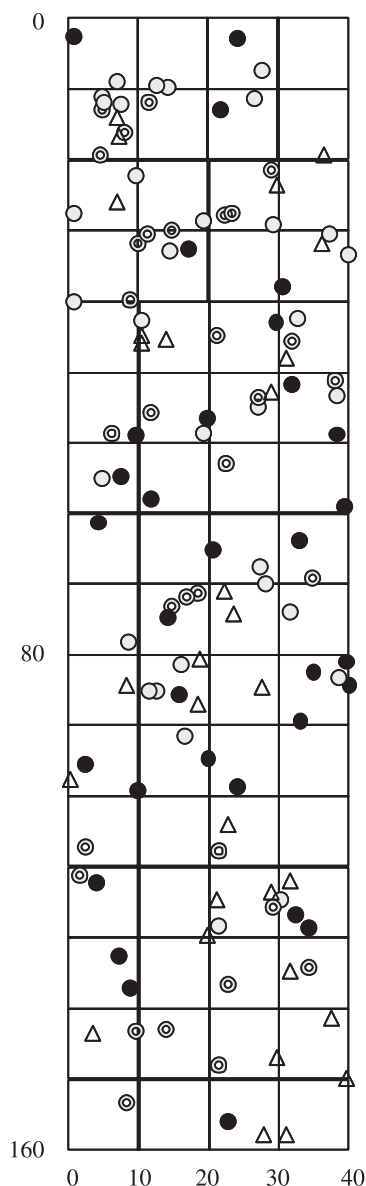
### RFLP analysis

Aliquots of 8  $\mu\text{l}$  of each amplified DNA were digested using the endonucleases *Cfo*I, *Hinf*I, *Mbo*I and *Hae*III. PCR products were size-fractionated on 3% agarose gels (38% agarose + 62% NU-sieve agarose (FMC BioProducts, Philadelphia, PA, USA), stained with ethidium bromide, and photographed under UV light.  $\Phi$ X174 digested by *Hinf*I was used to estimate fragment sizes. Gels were scanned using BioCapt 97.03 (Vilber Lourmat, France) and fragment sizes calculated using Bio1D++97.06 (Vilber Lourmat).

### Molecular identification of mycorrhizas

To allow identification of mycorrhizas, small pieces of fresh tissue were taken from at least one fruitbody for each of the 166 ECM taxa fruiting on the study site between September 1999 and January 2002 (Richard *et al.*, 2004). For 66% of the RFLP-typed species only one fruitbody was used; for 48 species (29%) two fruitbodies were used. For the eight remaining taxa, which belong to abundantly fruiting taxa poorly investigated from a taxonomic point of view, more than two fruitbodies were used as follows ( $n$  = number of fruitbodies tested): *Cortinarius elatior* Fr. ( $n$  = 4) and *Cortinarius pseudosalor* J. E. Lange ( $n$  = 3), *Inocybe tigrina* R. Heim ( $n$  = 4) and *Inocybe flocculosa* (Berk.) Sacc. ( $n$  = 3), *Leccinum lepidum* (Bouchet ex Essette) Quadr. ( $n$  = 4), *Russula decipiens* (Singer) Svrcek ( $n$  = 4), *Russula fragilis* (Pers. Fr.) Fr. ( $n$  = 3) and *Russula persicina* var. *rubrata* Romagn. ( $n$  = 4). DNA extraction, PCR amplification and RFLP digestion were carried out as for mycorrhizas. RFLP patterns from mycorrhizas and from fruitbodies were matched, and ECMs whose patterns did not correspond to any fruitbody were considered as unidentified taxa.

The most abundant taxa were sequenced to specify their taxonomic position: sequencing was restricted to *A. unedo*, a



**Fig. 1** Map of the transect studied with positions of the 120 plants sampled. Scale in metres. Triangles, old strawberry trees (*Arbutus unedo*); black circles, old Holm oaks (*Quercus ilex*); double circles, Holm oak saplings; grey circles, Holm oak seedlings.

typically Mediterranean species. Based on RFLP results, *A. unedo* symbionts that were represented by at least two mycorrhizas in our sampling were sequenced as described by Selosse *et al.* (2002), and identified according to BLAST analysis at the NCBI page <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>, using default settings. Sequences were deposited in GenBank (Table 2).

#### Data processing and statistical analyses

For each plant category, and for the two plant species, the richness of ECM communities was estimated using various

species diversity estimators based on abundance and frequency of taxa. Abundance was defined as the cumulative number of mycorrhizas of a given taxon divided by the total number of mycorrhizas, for a given plant category. Frequency was the number of plant individuals on which a given taxon was found divided by the total number of plant individuals, for a given plant category. Species diversity was estimated using: (i) richness, i.e. the total number of taxa,  $S$ ; (ii) Simpson's diversity index,  $D$ ; (iii) the Shannon–Wiener information index,  $H'$ ; and (iv) Fisher's alpha (Fisher *et al.*, 1943). The rarefaction method (Krebs, 1999) was used to compare one-to-one taxonomic richness of samples of various sizes. This method corrects for differences in sampling size by virtually reducing the size of all samples to that of the smallest one. Calculations were performed with the software BIODIVERSITY PRO 2 (<http://www.sams.ac.uk/activities/downloads/software/bdpro.zip>) and rarefaction curves obtained were compared graphically for the minimal sample size of the various data sets. Rarefaction analysis was used for comparison between the various plant categories (with minimal sample size  $n = 121$  ECM tips) and for comparison between above- and below-ground diversity (with minimal sample size  $n = 521$  ECM tips or fruitbodies). A visual comparison of the distributions of relative species abundance and frequency between above- and below-ground views of the ECM community was performed using rank–abundance curves.

Differences in abundance of *C. geophilum* (the only species that was abundant and frequent enough to perform parametric tests), according to plant category, were tested by one-way ANOVA with plant category as single factor using MINITAB 12.2 software (Minitab Inc., Paris, France).

Relatedness in the composition of ECM taxa among plant species and categories was compared using the Jaccard similarity index (Mueller-Dombois & Ellenberg, 1974),  $J = c/(a + b - c) \times 100$ , where  $a$  is the number of taxa found on the first plant category,  $b$  is the number of taxa found on the other, and  $c$  is the number of taxa shared by the two plant categories. A percentage similarity (PS) based on RFLP-type abundance was calculated in order to take distribution of taxa into account (Pielou, 1984):  $PS = c'/(a' + b') \times 100$ , where  $a'$  is the number of mycorrhizas formed by taxa found on the first plant category,  $b'$  is the number of mycorrhizas formed by taxa found on the other, and  $c'$  is the number of mycorrhizas formed by taxa colonizing the two plant categories.

## Results

### General description of the below-ground ECM community

In all, 2294 mycorrhizas were sampled from 120 plants (Table 1), among which a *Cenococcum* morphotype largely dominated. DNA extraction and RFLP typing were carried out on 558 ECMs, including 40 ascribed to this *Cenococcum*

**Table 2** Identification by ITS sequence of the most abundant RFLP types on *Arbutus unedo*

| Abundance rank on <i>A. unedo</i> roots* | Tentative identification† | GenBank accession number | Closest GenBank species  | BLAST expected value‡ |
|--|---------------------------|--------------------------|--|-----------------------|
| 1 (1)                                    | <i>C. geophilum</i>       | AY825508                 | AY394919 <i>Cenococcum geophilum</i>                               | 0.0                   |
| 2 (4)                                    | Thelephoraceae #5         | AY825526                 | AJ534912 <i>Tomentella</i> sp.                                     | 0.0                   |
| 3 (6)                                    | <i>Inocybe tigrina</i> †  | AY825515                 | AY310829 Uncultured ECM<br>AY751556 <i>Inocybe</i> sp.             | 1 e-124<br>1 e-123    |
| 4 (14)                                   | Thelephoraceae #3         | AY825524                 | AF272915 <i>Tomentella cinerascens</i>                             | 0.0                   |
| 5 (17)                                   | Sebacinaceae #1           | AY825518                 | AJ534907 Sebacinaceae sp.  | 0.0                   |
| 6 (18)                                   | Sebacinaceae #3           | AY825520                 | AF440646 <i>Sebacina</i> endomycorrhiza                            | 0.0                   |
| 7 (5)                                    | Sebacinaceae #4           | AY825521                 | AY243531 Uncultured mycorrhiza (sebacinoid)                        | 0.0                   |
| 8 (20)                                   | <i>Laccaria laccata</i> † | AY825516                 | AJ534898 <i>Laccaria</i> sp.                                       | 0.0                   |
| 9 (30)                                   | <i>Inocybe</i> #1         | AY825514                 | AY634142 Uncultured ECM ( <i>Inocybe</i> )                         | 1 e-100               |
| 10 (41)                                  | Thelephoraceae #1         | AY825522                 | U83467 Thelephoraceae sp.  | 1 e-174               |
| 11 (68)                                  | Thelephoraceae #4         | AY825525                 | U92537 <i>Tomentella</i> sp.                                       | 0.0                   |
| 12 (40)                                  | Sebacinaceae #2           | AY825519                 | AF440647 <i>Sebacina</i> endomycorrhiza                            | 0.0                   |
| 13 (15)                                  | <i>Russula nuragica</i> † | AY825517                 | AJ534906 <i>Russula</i> sp.  | 0.0                   |
| 14 (27)                                  | Clavulinaceae #1          | AY825509                 | AY534200 Uncultured ECM (Clavulinaceae)                            | 0.0                   |
| 15 (47)                                  | Thelephoraceae #7         | AY825528                 | AF272904 <i>Tomentella atramentaria</i>                            | 0.0                   |
| 16 (69)                                  | Thelephoraceae #2         | AY825523                 | AF430289 <i>Tomentella</i> sp.                                     | ECM 0.0               |
| 17 (66)                                  | Thelephoraceae #6         | AY825527                 | AF465184 Uncultured ECM (Thelephoraceae)                           | 0.0                   |
| 18 (16)                                  | <i>Cortinarius</i> #1     | AY825511                 | AB096872 ECM of <i>Salix reinii</i><br>AJ534923 <i>Inocybe</i> sp. | 6 e-90<br>3 e-88      |

The 18 RFLP types occurring more than once in the sampling were sequenced. Closest sequences from identified species, as found by BLAST analysis, are indicated.

\*Values within parentheses indicate the rank of RFLP types based on their relative abundance in the whole ECM community.

†*Inocybe tigrina*, *Laccaria laccata* and *Russula nuragica* were identified from perfect matches between RFLP patterns of mycorrhizas and fruitbodies.

‡BLAST expected value represents the number of sequence matches expected by random chance (the smaller the value, the better the match to the reported NCBI database sequence).

**Table 3** Ectomycorrhizal fungal diversity on the different host plant categories

| Parameter   | Below-ground  |               |               |                     |                      |            | Above-ground<br>All plants |
|---|---------------|---------------|---------------|---------------------|----------------------|------------|----------------------------|
|   | <i>Q.il.1</i> | <i>Q.il.2</i> | <i>Q.il.3</i> | <i>Quercus ilex</i> | <i>Arbutus unedo</i> | All plants |                            |
| Number of taxa*   | 37 (13)       | 25 (11)       | 76 (27)       | 112 (38)            | 46 (14)              | 140 (46)   | 166 (46)                   |
| Estimated number of taxa based on rarefaction analysis† | 34            | 20            | 54            | –                   | 44                   | –          | –                          |
| Simpson's diversity index                               | 0.579         | 0.244         | 0.852         | 0.613               | 0.815                | 0.657      | 0.953                      |
| Shannon–Wiener information index                        | 3.68          | 1.70          | 4.54          | 3.31                | 3.94                 | 3.69       | 5.48                       |
| Fisher's alpha  | 14.8          | 6.7           | 35.8          | 37.7                | 22.9                 | 47.8       | 36.5                       |

Above-ground diversity (based on fruitbody surveys) from Richard *et al.* (2004).

\*Taxa are RFLP types for below-ground diversity and species based on a morphological concept for above-ground diversity. Values in parentheses indicate number of taxa for which RFLP patterns from mycorrhizas and fruitbodies from the same study site successfully matched.

†Rarefaction analysis conducted on *Q.il.1*, *Q.il.2*, *Q.il.3* and *A. un.* (minimal sample size,  $n = 121$  root tips).

morphotype (Table 1). The 40 *Cenococcum* ECMs showed the same RFLP pattern, and three of them showed a unique *C. geophilum* ITS sequence (Table 2). The *Cenococcum* morphotype was therefore considered as homogeneous, accounting for 35.3% of the total ECM number and present on 97.5% of all investigated plant individuals (Table 1).

In all, including *C. geophilum*, ITS–RFLP analysis was conducted on 393 ectomycorrhizas (data not shown). The typing

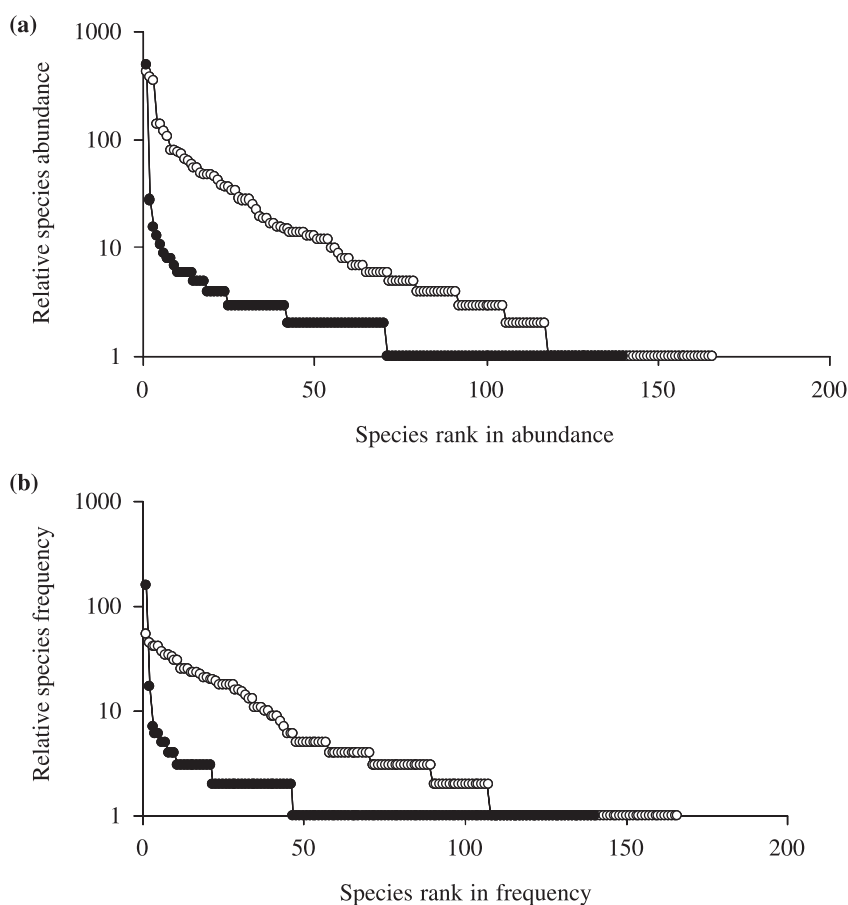
produced 140 different RFLP patterns (Table S1, available online as supplementary material), and resulted in high values of species richness estimators (Table 3). Identification of the mycorrhizal symbionts to species or species group was performed using ITS–RFLP matches with fruitbodies and direct sequencing of the ITS for the most common symbionts of *A. unedo* (Tables 2 and S1). No intraspecific polymorphism was observed whenever more than one fruitbody per species

**Table 4** Abundance of main fungal groups (excluding *Cenococcum geophilum*) on three *Quercus ilex* plant categories based on matching with fruitbody RFLP patterns

| Species/family     | Q.il.1      |           | Q.il.2      |           | Q.il.3      |           |
|--------------------|-------------|-----------|-------------|-----------|-------------|-----------|
|                    | Mycorrhizas | RFLP      | Mycorrhizas | RFLP      | Mycorrhizas | RFLP      |
| <i>Russula</i>     | 22 (13)     | 16.7 (6)  | 24.4 (10)   | 12.5 (3)  | 27.1 (45)   | 12 (9)    |
| <i>Cortinarius</i> | 5.1 (3)     | 8.3 (3)   | 0           | 0         | 7.2 (12)    | 8 (6)     |
| <i>Inocybe</i>     | 1.7 (1)     | 2.8 (1)   | 2.4 (1)     | 4.2 (1)   | 4.8 (8)     | 6.7 (5)   |
| <i>Amanita</i>     | 0           | 0         | 9.8 (4)     | 12.5 (3)  | 1.8 (3)     | 2.7 (2)   |
| Others*            | 3.4 (2)     | 5.6 (2)   | 12.2 (5)    | 16.7 (4)  | 11.4 (19)   | 12 (9)    |
| Unidentified       | 67.8 (40)   | 66.7 (24) | 51.2 (21)   | 54.2 (13) | 47.6 (79)   | 58.6 (44) |

Values are either percentage of total number of typed mycorrhizas (number of mycorrhizas in parentheses), or percentage of total number of RFLP types (number of RFLP types in parentheses).

\**Tricholoma*, *Laccaria*, *Hygrophorus*, *Lactarius*, *Inocybe*, *Hebeloma* and *Clavulinaceae*.



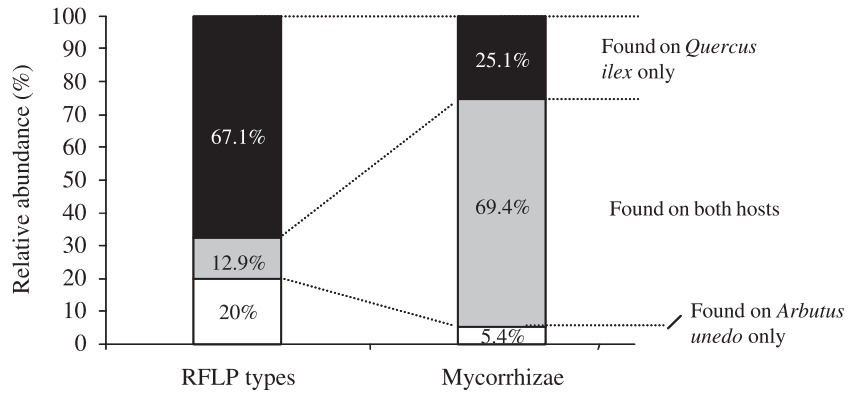
**Fig. 2** Dominance–diversity curves for above-ground (fruitbodies, open circles) and below-ground (ectomycorrhizas, filled circles) ECM communities based on either (a) relative abundance or (b) relative frequency of taxa. Left to right, most frequent to less frequent species. Abundance data are numbers of fruitbodies (above-ground) or mycorrhizas (below-ground) per species. Frequency data are numbers of 100 m<sup>2</sup> plots in which a species fruited (above ground) and number of plant individuals on which an RFLP type was found (below ground). Above-ground data (based on fruitbody surveys) are from Richard *et al.* (2004).

was examined, except for *R. fragilis* and *R. persicinavar. rubrata* which produced two different patterns (data not shown). Out of 140 RFLP types, 60 (42.9%) were identified at the genus level (Table S1).

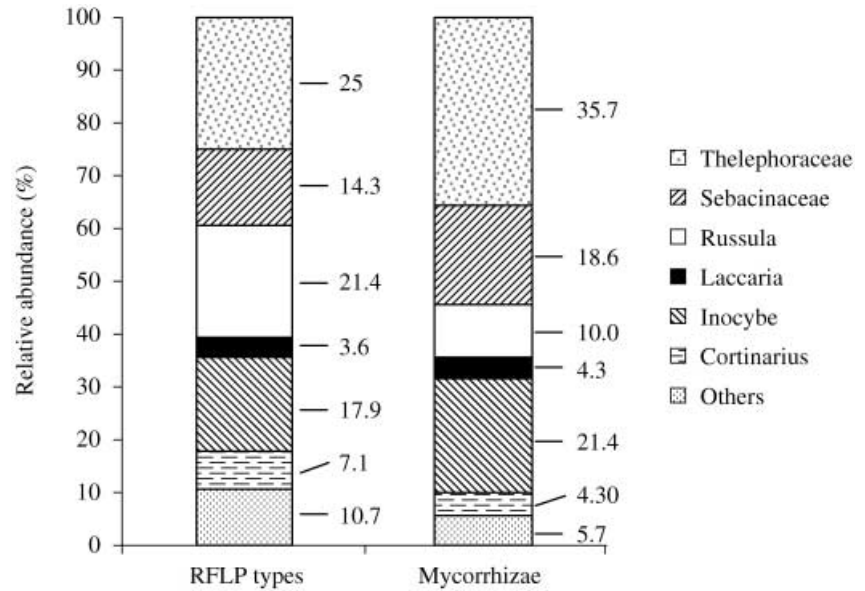
Of the 140 RFLP types, 70 were represented by a single mycorrhiza (Fig. 2a), so that the community harbored a large number of rare taxa. *Cenococcum geophilum* and *R. decipiens* were

frequent species, found on 117 and 17 plants, respectively (Fig. 2b). They also contributed to the overall number of mycorrhizas (839 and 28 for *C. geophilum* and *R. decipiens*, respectively; Table 3 and see Fig. 6a). Apart from these two mycorrhizas, only three taxa were represented by 10 or more mycorrhizas: *Russula acrifolia* Romagn. (16 mycorrhizas from four plants); a theleporoid species (13 mycorrhizas

**Fig. 3** Distribution of ectomycorrhizal (ECM) fungal diversity between *Quercus ilex* and *Arbutus unedo*, including *Cenococcum geophilum*, expressed as percentage of total number of RFLP types or as percentage of total number of sampled ECM.



**Fig. 4** Abundance of the main fungal groups (at genus level except for Thelephoraceae and Sebacinaceae) on *Arbutus unedo*, expressed as percentage of total number of RFLP types or as percentage of total number of sampled ectomycorrhizas (ECM). The dominant species *Cenococcum geophilum* was omitted for clarity.



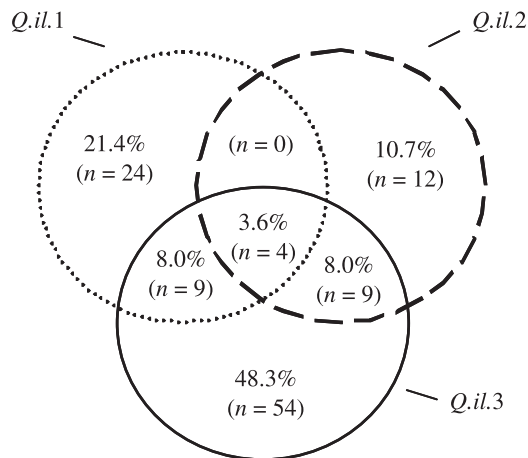
from seven plants); and a sebacinoid species (11 mycorrhizas from three plants; data not shown).

#### Distribution of the ECM community among the two host species

In all, 46 RFLP types were found on *A. unedo* and 112 on *Q. ilex* (Table 3). In a rarefaction analysis performed to account for differences in sample size (Table 3), the diversity for *A. unedo* was intermediate between that of senescent oaks and those of seedlings and saplings. Similarly, Shannon entropy, Simpson diversity index and Fisher's alpha were higher for *A. unedo* than for young *Q. ilex* (*Q.il.1* and *Q.il.2*), but lower than for old *Q. ilex* trees (*Q.il.3*, Table 3), suggesting that *Q.il.3* harbored more rare species than the three others. Only 18 of the 140 RFLP types occurred both on *A. unedo* and *Q. ilex* roots (Fig. 3). These two-host taxa represented 12.9% of the taxonomic diversity, but colonized 69.4% of all mycorrhizas sampled (Fig. 3) because of the abundance of *C. geophilum* on both hosts. Relative taxa frequencies had similar distributions

on the two hosts, with 50.9 and 60.9% of the RFLP types found only once on *Q. ilex* and *A. unedo* roots, respectively (data not shown). At the other extreme, only three RFLP types were found on at least four plant individuals of the same species (data not shown). Because of the low number of mycorrhizas representing each RFLP type (probably caused by the size of our sample), differences in abundance between hosts could not be tested statistically.

On *Q. ilex* roots, 48 RFLP types (42.9%) were identified to genus, species or family level, based on RFLP matches with fruitbodies (Table 4) or sequence analysis of taxa that were shared with *A. unedo* (Table 2). The genus *Russula* was the most represented (Table 4), accounting for 22–27.1% of the identified ECM and between 12 and 16.7% of the corresponding taxonomic diversity, depending on plant age. At the species level, apart from *C. geophilum*, *R. decipiens* (a species linked to the genus *Quercus*) was the most abundant (10.5% of the total number of *Q. ilex* mycorrhizas) and most frequent species (present on 28.3% of sampled *Q. ilex*, data not shown).



**Fig. 5** Distribution of ectomycorrhizal fungal diversity according to the three *Quercus ilex* categories: seedlings (*Q.il.1*); saplings (*Q.il.2*); old trees (*Q.il.3*). Values are percentage of total number of RFLP types ( $n$  = number of RFLP types).

On *A. unedo* roots the molecular analysis allowed identification of 28 taxa, accounting for 81.4% of the typed mycorrhizas and 60.9% of the species richness (data not shown). Thelephoraceae was the most represented, accounting for 25% of taxonomic diversity and 35.7% of the identified ECMs (Fig. 4). To a lesser extent, the genus *Inocybe* (21.4% of the ECMs) and the Sebacinaceae family (18.6% of the ECMs) were well represented (Fig. 4). The genera *Russula*, *Cortinarius* and *Laccaria*, as well as the Clavulinaceae family, were also present (Table 2). At the species level, the ECM community on *A. unedo* was strongly dominated by *C. geophilum*, present on all 30 sampled plants (Table 1). In addition to *C. geophilum*, only three species, two Thelephoraceae species and *I. tigrina*, were present on at least three plant individuals (data not shown).

#### ECM community and *Quercus ilex* age

The three *Q. ilex* categories were dominated by *C. geophilum* ECM (Table 1). In addition to *C. geophilum*, variable numbers of RFLP types were found (Table 3), and rarefaction analysis suggested that the ECM richness was higher under senescent trees than for *Q.il.1* and *Q.il.2* (Table 3). For each age, *R. decipiens* was among the most frequently recorded, and the only species found on more than three plant individuals (data not shown). Most RFLP types (90 out of 112) were found on one *Q. ilex* age only, while 19.6% were shared by at least two categories (Fig. 5). Only *C. geophilum*, *R. decipiens*, and two unidentified species were found on the three plant categories (Fig. 5). *Cenococcum geophilum* was significantly more abundant on *Q.il.1* and *Q.il.2* than on *Q.il.3* (Table 1). Based on taxonomic diversity, *Q. ilex* of various ages shared few fungal partners (between 6.9 and 14.8%; Table 5). However, based on RFLP type abundances, similarities ranged from

**Table 5** Comparison of ectomycorrhizal fungal communities between plant species and plant categories based on presence/absence of taxa, using a Jaccard similarity index ( $J$ ) or quantitative comparisons (abundance of taxa, considering the dominant *Cenococcum geophilum* or not), using a percentage of similarity (PS)

| Category                        | Similarity estimator (%) |                      |                      |
|---------------------------------|--------------------------|----------------------|----------------------|
|                                 | $J$                      | PS (+ <i>C.g.</i> )* | PS (– <i>C.g.</i> )† |
| <i>Q.il.1</i> and <i>Q.il.2</i> | 6.9                      | 81.7                 | 20.0                 |
| <i>Q.il.1</i> and <i>Q.il.3</i> | 13.0                     | 63.9                 | 31.1                 |
| <i>Q.il.2</i> and <i>Q.il.3</i> | 14.8                     | 71.9                 | 17.9                 |
| <i>Q.il.1</i> and <i>A.un.</i>  | 10.7                     | 59.6                 | 21.2                 |
| <i>Q.il.2</i> and <i>A.un.</i>  | 4.4                      | 71.1                 | 5.5                  |
| <i>Q.il.3</i> and <i>A.un.</i>  | 13.0                     | 53.8                 | 28.9                 |

\*Including *Cenococcum geophilum*.

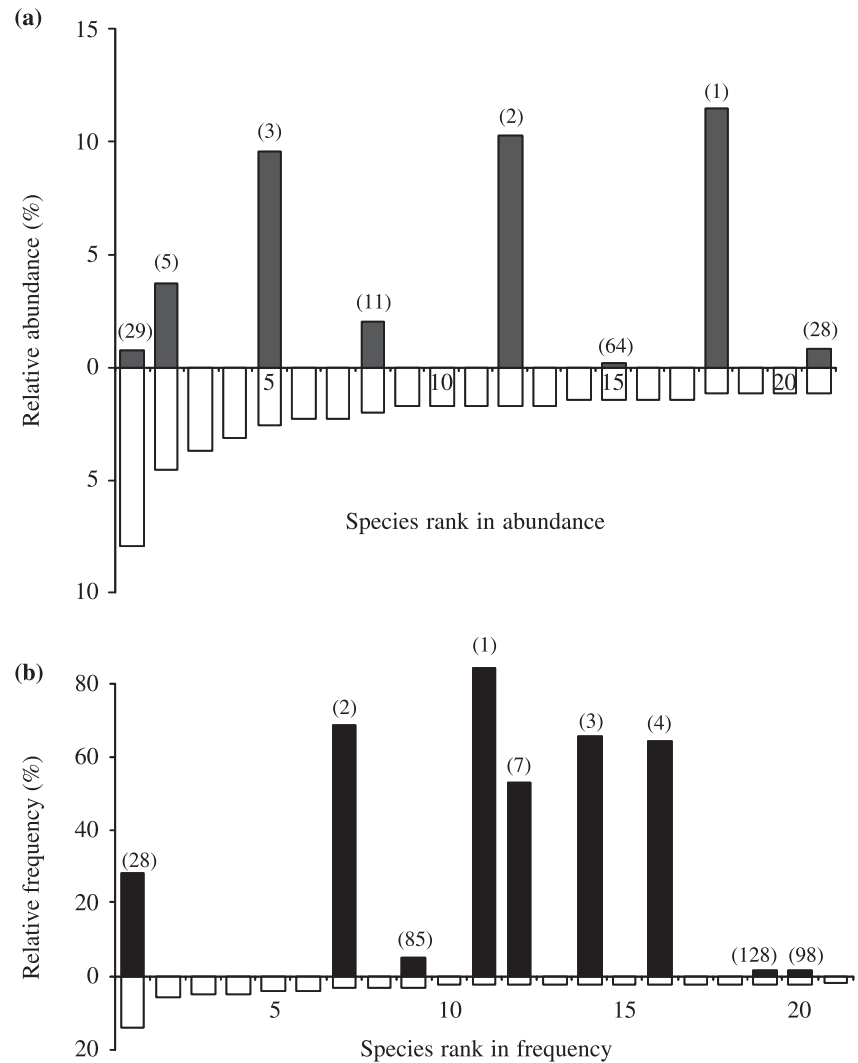
†Without *Cenococcum geophilum*.

63.9 to 81.7% (or at least 17.9% excluding *C. geophilum*), with successive age categories being more similar than the distal ones, *Q.il.1* and *Q.il.3* (Table 5).

#### Comparison between above- and below-ground views

In all, 260 ECM taxa were revealed on the studied transect by combining the present ECM typing and the 166 species from fruitbody identifications by Richard *et al.* (2004) (Table 3). Rarefaction analysis (data not shown) suggested that, for the minimal samples size  $n = 521$ , below-ground richness was higher (127.3 taxa) than above-ground diversity (92.4 taxa). Only 17.7% (46 species) of these taxa were found in both analyses, while 36.2% (94 RFLP types) were found only below-ground (Table 3). In addition, nonfruiting species do exist on this site, as exemplified by *C. geophilum*. Reciprocally, 46.1% of the total taxonomic diversity (120 species) that fruited was not found on roots (Table 3).

Dominance–diversity curves showed very similar distributions of above- and below-ground diversities. The ECM community was strongly dominated by rare species (Fig. 2a): more than 60% of the taxa produced less than one fruitbody per 1000 m<sup>2</sup>, while 50% of the taxa were represented by only one mycorrhiza. Similarly, both distributions showed a few very abundant species (Fig. 2a): three dominant species (*Laccaria laccata*, *I. tigrina* and *Lactarius chrysorrheus*) produced 32.9% of all fruitbodies (Richard *et al.*, 2004), while *C. geophilum* accounted for more than a third of all mycorrhizas (Table 1). An ordination based on either relative abundance (Fig. 6a) or frequency (Fig. 6b) of RFLP types was used to visualize correspondences between above- and below-ground views. Of the 22 most abundant ECM RFLP types (including *C. geophilum*), only eight produced epigeous conspicuous fruitbodies, including the three most productive ones, *L. laccata*, *I. tigrina* and *L. chrysorrheus* (Fig. 6a). Using mycorrhizas and fruitbody frequencies, a similar relative discrepancy was observed,



**Fig. 6** Most abundant and most frequent ectomycorrhizal taxa, excluding the highly abundant *Cenococcum geophilum* for clarity. Numbers in brackets are species abundance ranks in the above-ground fruitbody community (from Richard *et al.*, 2004). (a) The 21 most abundant RFLP types: values are relative abundances of taxa using either number of fruitbodies (above, filled bars, from Richard *et al.*, 2004) or number of mycorrhizas (below, open bars). (b) The 21 most frequent RFLP types: values are relative frequencies of taxa using either number of 100 m<sup>2</sup> plots where fruitbodies occurred (above, filled bars, from Richard *et al.*, 2004) or number of plants on which mycorrhizas occurred (below, open bars).

with only nine taxa found above ground (including the four most frequent) belonging to the 22 most represented ECM RFLP types (Fig. 6b). A single fruiting species, *R. decipiens*, was present among the seven most frequent RFLP types found in this study (Fig. 6b). This taxon was not abundant (ranking 29th) and relatively infrequent (ranking 28th) above ground (data not shown). Symmetrically, some very infrequent species producing few fruitbodies were more represented below ground, such as *Cortinarius* subsp. *Phlegmacium*-3 and *Inocybe* subsp. *Inocybium*-3. They produced, respectively, one and three fruitbodies over 3 yr (Richard *et al.*, 2004), but their ECM occurred on three different plant individuals (Fig. 6b).

## Discussion

The present study confirms the remarkable species richness of the fungal community measured by fruitbody surveys of epigeous macromycetes at the same site (Richard *et al.*, 2004). High values of species diversity estimators were obtained, as

illustrated by Fisher's alpha (Table 3), an estimator linking the number of taxa to the number of individuals sampled, which is not unduly affected by sample size (Tokeshi, 1993). Assuming that each RFLP type corresponds more or less to one species (see below), a total of 140 species were detected based on analysis of mycorrhizas (one RFLP type per 2.5 tips investigated, excluding *C. geophilum*), compared with 166 species that have been found using fruitbody surveys (Richard *et al.*, 2004). Based on these two approaches together, there were at least 260 ECM fungal species at the site between 1999 and 2002. This is more than in most previously described late successional stands covering similar areas (Jonsson *et al.*, 1999; Bidartondo *et al.*, 2000, 2001), although similar values were found in old temperate coniferous forests, either by ECM typing (Dahlberg *et al.*, 1997; Luoma *et al.*, 1997) or fruitbody surveys (Villeneuve *et al.*, 1989; O'Dell *et al.*, 1999; Smith *et al.*, 2002).

Our results suggest that ITS-RFLP data are robust for characterizing community diversity, for two reasons. First,

from 158 morphologically defined species that were used in the DNA analysis, 144 (91% of total) yielded a single species-specific RFLP type (Table S1 and data not shown). Second, intraspecific variation was a minor problem. Of the 58 species represented by at least two fruitbodies, 56 (96%) yielded a single RFLP type for all fruitbodies with the exceptions of *R. fragilis* and *R. persicina* var. *rubrata* (Table S1 and data not shown). Together our results highlight the fact that ITS–RFLP data are a valuable tool for grouping ECM species, and for identification of the mycorrhizal symbionts with the fruitbody RFLP-matching approach. These results are similar to those reported by Horton (2002), who also investigated the use of ITS–RFLP patterns to assess diversity of ECM fungi collected across a 7 km coniferous forest. In addition, Kårén *et al.* (1997) already reported that intraspecific variation was not a problem on a local scale. In the two polymorphic species of *Russula*, the RFLP variation found in the ITS is the result of variation in two of the four endonucleases (data not shown). Currently, we do not know if the variation observed is a reflection of cryptic species.

The below-ground method revealed the same distribution pattern as the above-ground survey with respect to the relative proportion of abundant vs rare taxa. The below-ground community was characterized by a few common types and a large number of rare types (Fig. 2). This pattern was also observed using fruitbodies (Fig. 2; Richard *et al.*, 2004). Below ground, 50% of the RFLP types collected were represented by one mycorrhiza. The two dominant species were *C. geophilum* (Table 1) and *R. decipiens* (Fig. 6a). *Cenococcum* alone contributed to 35% of the ectomycorrhizas. Of the 120 total plants, 117 (98%) were colonized by this fungus (Table 1). *Russula decipiens* was found on 17 oaks (data not shown). However, several questions remain concerning the below-ground diversity because of the large number of rare types observed at our site. Which proportion of the local community is really sampled? Would comparable patterns be obtained at another time? Are all abundant species included? Our ability to detect community similarity (e.g. *Arbutus* vs *Quercus*) based on species abundance is also limited by the inherent distribution of the diversity.

Fungal species richness was comparable above and below ground, but the two levels exhibited little overlap (< 20%; Table 3) in fungal species composition. This result confirms and extends earlier observations on the complementarity of the two levels in obtaining a comprehensive view of community composition (Gardes & Bruns, 1996; Jonsson *et al.*, 2000; Peter *et al.*, 2001). For instance, without the below-ground approach we would have missed *C. geophilum*, an ascomycetes species that does not produce fruitbodies at all. It was particularly abundant on oak seedlings and saplings (Table 1). Its high dominance and frequency at our site may arise in part from its ability to sustain xeric conditions by formation of sclerotia (Lilleskov *et al.*, 2004). The role of this fungus in ecosystem functioning is also intriguing – could it

provide drought protection to plant roots, as suggested by Jany *et al.* (2003)? Or, alternatively, could *C. geophilum* be purely opportunistic, with little relevance to tree physiology? The observation that *C. geophilum* often dominates in ECM communities, for example in Spanish *Q. ilex* forests (De Román & De Miguel, 2002); in the Californian chaparral (Borchers & Perry, 1990); or in temperate *Fagus sylvatica* forests (Blaise & Garbaye, 1983), leads us to question the existence of ecotypes or cryptic biological species (Shinohara *et al.*, 1999).

Combining the species composition viewed above and below ground, the following patterns were observed. Apart from *Cenococcum*, the community appeared to be dominated by members of the genus *Russula* and, to a lesser extent, by the genus *Inocybe* as well as members of the Thelephoraceae and Sebacinaceae (Fig. 4; Richard *et al.*, 2004). In Spanish managed *Q. ilex* forests, thelephoroid morphotypes accounted for a quarter of the root tips investigated by De Román & De Miguel (2002). Russulaceae and Thelephoraceae also dominated the community in two other Californian Mediterranean ecosystems, the chaparral (Horton *et al.*, 1999) and the bishop pine forest (Gardes & Bruns, 1996), whereas Sebacinaceae were among the most frequently encountered taxa in *Eucalyptus* sclerophyllous forests in Australia (Glen *et al.*, 2002). An intriguing feature is the absence of hypogeous fungi (at least among dominant taxa on *A. unedo*, Table 2 and S1), which is perhaps caused by environmental conditions. For instance, the lack of species of *Tuber* may be explained by acidic soil conditions.

Tree diversity has been suggested to favour ECM diversity on a local scale (Nantel & Neumann, 1992; Kernaghan *et al.*, 2003). We tested the hypothesis that the hosts contribute to ECM fungal diversity. Only 12.9% of the taxa were shared (Fig. 3), less than what was found in mixed forest stands by Horton & Bruns (1998), Cullings *et al.* (2000) and Kennedy *et al.* (2003), where multihost fungi dominated, accounting for 30 to 90% of the ECM fungal community in all three studies. Unfortunately our sampling is insufficient to provide statistically significant data, because of the high species richness of the community. Most species were too infrequent to draw conclusions about their distribution, a problem that is often limiting in studies of ECM communities (Horton & Bruns, 2001; Taylor, 2002). Nevertheless, even if restricted to a limited number of fungal taxa, sharing of symbionts may have ecological consequences as *Q. ilex* seedlings successfully establish and survive in *A. unedo*-dominated chaparral (Gamisans, 1999). This pattern suggests that *A. unedo* shrubs may provide conducive conditions for *Q. ilex* seedlings in early stages of forest succession, perhaps by providing a compatible fungal network.

Despite the important width of the age sequence, the ECM community was quite similar at the various developmental stages of *Q. ilex* investigated. We observed: (i) similar rank–abundance curves (reflecting high taxonomic diversity and a

dominance of rare taxa, Table 3); (ii) among the identified taxa, similar dominance of genera such as *Russula*, *Cortinarius* and *Amanita* (Table 4); and (iii) a high abundance of *C. geophilum* (Table 1). Our findings support the conclusion that established seedlings recruit ECM symbionts in an opportunistic way among mycobionts colonizing the old surrounding trees. Similar observations were made in multi-aged stands dominated by conifers such as *Pinus sylvestris* (Jonsson *et al.*, 1999) or *Tsuga heterophylla* (Kranabetter, 1999; Kranabetter & Friesen, 2002).

An intriguing question is whether or not the sharing of ECM partners between seedlings and old trees is under natural selection. Seedlings may take benefit from established ECM fungi that already have large extraradical soil-exploring mycelia built at the oldest trees' expense (Högberg *et al.*, 1999). In addition, shared symbionts may even transfer carbon from high-canopy trees to understorey seedlings (Simard *et al.*, 1997; Lerat *et al.*, 2002), counterbalancing low light influx. For the related species *Quercus rubra*, seedling nutrition and mycorrhization (infection level and diversity) are improved in the vicinity of adult conspecifics (Dickie *et al.*, 2002). Symbiont sharing between seedlings and older *Q. ilex* could thus result in favouring of kin, as most *Q. ilex* acorns remain around the mother tree due to barochory (Darley-Hill & Carter Johnson, 1981).

## Conclusions

This first report on the below-ground ECM diversity in a Mediterranean hardwood old-growth forest revealed a striking diversity of ECM fungi. The ascomycete *C. geophilum* and members of the Russulaceae, Cortinariaceae (genus *Inocybe*), Thelephoraceae and Sebacinaceae are the most abundant taxa on roots. Exhaustive inventory of the diversity is a real challenge because of the large number of rare types. Our results suggest that both above- and below-ground levels have to be explored to obtain a comprehensive overview of the composition of the ECM fungal community. The composition and diversity of the ECM community does not depend on host age. Our results also question the ecological importance of symbiont sharing between *Q. ilex* and *A. unedo* in old-growth forest dominated by *Q. ilex*.

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## Supplementary material

The following material is available as supplementary material at <http://www.blackwellpublishing.com/products/journals/suppmat/NPH/NPH1382/NPH1382sm.htm>. These supplementary data include (i) for each ITS-RFLP type, DNA fragment sizes in base pairs and (ii) for the 60 taxonomically identified taxa, genus- or species-level identification obtained by comparing ITS-RFLP types to those from voucher specimens of sporocarps or by BLAST analysis of the ITS sequences.

**Table S1** ITS-RFLP types of ectomycorrhizas collected on the 120 sampled plants.

## References

- Agerer R. 1987–93. *Colour Atlas of Ectomycorrhizae*. Munchen, Germany: Einhorn Verlag Eduard Dietenberger.
- Bidartondo M, Kretzer AM, Bruns TD. 2000. High root concentration and even ectomycorrhizal diversity near *Sarcodes sanguinea* (Ericaceae): a cheater that stimulates its victims? *American Journal of Botany* 87: 1783–1788.
- Bidartondo MI, Baar J, Bruns TD. 2001. Low ectomycorrhizal inoculum potential and diversity from soils in and near ancient forests of bristlecone pine (*Pinus longeva*). *Canadian Journal of Botany* 79: 293–299.
- Blaise T, Garbaye J. 1983. Effets de la fertilisation sur les ectomycorhizes d'une hêtraie. *Acta Oecologica* 18: 165–169.
- Borchers SL, Perry DA. 1990. Growth and ectomycorrhiza formation of Douglas-fir seedlings grown in soils collected at different distances from pioneering hardwoods in southwest Oregon clear-cuts. *Canadian Journal of Forest Research* 20: 712–721.
- Cullings KW, Vogler DR, Parker VT, Finley SK. 2000. Ectomycorrhizal specificity in a mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone national park. *Applied and Environmental Microbiology* 66: 4988–4991.
- Dahlberg A, Jonsson L, Nylund JE. 1997. Species diversity and distribution of biomass above and below-ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Canadian Journal of Botany* 75: 1323–1335.
- Darley-Hill S, Carter Johnson W. 1981. Acorn dispersal by the blue jay (*Cyanocitta cristata*). *Oecologia* 50: 231–232.
- de Román M, de Miguel AM. 2002. Post-fire dynamics of the ectomycorrhizal community in a *Quercus ilex* subsp. *ballota* forest. In: Trabaud L, Prodon R, eds. *Fire and Biology Processes*. Leiden, the Netherlands: Backhuys, 131–136.
- Dickie IA, Koide RT, Steiner KC. 2002. Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. *Ecological Monographs* 72: 505–521.
- Fisher RA, Corbet AS, Williams CB. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. *Journal of Animal Ecology* 12: 42–58.
- Fusconi A, Bonfante-Fasolo P. 1984. Ultrastructural aspects of host–endophyte relationships in *Arbutus unedo* L. mycorrhizae. *New Phytologist* 96: 397–410.

- Gamisans J. 1999. *La Végétation de la Corse*, 2nd edn. Aix-en-Provence, France: Edisud.
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
- Gardes M, Bruns TD. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Canadian Journal of Botany* 74: 1572–1583.
- Giovannetti M, Lioi L. 1990. The mycorrhizal status of *Arbutus unedo* in relation to compatible and incompatible fungi. *Canadian Journal of Botany* 68: 1239–1244.
- Glen M, Tommerup IC, Bougher NL, O'Brien PA. 2002. Are Sebacinaceae common and widespread ectomycorrhizal associates of *Eucalyptus* species in Australian forests? *Mycorrhiza* 12: 243–247.
- Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA. 1999. Natural <sup>13</sup>C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences, USA* 96: 8534–8539.
- Horton TR. 2002. Molecular approaches to ectomycorrhizal diversity studies: variations in ITS at a local scale. *Plant and Soil* 244: 29–39.
- Horton TR, Bruns TD. 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). *New Phytologist* 139: 331–339.
- Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10: 1855–1871.
- Horton TR, Bruns TD, Parker VT. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Canadian Journal of Botany* 77: 93–102.
- Jany JL, Martin F, Garbaye J. 2003. Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. *Plant and Soil* 255: 487–494.
- Jonsson L, Dahlberg A, Nilsson M-C, Kären O, Zackrisson O. 1999. Continuity of ectomycorrhizal fungi in self-regenerating boreal *Pinus sylvestris* forests studied by comparing mycobiont diversity on seedlings and mature trees. *New Phytologist* 142: 151–162.
- Jonsson L, Dahlberg A, Brandrud T-E. 2000. Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to experimental nitrogen addition: above- and below-ground views. *Forest Ecology and Management* 132: 143–156.
- Kären O, Högberg N, Dahlberg A, Jonsson L, Nylund J-E. 1997. Inter- and intra-specific variation in the ITS region of r-DNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytologist* 136: 313–325.
- Kennedy PG, Izzo AD, Bruns TD. 2003. High potential for common mycorrhizal networks between understory and canopy trees in a mixed evergreen forest. *Journal of Ecology* 91: 1071–1080.
- Kernaghan G, Widden P, Bergeron Y, Légaré S, Paré D. 2003. Biotic and abiotic factors affecting ectomycorrhizal diversity in boreal mixed-woods. *Oikos* 102: 497–504.
- Kranabetter JM. 1999. The effect of refuge trees on a paper birch ectomycorrhiza community. *Canadian Journal of Botany* 77: 1523–1528.
- Kranabetter JM, Friesen J. 2002. Ectomycorrhizal community structure on western hemlock (*Tsuga heterophylla*) seedlings transplanted from forests into openings. *Canadian Journal of Botany* 80: 861–868.
- Krebs CJ. 1999. Ecological methodology. Menlo Park, CA, USA: Benjamin Cummings.
- Laganà A, Loppi S, De Dominicis V. 1999. Relationship between environmental factors and the proportions of fungal trophic groups in forest ecosystems of the central Mediterranean area. *Forest Ecology and Management* 124: 145–151.
- Lerat S, Gauci R, Catford JG, Horst V, Piché Y, Lapointe L. 2002. <sup>14</sup>C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings via arbuscular mycorrhizal fungi in natural stands. *Oecologia* 132: 181–187.
- Lilleskov EA, Bruns TD, Horton TR, Taylor DL, Grogan P. 2004. Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. *FEMS Microbiology Ecology* 49: 319–332.
- Luoma DL, Eberhart JL, Amaranthus MP. 1997. Biodiversity of ectomycorrhizal types from southwest Oregon. In: Kaye TN, Liston A, Love RM, Luoma DL, Meinke RJ, Wilson MV, eds. *Conservation and Management of Native Plants and Fungi*. Corvallis, OR, USA: Native Plant Society of Oregon, 249–253.
- Molina R, Trappe JM. 1982. Lack of mycorrhizal specificity by the ericaceous hosts *Arbutus menziesii* and *Arctostaphylos uva-ursi*. *New Phytologist* 90: 495–509.
- Molina R, Massicotte HB, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community–ecological consequences and practical implications. In: Allen MF, ed. *Mycorrhizal Functioning, An Integrative Plant–Fungal Process*. New York, USA: Chapman & Hall, 357–423.
- Mueller-Dombois D, Ellenberg H. 1974. *Aims and Methods of Vegetation Ecology*. New York, USA: John Wiley & Sons.
- Münzenberger B, Kottke I, Oberwinkler F. 1992. Ultrastructural investigations of *Arbutus unedo*–*Laccaria amethystea* mycorrhiza synthesized *in vitro*. *Trees* 7: 40–47.
- Nantel P, Neumann P. 1992. Ecology of ectomycorrhizal-basidiomycete communities on a local vegetation gradient. *Ecology* 73: 99–117.
- O'Dell TE, Ammirati JF, Schreiner EG. 1999. Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. *Canadian Journal of Botany* 77: 1699–1711.
- Panaïotis C, Carcaillet C, M'hamedi M. 1997. Determination of the natural mortality age of an holm oak (*Quercus ilex* L.) stand in Corsica (Mediterranean Island). *Acta Oecologica* 18: 519–530.
- Peter M, Ayer F, Egli S, Honegger R. 2001. Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. *Canadian Journal of Botany* 79: 1134–1151.
- Pielou EC. 1984. *The Interpretation of Ecological Data. A Primer on Classification and Ordination*. New York, USA: John Wiley & Sons.
- Quézel P. 1985. Definition of the Mediterranean region and the origin of its flora. In: Gómez-Campo C, ed. *Plant Conservation in the Mediterranean Area*. Dordrecht, the Netherlands: Dr W. Junk, 9–24.
- Quézel P, Médail F. 2003. *Ecologie et Biogéographie des Forêts du Bassin Méditerranéen*. Paris, France: Elsevier.
- Richard F, Moreau P-A, Selosse M-A, Gardes M. 2004. Diversity and fruiting patterns of ectomycorrhizal and litter saprobic fungi in an old-growth Mediterranean forest dominated by *Quercus ilex* L. *Canadian Journal of Botany* 82: 1711–1729.
- Scarascia-Mugnozza G, Oswald H, Piussi P, Radoglou K. 2000. Forests of the Mediterranean region: gaps in knowledge and research needs. *Forest Ecology and Management* 132: 97–109.
- Selosse M-A, Bauer R, Moyersoen B. 2002. Basal hymenomycetes belonging to the Sebacinaceae are ectomycorrhizal on temperate deciduous trees. *New Phytologist* 155: 183–195.
- Shinohara ML, LoBuglio KF, Rogers SO. 1999. Comparison of ribosomal DNA ITS regions among geographic isolates of *Cenococcum geophilum*. *Current Genetics* 35: 527–535.
- Signorello P. 1996. Indagini micocenologiche sulle cenosi a *Quercus ilex* L. dell'Etna. *Micologia Italiana* 1: 74–80.
- Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388: 579–582.
- Smith JE, Molina R, Huso MMP, Luoma DL, McKay D, Castellano MA, Lebel T, Valachovic Y. 2002. Species richness, abundance, and composition of hypogeous and epigeous ECM fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, USA. *Canadian Journal of Botany* 80: 186–204.

- Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis*, 2nd edn. London: Academic Press.
- Taylor AFS. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant and Soil* 244: 19–28.
- Tedersoo L, Kõljalg U, Hallenberg N, Larsson K-H. 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* 159: 153–165.
- Tokeshi M. 1993. Species abundance patterns and community structure. *Advances in Ecological Research* 24: 111–186.
- Trappe JM. 1962. Fungus associates of ectotrophic mycorrhizae. *Botanical Reviews* 28: 538–606.
- Villeneuve N, Grandtner MM, Fortin JA. 1989. Frequency and diversity of ectomycorrhizal and saprophytic macrofungi in the Laurentide Mountains of Quebec. *Canadian Journal of Botany* 67: 2616–2629.
- Vogt KA, Bloomfield J, Ammirati JF, Ammirati SR. 1992. Sporocarp production by basidiomycetes, with emphasis on forest ecosystems. In: Carroll CG, Wicklow DT, eds. *The Fungal Community: its Organization and Role in the Ecosystem*. New York, USA: Marcel Dekker, 563–581.
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for polygenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR Protocols: A Guide to Methods and Applications*. New York, USA: Academic Press, 315–322.



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