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Similar taxonomic richness but different communities of ectomycorrhizas in native forests and non-native plantation forests

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Abstract This investigation sought to examine if there was a difference between the ectomycorrhizal (ECM) communities in plots of native oak and introduced Scots pine and Sitka spruce forest. The ECM communities in four plots of each forest type were described, from five soil cores collected in each plot, by morphotyping, internal transcribed spacer (ITS)–restriction fragment length polymorphism matching of mycorrhizas and sporocarps and ITS sequencing. Fifty-one distinct taxa were distinguished; 25 were identified to species level, 11 to genus and 15 remained unidentified. Seventy-one ECM species were recorded as sporocarps from the forest plots; most (43 species) were found in the Sitka spruce plots. The below-ground ECM communities of the different forest types did not differ significantly with respect to species richness of taxa on roots, but differed in species composition. Multivariate analysis produced a clear separation of the communities of the different forest types using below-ground data, but the above-ground sporocarp data did not separate the forest types. Moreover, results of a Mantel test found no relationship between the above- and below-ground similarity matrices. The oak plots had the most distinctive ECM community, with *Laccaria amethystina* and *Elaphomyces granulatus* being frequent. The Sitka spruce plots showed the lowest intra-forest type similarity and were often dominated by "nursery type" ectomycorrhizas. There was only 10% similarity between the above- and below-ground ECM species in these plots, different colonisation methods of ectomycorrhizal taxa and insufficient below-ground sampling being possible reasons for this disparity. Our results indicate that plantations of non-native Sitka spruce can support similar levels of ECM diversity as native forests.

Keywords RFLP · Morphotyping · *Quercus* spp. · *Picea sitchensis* · *Pinus sylvestris* · Nursery · Above ground

Introduction

Up to 85% of angiosperm and 90% of gymnosperm species show mycorrhizal associations (Wang and Qiu 2006). Ectomycorrhizas are particularly important for plant hosts that grow in nutrient-poor soil conditions (Smith and Read 2008), for example, boreal coniferous forests, and also where tree species are introduced into new habitats where they may not be suited to the particular soil and environmental conditions (Nuñez et al. 2009). The removal of native forest and replacement with exotic tree species generally reduces the distribution of native ectomycorrhizal (ECM) fungi, unless those fungi can colonise the replacement tree species. The majority of ECM fungi are host generalists (Molina et al. 1992), and these are less likely to be adversely affected by replacement of native forest habitat. However, other ECM fungi such as members of the genera *Suillus* and *Alnicola* show high host specificity with the tree genera *Pinus* (Bruns et al. 2002) and *Alnus* (Moreau et al. 2006), respectively. Previous research has shown that the invasive success of exotic tree species often hinges on their ability to form ECM associations with native ECM fungi (Mikola 1973), thus creating novel ECM associations, especially when trees are introduced without

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their usual ECM biota (Cullings et al. 2000; Trocha et al. 2011). However, it has also been found that co-introduction of an exotic tree species with its own ECM community can facilitate its survival (Tedersoo et al. 2007; Vellinga et al. 2009; Dickie et al. 2010). Many exotic tree species in the UK have ECM assemblages that include many cosmopolitan and host-generalist species (Alexander and Watling 1987; Humphrey et al. 2003; Palfner et al. 2005).

The likely climax vegetation for the majority of Ireland is oak forest of sessile oak (*Quercus petraea* L.) or pedunculate oak (*Quercus robur* L.) (Cross 2006), but forest clearance mainly during the fourteenth and again in the eighteenth century (Cole and Mitchell 2003) reduced the cover of native deciduous forests to 1% of Ireland's land area, the third lowest in Europe after The Netherlands and the Czech Republic (Forest Resources Assessment 2010). However, a further 9% of the land area is under plantation forestry, 60% of which is dominated by the exotic conifer Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. This species grows particularly well in Ireland because it is adaptable to the prevailing climate and soil conditions (Joyce and O'Carroll 2002) and, if properly managed on fertile sites, can show growth rates similar to or in excess of those recorded in its home range (Farrelly et al. 2011). It is planned that by 2030, plantation forestry will increase to over 17% of land area, with Sitka spruce likely continuing to be the most planted tree species (Department of Agriculture, Forestry and Food 1996). In the UK, where Sitka spruce is also an important component of plantation forestry (UK Forestry Commission 2003), work by Alexander and Watling (1987) indicated that Sitka spruce in Scotland supports a generalist ECM fungal biota, sharing many of the ECM fungi normally found on birch and Scots pine. Moreover, Humphrey et al. (2000) and Ferris et al. (2000) have shown from sporocarp surveys that Sitka spruce and Scots pine (*Pinus sylvestris* L.) plantations can have similar levels of ECM species richness to native Scots pine and oak forest.

Investigations of fungal diversity, but especially below-ground ECM diversity, have been fewer in Ireland than in Britain (O'Hanlon and Harrington 2011a). Heslin et al. (1992) examined the below-ground ECM communities of some mature Irish first rotation Sitka spruce monoculture and Sitka spruce-Japanese larch (*Larix kaempferi*) mixtures and found that the ECM community was relatively poor (comprising nine morpho-types) especially when compared with Sitka spruce in its home range in North America. Sitka spruce shared many of its ectomycorrhizas with other coniferous tree species such as Japanese larch and lodgepole pine (*Pinus contorta*), indicating that it may have a generalist ECM mycota in Irish plantations. Further work examining the ECM associations of Sitka spruce in Irish nurseries (Grogan et al. 1994) found that the communities on nursery Sitka spruce seedlings are very similar to those recorded on mature Sitka spruce by Heslin et al. (1992), indicating that Irish Sitka spruce forests may retain their nursery ECM communities long after outplanting. In the UK, morphotype analysis of ECM fungi on roots of mature Sitka spruce plantations have been more numerous, finding from 16 to 25 ECM taxa, with the community dominated by the ectomycorrhiza *Tylospora fibrillosa* (Thomas et al. 1983; Taylor and Alexander 1989; Palfner et al. 2005). Other studies have examined the ECM assemblages on nursery-grown Sitka spruce seedlings (Thomas and Jackson 1979; Flynn et al. 1998), although these are known to be less species rich than forest ECM communities (Jones et al. 1997; Menkis et al. 2005).

This article describes and compares the above- and below-ground ECM communities of native oak *Q. petraea* and *Q. robur* forests, with those of introduced Sitka spruce and Scots pine forests. Sitka spruce was introduced ca. 100 years ago using seed of Queen Charlotte Island, Washington and Oregon provenance (Joyce and O'Carroll 2002). Scots pine is also an introduced species, which, although not nearly as important in area terms as Sitka spruce, has a much longer planting history (300 years) in Ireland (Roche et al. 2009). Due to the differing residency times, it might be expected that the species richness of the forest types would follow a trend of oak > Scots pine > Sitka spruce. In order to test this, the below-ground communities of the forest types were examined using a combination of morphological and molecular identification methods, with the similarity of below-ground ECM communities to the above-ground sporocarp communities of the forests also investigated.

Methods

The plots

The sampling sites consisted of three forest types chosen on the basis of the dominant tree species. Four oak, four Scots pine and four Sitka spruce sites were distributed across six counties in the Republic of Ireland (Table 1). The climate of all of the sites is a temperate maritime climate, with annual temperatures of 9°C and annual rainfall of about 1,000 mm (MET Eireann 2011). A 100 m^2 (2×50 m) rectangular permanent plot was laid out in each site. Plots were positioned so as to avoid any understory trees of different

Table 1 Forest plots where sampling took place

Plot name	Location	Ectomycorrhizal tree species	Soil parent material	Soil pH
Abbeyleix	52°53′ N, 07°22′ W	Qr, Bp, Fs, Ld,	Limestone glacial till	5.67
Kilmacrea	52°54′ N, 06°10′ W	Qp, Ca	Ordovician shale	5.01
Raheen	52°53′ N, 08°31′ W	Qp, Ca, Fs,	Sandstone glacial till	4.83
Tomies	52°02′ N, 09°35′ W	Qp	Devonian Sandstone	5.56
Annagh	53°06′ N, 07°58′ W	Ps, Bp,	Basin peat	4.86
Bansha	52°27′ N, 08°06′ W	Ps, Qp	Devonian sandstone	4.41
Brittas	53°09′ N, 07°32′ W	Ps, Qp	Quartzite, sandstone	4.45
Torc	52°00′ N, 09°31′ W	Ps	Devonian sandstone	4.55
Bohatch	52°57′ N, 08°27′ W	Pi, Pa	Blanket peat	6.72
Chevy Chase (young)	53°02′ N, 08°41′ W	Pi, Bp	Devonian sandstone	6.59
Chevy Chase (mature)	53°02′ N, 08°41′ W	Pi	Devonian sandstone	6.63
Dooary	52°56′ N, 07°15′ W	Pi	Carboniferous shale	7.16

The ectomycorrhizal tree species within 5 m of the plots are listed with the dominant tree species listed first. Soil description was taken from the geological maps of Ireland (Gardener and Radford 1980). Soil pH was measured using a pH meter in a 5:1 H_2O soil dilution (Anonymous 2006)

Qr Quercus robur, *Bp Betula pubescens*, *Fs Fagus sylvatica*, *Ld Larix decidua*, *Qp Quercus petraea*, *Ca Corylus avellana*, *Ps Pinus sylvestris*, *Pi Picea sitchensis*, *Pa Picea abies*

species to the dominant species. Each plot was visited over 3 years (2007–2009) on at least three occasions for sporocarp collection (O'Hanlon 2011) and in the year 2009 for soil core collection for ECM analysis.

Sampling and enumeration of ectomycorrhizas

A total of five soil cores, one from each sub-plot, were taken from the upper soil layer in each plot using a 10-cm diameter soil corer, which measured 15 cm in length. In each sub-plot, one core was taken 1 m from the trunk of a target tree (i.e. oak, Scots pine or Sitka spruce) and in an area where non-target trees were as far away as possible. This was done to reduce the effect of neighbouring non-target tree species on the ECM communities of the target tree. The cores were kept at 4°C until counting, and analysis could be undertaken. The cores were soaked in tap water for 15 min and washed through a 1-mm sieve. Senescent roots, coarse woody debris and large root segments were removed manually. The remaining roots were cut into 2-cm segments and divided randomly into six sub-samples (Petri dishes). The length of the ECM roots segments in each Petri dish was estimated using the grid-line intersect method (Tennant 1975). The length of the roots was used to allow for comparison of ECM taxonomic richness between the forest types at a similar sampling intensity. Root tips were not used for this comparison, as a preliminary study in 2008 (data not shown) found that the counting of individual tips consumed more time than the line intersect method to quantify root length. It is also known that some ECM fungi preferentially infect older sections of root systems (e.g. *Lactarius pubescens*; Gibson and Deacon 1988); therefore, comparisons based on root tips would exclude the measurement of these mature non-tip root segments. Each Petri dish was examined at ×30 magnification using a Nikon stereo microscope, and morphologically similar morphotypes were distinguished. Taxa richness (per plot) is the number of distinct ECM taxa recorded from the five cores taken from the plot. Taxa abundance was estimated as per cent frequency (per plot); this was calculated as:

$$\frac{\text{Presence of the taxa in 30 subsamples}}{30} \times 100$$

Each morphotype was microscopically examined at ×400 and ×1,000 by taking pieces of the mantle and describing the inner and outer mantle according to Agerer (1987–2002). Digital images of the outer surface and basal layers of the mantle were used in identification, with reference to descriptions by Agerer (1987–2002). Secondary morphological characteristics such as clamp connections and presence of specialised cells such as cystidia were also noted. Terminology for the descriptions of ectomycorrhizas follows Agerer (1987–2002). Samples of the mycorrhizal root tips were stored in 2% glutaraldehyde for future morphological examination and at −80°C for molecular analysis at a later date at the Department of Life Sciences, University of Limerick. Types that could not be identified morphologically or by molecular methods were given a unique code, e.g. 3 a1 and are referred to hereafter as their code name.

The percentage similarity between below- (morphotype) and above-ground (sporocarp) ECM assemblages was calculated using a modified form of the Jaccard index:

$$\frac{\text{Taxa in common}}{\text{Taxa in common} + \text{Taxa only found aboveground} + \text{Taxa only found belowground}} \times 100$$

Molecular identification of ECM types

DNA was extracted from ECM sporocarps and ECM root tips using the Qiagen Plant Mini Kit (Qiagen, California, USA). The manufacturer's instructions were followed without modifications. The ITS region of ribosomal DNA was amplified using the method described by Gardes and Bruns (1993) using combinations of the primers ITS1-F and ITS4 (White et al. 1990; Gardes and Bruns 1993). The PCR mix was composed of 2.5 μl of extracted DNA, 4.175 μl PCR grade water, 1 μl 10× buffer, 1 μl dNTPs stock (containing premixed bases), 0.65 μl $MgCl_2$, 0.3 μl forward primer, 0.3 μl reverse primer and 0.075 μl High-Fidelity Taq polymerase (Roche Applied Sciences, West Sussex, UK) to make a 10-μl master mix. The final concentrations of the reagents in the master mix were 200 μM dNTPs, 2.5 μM $MgCl_2$, 0.3 μM forward primer, 0.3 μM reverse primer and 0.26 U of High-Fidelity Taq polymerase. Amplification was carried out in a G-Storm GS2 thermocycler (G-Storm Ltd., Surrey, UK) using the following three-stage amplification conditions: stage 1, 94°C for 120 s; stage 2, 35 cycles at 94°C for 15 s, at 55°C for 30 s and at 72°C for 60 s; and stage 3, 72°C for 7 min, after which the PCR was terminated.

For restriction fragment length polymorphism (RFLP) analysis of the samples, the PCR fragments were digested with the restriction endonucleases *Alu*I and *Hinf*I (Roche Applied Sciences, West Sussex, UK). Ten microlitres of amplified PCR product was incubated for 1 h with 1.5 μl of 10× buffer (supplied with restriction enzyme), 8.4 μl PCR grade water and 0.1 μl restriction enzyme (final concentration of restriction enzyme 1 unit), at 37°C. The resulting PCR-RFLP fragments were separated on a 2% Sybr-Safe agarose gel with a negative control. The molecular sizes of the fragments were calculated using the band analysis feature of the GeneTools software (Syngene UK, Cambridge UK) and recorded by measurement against a DNA molecular weight marker (DNA molecular weight marker VIII, Roche Applied Sciences, West Sussex, UK). This information was then entered into a database using the GERM (Good Enough RFLP Matcher) Excel tool (Dickie et al. 2003). Matches between sporocarps and ECM tips were calculated using the recommended settings for matches as advocated by Dickie et al. (2003); backward and forward matches of the bands were accepted only if the molecular weights were within 25 base pairs (bp) of each other. If the sum of the differences between observed and expected bands exceeded 100 bp, then the samples were not a match.

PCR products were purified prior to sequencing using the QiaQuick PCR purification kit (Qiagen, California, USA). All ECM samples that amplified were sent for sequencing to Eurofins MWG Operon, London, UK. The resulting sequences were entered into Bio-Edit Software (Hall 1999), and all sequences were compared to identify matches with the optimal global alignment option and their identity similarity index calculated according to the "IDENTIFY" matrix. Sequences with similarity values >0.98 were accepted as the same species. The identify matrix setting was used because it has high penalties for mismatches (BIOedit manual; Hall 2005) and is therefore useful for separating species within the same genus. The unedited sequence data were entered into the UNITE data base (Kõljalg et al. 2005) and a Blast-n search was carried out using both the UNITE database and the GenBank database. Sequences with identities above 98% were treated as species-level matches, while identification at the genus level was based on Blast consensus of over 90% (Barreotavena et al. 2010). The name suggested by UNITE, a curated database for ECM fungi (Kõljalg et al. 2005), was used preferentially and that of GenBank only if there was no entry in UNITE. The sequences were deposited in NCBI GenBank with the GenBank accession numbers HQ703019–HQ703027.

Statistical analysis

To compare the morphotype richness of the different forest types at a similar sampling intensity, sample-based rarefaction curves with 95% confidence intervals were calculated, using the computer program EstimateS (Colwell 2004). In these analyses, plots were sampled randomly with replacement over 1,000 permutations of the data because otherwise confidence intervals are meaningless in the upper end of the rarefaction curve (Colwell et al. 2004). The length of roots sampled was used to compare the morphotype richness of the different forest types at a similar sampling intensity.

To assess relative ECM distributions within the forest type communities, rank abundance plots (Whittaker 1965) were constructed following Magurran (2004). The relative frequency of $Taxa_X$ in forest $type_N$ was calculated as:

$$\frac{\text{Presence of taxa}_X \text{ in all subsamples in forest type}_N}{\text{Presence of all taxa in all subsamples in forest type}_N}$$

Detrended correspondence analysis (DCA) (PC-Ord version 4.36; MjM Software Design, Gleneden Beach,

OR, USA) was performed to assess the similarities between the ECM assemblages (sporocarps and ectomycorrhizas) from the different forest plots using per cent frequency data for sporocarps (Appendix 3) and ectomycorrhizas (Appendix 1). Multi-response permutation procedure (MRPP) analysis, employing relative Euclidian distance as similarity measure, was used to test if ECM assemblages from plots of the same forest type were more similar to each than they were to assemblages from a different forest type.

To see if the relationship between the plots based on composition of the below-ground ECM assemblages was mirrored in the distribution of sporocarps above ground, Mantel tests were employed using the chi-square distance measure. Two similarity matrices were compared: the above- and below-ground plot similarities based on per cent frequency of species. Similarity matrices were created using the same distance measure as used in the DCA (chi-square distance index; McCune and Grace 2002) and were compared using PC-Ord and evaluated for significance using a Monte Carlo test with 9999 randomisations.

Results

ECM morphotypes

In total, 51 distinct taxa were collected from 12 forest plots (Appendix 1). This included 16 taxa identified solely by morphotyping methods, three taxa identified by RFLP matching to sporocarps, 17 taxa identified by sequencing (Table 2) and 15 taxa remaining unidentified. Twenty-one ECM taxa were found in oak, 20 in Scots pine and 18 in Sitka spruce. Fifteen ECM taxa could not be identified to genus level and so were given code names (i.e. morphotype 5c9). Both morphological and RFLP analysis allowed for the separation of these types into distinct taxa, but it was not possible to assign them to genus or species either by RFLP matching to sporocarps or sequencing of the ITS region. The 15 taxa are described in Appendix 2 with corresponding Figs. A1–A36.

ECM abundance and richness

In all of the forest types, there were two to four dominant taxa and with the remainder taxa present at low frequencies (Fig. 1).

Table 2 Successfully sequenced ECM fungi using the primer ITS1-F

Sample name	Sequence length	Closest accession match	Accession name	Match (%)
4i5[e]	97	UDB001476	*Amanita citrina*	95
3c5[e]	567		*Amphinema* sp.	>90
3g7[e]	591		*Amphinema* sp.	>90
3f3[e]	664	UDB000127	*Cortinarius obtusus*	99
Cortinarius rubellus[f]	661	UDB002427	*Cortinarius rubellus*	99
Cortinarius scandens [f]	142		*Cortinarius* (*Telamonia* sp.)	92
4d1[e]	596	UDB000592	*Entoloma serrulatum*	98
Entoloma conferendum var. *pusillum*[f]	786	GQ397990	*Entoloma sp.* cf *cetratum*	94
3a9[e]	824	AM882850	*Inocybe cincinnata*	99
4e3[e]	665	UDB000861	*Lactarius hepaticus*	100
4g3[e]	652	UDB001617	*Pseudotomentella griseopergamacea*	99
4d5[e]	75	UDB000353	*Russula caerulea*	91
Russula fellea[f]	834	UDB000110	*Russula fellea*	100
Russula foetans[f]	806	DQ422024	*Russula illiota*	99
3e3[e]	802	AY254880	*Russula ochroleuca*	99
3g3[e]	802	AY254880	*Russula ochroleuca*	99
5i1[e]	810	AY254880	*Russula ochroleuca*	99
4a3[e]	802	AY061709	*Russula puellaris*	99
Russula fragilis[f]	27	UDB001637	*Russula sardonia*	84
4j5[e]	670	UDB000664	*Suillus variegatus*	100
4b1[e]	772	UDB001656	*Tomentella badia*	99
3j3[e]	719	UDB003349	*Tomentella sublilacina*	98
3b5[e]	56		*Tylospora* sp.	95

Closest database matches are listed with species being taken as identical if matches share 98% base pair similarity. In cases where the match is not very similar (similarity ≤95%), the accession number is not given. Superscript e indicates sequence from ectomycorrhizal morphotype, and superscript *f* indicates sequence from sporocarp

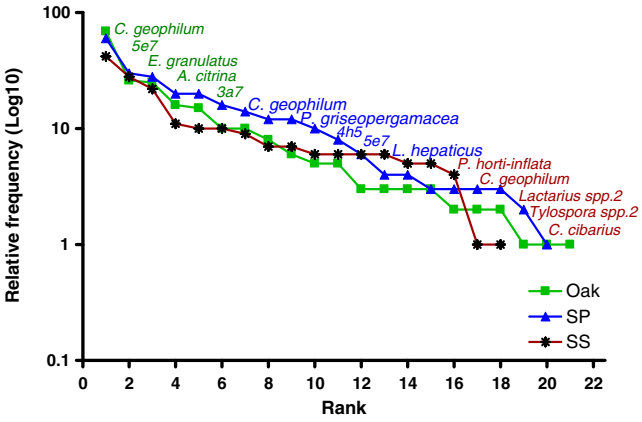

Fig. 1 Distribution of relative frequencies of ectomycorrhizal taxa in each forest type along with the five most abundant morphotypes from each forest type: common logarithms of the frequency of the ectomycorrhizal type in the sub-samples as a function of its rank. *Green squares and text* Oak forest type, *blue triangles and text* Scots pine (SP) forest type and *brown stars and text* Sitka spruce (SS) forest type

Two taxa (*Cenococcum geophilum* and *Russula ochroleuca*) were common to all forest types; three taxa (*Laccaria amethystina*, *Laccaria laccata*, type 5e7) were common to oak and Scots pine. *Piceirhiza nigra* was common to Sitka spruce and Scots pine (Fig. 2). The remaining taxa were found in only one forest type.

As the amount of sampling was not equal between the forest types (based on length of roots sampled: oak= 13,731 cm, Scots pine=19,685 cm, Sitka spruce= 14,157 cm), sample-based rarefaction was used to equalise the sampling intensity between the forest types. At 13,700 cm of root length (the lowest amount sampled for a forest type, oak), the rarefied mean (and 95% lower and upper confidence bounds) number of ECM taxa was 18 (±7), 15 (±6) and 16 (±7) for oak, Scots pine and Sitka spruce forests, respectively (Fig. 3). This compares with the actual taxa richness of the oak, Scots pine and Sitka spruce forest types, which was 21,

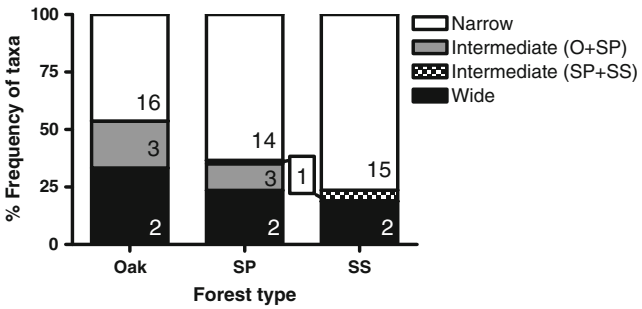

Fig. 2 Overview of percent frequency and species richness of ectomycorrhizal morphotypes on roots of oak, Scots pine and Sitka spruce plots. *Figures in bars* indicate the number of morphotypes that were classified as wide (ECM fungi that were found in the oak, Scots pine and Sitka spruce forest type), intermediate (ECM fungi found in two of the three forest types) and narrow (ECM morphotypes occurring on only one forest type)

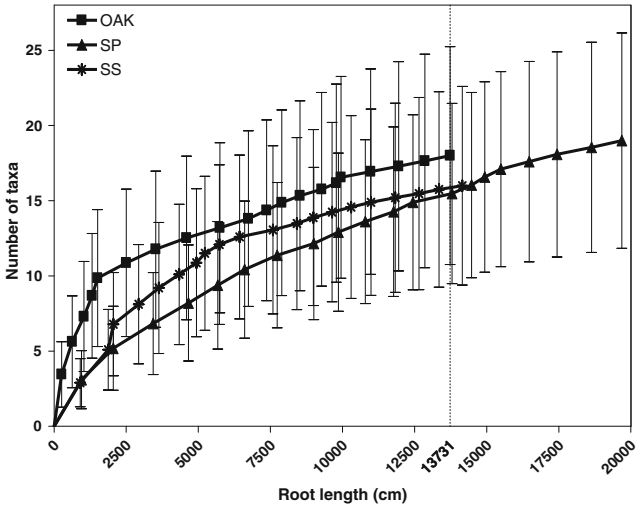

Fig. 3 Sample-based rarefaction curves of mean (*symbols*) morphotypes with 95% upper and lower confidence intervals (*whiskers*) for oak (*square symbol*), SP = Scots pine (*upright triangle symbol*) and SS = Sitka spruce (*star symbol*). *Vertical dashed line* at x=13,731 indicates maximum common sampling intensity. Samples were sampled randomly with replacement in this analysis using 1,000 permutations for each sample size

20 and 18 taxa, respectively. Visual examination of the curves and 95% confidence intervals show that the number of ECM taxa at a standard sample size is not significantly different (P>0.05) between the three forest types based on the overlapping confidence intervals (Fig. 3).

Similarity of below- and above-ground ECM assemblages

Seventy-one ECM species were collected as sporocarps from the sites; 40 species were found in oak, 28 in Scots pine and 43 in the Sitka spruce forest type (Appendix 3). *L. amethystina* and *L. laccata* were the two most commonly found ECM sporocarps, being found in 10 of the 12 sites. These were followed by *Cortinarius acutus*, *R. ochroleuca*, *Cortinarius flexipes* and *Lactarius tabidus*, and the rest of the species were found as sporocarps in less than half of the sites (Appendix 3). All of the aforementioned species, except *L. tabidus*, were also prolific sporocarp producers, with 205, 212, 71, 120 and 76 sporocarps, respectively, collected across all sites. *L. laccata*, *L. amethystina* and *R. ochroleuca* were the only ECM species that produced sporocarps in all years of the study (data not shown). The most species-rich genera above ground were *Cortinarius*, *Russula* and *Lactarius* with 15, 12 and 11 species, respectively. Based on the sporocarp data, 12 species were shared between all forest types, four between oak and Scots pine, six between oak and Sitka spruce and six between Scots pine and Sitka spruce.

In all plots except two Scots pine plots, there was a higher taxa richness of ECM fungi found above than below

ground, particularly in the oak plots (Fig. 4). The Jaccard similarity between the above- and below-ground ECM assemblages was low in all sites. The four oak plots had a mean similarity value of 12±9%, the Scots pine plots 7±1% and the Sitka spruce plots 9±14% between their above- and below-ground ECM taxa (Table 3). Moreover, the results of a Mantel test (using a quantitative similarity index; chi square) indicated that there was no significant relationship between the above- and below-ground ECM similarity matrices for the plots (Mantel statistic r=0.09; P=0.36).

A number of species were more frequent either as above-ground sporocarps or below-ground ectomycorrhizas. Ectomycorrhizas of *Suillus variegatus* were frequently found, yet sporocarps were rarely found. *Cortinarius obtusus* and *Lactarius hepaticus* were of similar frequency above- and below-ground. *Cantharellus cibarius*, *Craterellus tubaeformis*, *Cortinarius rubellus*, *L. amethystina*, *L. laccata* and *R. ochroleuca* had higher frequencies of occurrence above than below ground.

ECM community analysis

DCA showed that the below-ground ECM assemblages separated well according to forest type (Fig. 5). This was most marked for the oak plots, but there was some overlap between the Scots pine and Sitka spruce plots. MRPP analysis confirmed statistically that ECM assemblages from plots of the same forest type were more similar to each than they were to assemblages from a different forest type (A=0.03; P=0.04). The ordination explained a total 28% of the variation between the original and ordination space.

The ECM community of the oak forest type was distinguished by having a high frequency of *C. geophilum*, morphotype 5e7 and *Elaphomyces granulatus*. Morphotype 5e7 produces contact-type ectomycorrhizas and was present in three of the four oak plots. *E. granulatus* was present in two of the oak plots as ectomycorrhizas and one more of the plots as hypogeous sporocarps. *L. amethystina* ectomycorrhizas were present in three of the four oak plots, with sporocarps of this species being found in only one of the oak plots. The most common ectomycorrhizas in the Scots pine plots were *C. geophilum*, *Pseudotomentella griseopergamacea* and morphotype 4h5 and 5e7. *C. geophilum* was present in three pine plots at high frequencies (50–77% of sub-samples), yet completely absent from the pine plot at Annagh. *C. geophilum* and *Piceirhiza horti-inflata* were the two most common ectomycorrhizas in the Sitka spruce plots. Both of these were present in three of the Sitka spruce plots, but there was a large variation in frequency between plots (Appendix 1). The spruce plots shared few ECM types between them; 13 of 18 taxa were found in just one spruce plot.

DCA ordination was also carried out on the above-ground sporocarp assemblages (Fig. 6). No clear separation of the plots according to forest type was found, a result confirmed by a non-significant MRPP test (A=0.001; P=0.43), indicating that the plots were not separated in a similar fashion based on forest type and above-ground ECM assemblages. The lack of differentiation of the plots was due to the common occurrence in all forest types of sporocarps of species such as *Amanita rubescens*, *Clavaria rugosa*, *C. acutus*, *C. flexipes*, *L. tabidus*, *L. laccata*, *L. amethystina*, and *R. ochroleuca* (Fig. 6 and Appendix 3).

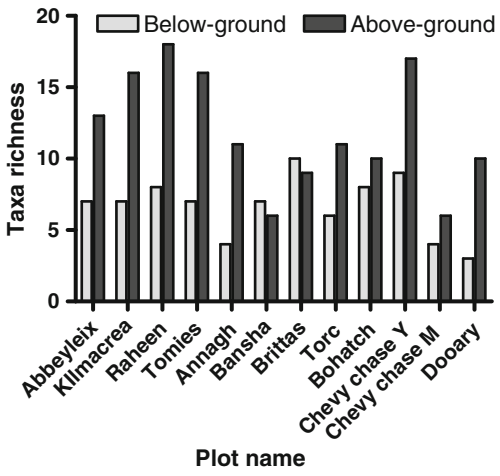

Fig. 4 Below- and above-ground ectomycorrhizal taxa richness of the plots. *Plots 1–4* are oak plots, *5–8* are Scots pine plots and *9–12* are Sitka spruce plots

Discussion

Although there were marked differences in the composition of the ECM communities between the three forest types, there was no significant difference in ECM taxa richness. This shows that Sitka spruce and Scots pine plantations in Ireland can support similar levels of ECM fungi as native oak forests. Above-ground sporocarp studies in Ireland (O'Hanlon 2011; O'Hanlon and Harrington 2011b) and the UK (Humphrey et al. 2000; Ferris et al. 2000) have highlighted the ability of plantation Sitka spruce and Scots pine forests to support many ECM fungi. In the absence of co-invasion by ECM symbionts, the ability of exotic tree species to form ECM linkages with native ECM fungi can be important for tree species survival and growth (Nuñez et al. 2009; Pringle et al. 2009), and the ECM generalist nature of Sitka spruce (Alexander and Watling 1987) may be partly responsible for its good survival and growth rates in Ireland and the UK. The taxonomic richness and community structure of the Sitka spruce forest type in this study is similar to that found in Sitka spruce in its home range. Both Helm et al. (1996) and Wurzberger et al. (2004)

Table 3 Modified Jaccard similarity index (for more information see Methods) between above- and below-ground ECM communities in the different plots and forest types

Forest type	Jaccard similarity (%)				Mean (±SD)
Oak	Abbeyleix (18)	Kilmacrea (21)	Raheen (0)	Tomies (10)	12 (±9)
Scots pine	Annagh (7)	Bansha (8)	Brittas (6)	Torc (6)	7 (±1)
Sitka spruce	Bohatch (29)	Chevy chase mature (0)	Chevy chase young (8)	Dooary (0)	9 (±14)

Each plot is listed along with the per cent similarity value (in parenthesis). The final column lists the mean (and standard deviation) Jaccard value for each forest type

in Alaska and Bothwell et al. (2001) on Vancouver Island found similar taxonomic richness (6, 12 and 8 taxa respectively) and community structure (dominated by *C. geophilum*) on Sitka spruce roots. In comparison to the ECM communities of other Pacific Northwest coniferous forests, it would seem that the Sitka spruce ECM community is comparatively species poor and has a noticeable dominance of a few taxa. Research in mature hybrid spruce (*Picea glauca* × *Picea sitchensis*) by Kranabetter (2004) and Douglas fir forests by Goodman and Trofymow (1998) in British Columbia found a much higher taxa richness (35 taxa and 69 taxa, respectively) and a more even distribution of taxa across the community than that in Sitka spruce forests in this and the previously listed studies. Although differences in host tree species, forest age and sampling strategy make comparisons between these studies difficult.

The community structure with regard to the abundance of ECM taxa was similar in all of the forest types examined, following the log-normal distribution similar to ECM communities in other coniferous (Horton and Bruns 2001; Kranabetter 2004; Luoma et al. 2006; Cline et al. 2005) and deciduous forests (Courty et al. 2008). This distribution is due to the samples being dominated by a few ECM taxa with high abundances, with the remainder taxa present at low frequencies. It is thought that the large number of taxa present at low abundances is an "insurance measure" carried out by trees, whereby the tree supports excess ECM species in case ecological conditions change and the dominant ECM fungi are not suited to the new

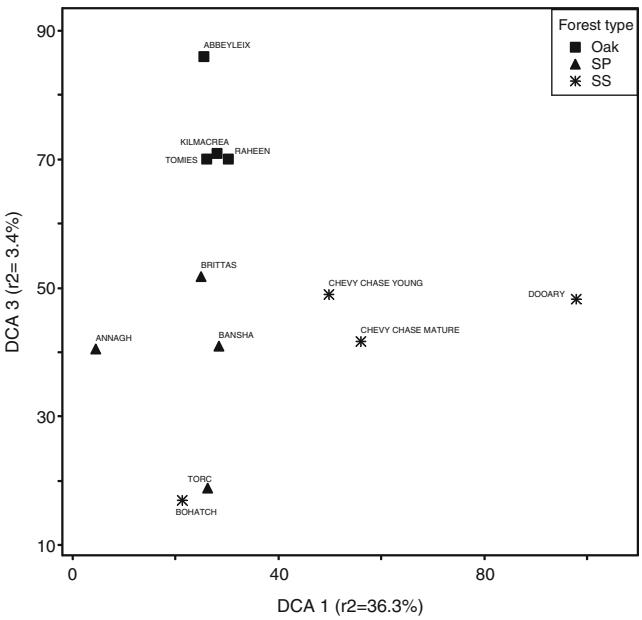

Fig. 5 Detrended correspondence analysis (DCA) ordination of the below-ground ectomycorrhizal taxa abundances from the plots. Plots with a different forest type (based on dominant tree species) are marked with *different symbols*. Eigenvalues of first, second and third DCA axes are 0.93, 0.65 and 0.45, respectively. The percentage of variation represented by the axes was calculated using the relative Euclidian distance measure, with the ordination representing 28.2% of the variation between the ordination and original space

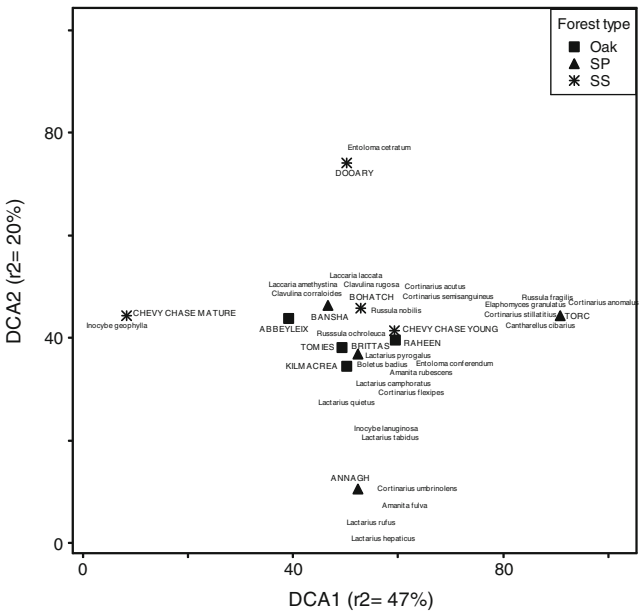

Fig. 6 Detrended correspondence analysis (DCA) ordination of the above-ground ectomycorrhizal taxa abundances from the plots. Plots with a different forest type (based on dominant tree species) are marked with *different symbols*. The species that were found in two or more forest types are also indicated on the ordination. Eigenvalues of first, second and third DCA axes are 0.77, 0.63 and 0.34, respectively. The percentage of variation represented by the axes was calculated using the relative Euclidian distance measure, with the ordination representing 76% of the variation between the ordination and original space

conditions (Druebert et al. 2009; Pena et al. 2010). In the case of all three forest types, *C. geophilum* was found to be one of the most frequent ECM taxa, a finding common to many other ECM surveys in different forest types (Goodman and Trofymow 1998; Luoma et al. 2006; Ishida et al. 2007; Buee et al. 2007; Pickles et al. 2010; Lang et al. 2011).

ECM community analysis using below-ground data showed that each forest type had a statistically distinctive ECM community; this was because most ECM taxa recorded from a forest type were confined to that forest type, and relatively few taxa were shared between the three forest types. Oak forest plots showed the highest similarity to each other as regards the ECM communities, and the Sitka spruce plots the least. The most common ECM morphotype in the oak sites was *C. geophilum*, a finding shared with studies in oak forests in many countries (Valentine et al. 2004; Richard et al. 2005; Buee et al. 2007). Ectomycorrhizas of *E. granulatus*, *Amanita citrina*, *L. amethystina* and a number of unidentified morphotypes were common in the oak forest plots. These species are common as sporocarps in oak forests in Ireland (O'Hanlon 2011) and elsewhere (Buee et al. 2011). The Scots pine plots also had a distinctive ECM community. *C. geophilum* and *P. griseopergamacea* were the most common species, while *L. hepaticus* was found as both ectomycorrhizas and sporocarps. The Scots pine plots were much poorer in ECM species than native Scot pine plots in Scotland, particularly *Cortinarius* and *Suillus* (Pickles et al. 2010).

The Sitka spruce forest type was the most species rich in terms of above-ground ECM diversity, although this was not reflected to the same extent in the diversity of ectomycorrhizas below ground. Certain species (e.g. *Cortinarius cinnamomeus*, *C. rubellus*) were confined to Sitka spruce plots. Sitka spruce plots were the most variable of all the forest types with regard to their below-ground ECM communities. This mirrored similar variability in above-ground sporocarp communities (O'Hanlon 2011). Possible reasons for this high intra-plot variability include differences in soil characteristics that are known to influence the composition of ECM communities (Erland and Taylor 2002), the previous vegetation cover of the sites (Jumpponen et al. 1999) and the ECM generalist nature of Sitka spruce (Alexander and Watling 1987). The species richness of ECM assemblages in Sitka spruce forests in Ireland and native Sitka forests in North America (Wurzburger et al. 2004) were broadly similar; however, the composition of the ECM assemblages of the two regions were found to be different, with the native forest supporting ectomycorrhizal taxa such as *Hebeloma*, *Clavulina* and *Piloderma* species, while this study found *Piceirhiza* spp., *Tylospora* sp. and *Amphinema* sp. were most common. Similar to previous examinations of the ectomycorrhizas of exotic Sitka spruce plantations (Thomas et al. 1983; Taylor and Alexander 1989; Heslin et al. 1992; Flynn et al. 1998; Palfner et al. 2005), this study has found that the ECM community of plantation Sitka spruce forests in Ireland is made up of taxa normally abundant in nursery seedlings (e.g. *P. horti-inflata*, *P. nigra*, *Tylospora* spp. and *Amphinema* spp.) (Grogan et al. 1994; Menkis et al. 2005). Under natural regeneration circumstances, these pioneer (sensu Newton 1992) ectomycorrhizas would most certainly be replaced by multi-stage or late stage ectomycorrhizas (Thomas et al. 1983; Visser 1995; Jones et al. 1997). However, the lack of inoculum of these ectomycorrhizas and short growth period of these forests areas could impede this natural succession of ECM fungi.

The distinctive below-ground ECM communities differentiated by forests type were not mirrored by similar above-ground sporocarp assemblages. This is because some species that were widely distributed as sporocarps between the forest types were not very widely found on roots. For example, *L. laccata*, *L. amethystina* and *R. ochroleuca* were widely distributed in all three forest types but were not found commonly as ecotomycorrhizas. Conversely, some ECMs that were common on roots were not found in the sporocarp assemblages. The overall similarity between the above- and below-ground ECM taxa was low; on average, <10% of the species occurred as both sporocarps and ectomycorrhizas on roots. It is common to find disparities between above- and below-ground ECM assemblages (Gardes and Bruns 1996; Dahlberg et al. 1997; Peter et al. 2001; Richard et al. 2005; Porter et al. 2008). Many of the disparities are due to actual differences in distributions related to ECM sporocarp production patterns and the fact that many common types do not form sporocarps or form inconspicuous sporocarps that can be overlooked (e.g. *Tomentella* spp.). The functional morphology of other species would make them difficult to record in the relatively short (3 years) time period of our sporocarp survey. Recent work by Peay et al. (2011) has attempted to explain the sequence of ECM fungi that appear in forests and also the irregular fruiting patterns of other ECM species through the functional morphology hypothesis. Some species (such as some *Cortinarius* species) rely more on long distance exploration hyphae to colonise new roots than on spore dispersal (Agerer 2001) and, therefore, only irregularly produce sporocarps (Straatsma et al. 2001). Other species such as *Laccaria* species (Gherbi et al. 1999) and some *Russula* species (Redecker et al. 2001) rely more on spore propagation than mycelia contact to colonise new roots and, therefore, are frequently present as sporocarps during the sampling season in coniferous (Dahlberg et al. 1997) and deciduous (Smith et al. 2007) forests.

Insufficient sampling intensity below ground is another likely cause of the large disparities between the ECM communities found in this ECM root and sporocarp analysis. Below-ground ectomycorrhizal communities are known to show horizontal (Dickie and Reich 2005; Pickles

et al. 2010), vertical (Dickie et al. 2002; Baier et al. 2006; Genney et al. 2006; Scattolin et al. 2008) and temporal (Izzo et al. 2005; Koide et al. 2007; Courty et al. 2008) variation in forest ecosystems. Our below-ground sampling scheme only described the ECM communities, which were found at one distinct location in space and time. Conversely, our above-ground data recorded ECM species that may have been present as ectomycorrhizas in many vertical and horizontal locations along the soil profile, including ECM species that may have been ectomycorrhizas on host species other than our target hosts. Moreover, we collected sporocarp data across three seasons whilst only collecting ectomycorrhizas over one season. The difficulty with trying to give equal time and resources to the above- and below-ground study of ectomycorrhizas has been commented upon by Taylor (2002), and it is estimated that equal sampling of the above- and below-ground communities is not feasible, at least not on an equal area basis.

In summary, this research has described, for the first time, part of the ECM assemblages of native oak and non-native Scots pine forests and revealed further information about the non-native Sitka spruce forest ECM communities in Ireland. That the exotic species Sitka spruce can support as high ECM taxa richness as native oak forests indicates that these plantation forests may provide a suitable habitat for native ECM fungi. Further research into the ECM communities of other native and non-native forests in Ireland would reveal even more about ECM diversity in a country with low historic levels of forest cover and increasing current levels of exotic forests.

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