Ectomycorrhizal fungal communities at forest edges

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Summary

1 Ectomycorrhizal fungi are spatially associated with established ectomycorrhizal vegetation, but the influence of distance from established vegetation on the presence, abundance, diversity and community composition of fungi is not well understood.

2 We examined mycorrhizal communities in two abandoned agricultural fields in Minnesota, USA, using *Quercus macrocarpa* seedlings as an *in situ* bioassay for ectomycorrhizal fungi from 0 to 20 m distance from the forest edge.

3 There were marked effects of distance on all aspects of fungal communities. The abundance of mycorrhiza was uniformly high near trees, declined rapidly around 15 m from the base of trees and was uniformly low at 20 m. All seedlings between 0 and 8 m distance from forest edges were ectomycorrhizal, but many seedlings at 16–20 m were uninfected in one of the two years of the study. Species richness of fungi also declined with distance from trees.

4 Different species of fungi were found at different distances from the edge. 'Rare' species (found only once or twice) dominated the community at 0 m, *Russula* spp. were dominants from 4 to 12 m, and *Astraeus* sp. and a Pezizalean fungus were abundant at 12 m to 20 m. *Cenococcum geophilum*, the most dominant species found, was abundant both near trees and distant from trees, with lowest relative abundance at intermediate distances.

5 Our data suggest that seedlings germinating at some distance from established ectomycorrhizal vegetation (15.5 m in the present study) have low levels of infection, at least in the first year of growth. Distance from established vegetation represents an important gradient for ectomycorrhizal fungi, with different species occupying distinct niches along this gradient. This provides support for niche differentiation as a factor contributing to ectomycorrhizal fungal diversity.

6 Ectomycorrhizal infection of seedlings is spatially complex, with high infection and high fungal diversity near trees, high infection but lower diversity at intermediate distances, and low infection and low fungal diversity distant from trees. This spatial complexity should be considered as a factor potentially influencing the establishment of ectomycorrhizal vegetation.

Key-words: ascomycetes, Cedar Creek LTER, diversity, ectomycorrhiza, facilitation, fungal communities, old field succession, *Quercus* (Oak), resource partitioning, seedling establishment

Introduction

Ectomycorrhizal fungi are not uniformly distributed in terms of their presence, abundance or community composition. This has important implications for plant ecology, as the vegetation with which they are associated is unlikely to survive where these fungi are absent. In particular, a lack of ectomycorrhizal fungi may slow the invasion of disturbed sites by trees or may play a role in the community dynamics of mixed ectomycorrhizal/arbuscular mycorrhizal forests. On a global scale, their lack has been a major limitation to reforestation in areas with no native ectomycorrhizal vegetation (Marx 1991). On a local scale, seedlings germinating distant from such vegetation may have very low levels of infection, while ectomycorrhizal plants may facilitate the establishment of seedlings by supporting patches of fungi (Baylis 1980;

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Borchers & Perry 1987; Perry *et al*. 1989; Dickie *et al*. 2002a; Dickie *et al*. 2004). Such facilitation is likely to be apparent in sites where ectomycorrhizal fungi are absent following natural (Allen 1987; Terwilliger & Pastor 1999) or anthropogenic disturbance (Perry *et al*. 1982; Alexander *et al*. 1992; Kranabetter 1999; Jones *et al*. 2003). Limitation of seedling establishment due to low availability of fungi may also be apparent in systems where ectomycorrhizal plants are spatially dispersed in a matrix of non-ectomycorrhizal vegetation such as oak and pine savannas or oak/maple forests (Dickie *et al*. 2002a).

Communities of ectomycorrhizal fungi also show distinct patterns of fungal distributions relative to distance from trees, with certain species found only proximal or distal to the tree stem both in terms of fruit bodies (Mason *et al*. 1982; Last *et al*. 1984) and mycorrhizal infection of seedlings (Deacon *et al*. 1983; Borchers & Perry 1990; Dickie *et al*. 2002a). Niche partitioning by fungi along distance gradients from trees has been proposed as a mechanism explaining the typically high diversity of ectomycorrhizal fungal communities (Bruns 1995). Differences in fungal communities may directly influence the ability of seedlings to obtain nutrients from different sources (Lilleskov *et al*. 2002). In addition, the species richness of fungi on seedlings has been found to be lower distant from trees than near trees (Kranabetter & Wylie 1998; Durall *et al*. 1999; Hagerman *et al*. 1999; Kranabetter 1999; Dickie *et al*. 2002a; Lindahl 2002). Mycorrhizal diversity may be beneficial to plants in nutrient-poor soils (Jonsson *et al*. 2001), as different species of fungi may access different nutrient sources. Low diversity of ectomycorrhizal fungi may therefore be an additional limitation on seedlings germinating distant from established trees.

The objectives of the present study were (i) to determine how mycorrhizal infection of seedlings is influenced by distance from established trees, and (ii) to determine how communities of fungi differ with distance from trees. One limitation of many prior studies has been treating proximity to trees as a dichotomy rather than a continuous variable, such that it is not presently known at what distance from trees infection of seedlings declines or whether the change is gradual or abrupt. We therefore used transects at the border between oak woodlands and abandoned agricultural fields (old fields) to examine the nature of the change in infection as a function of distance. We examined four aspects of mycorrhizal infection of seedlings: its presence, extent and species richness, and the community composition as measured by frequency and relative abundance of different fungal species.

Methods

STUDY SITES

We used two fields, Field 56 and Field 57, at the Cedar Creek Natural History Area and Long-term Ecological

Research (LTER) site of the University of Minnesota, located in Anoka County, MN, USA. Field 56 (UTM 15T 485447 mE, 5028523 mN, 282 m elevation) was abandoned from agricultural use around 1958 and Field 57 (UTM 15T 485739 mE, 5028234 mN, 278 m) was abandoned around 1943. The northern edge of both fields bordered established oak woodlands with a relatively straight edge between the two habitats, running east–west. The area is flat and soils are very sandy, excessively drained Typic Udipsamment, Nymore series soils (94% sand, 5% silt, 1% clay), formed from glacial outwash sediments (Grigal *et al*. 1974) with average pH 5.4 (0–20 cm depth, 1 : 2 H_2O extract). Herbaceous vegetation in Field 56 was dominated by *Poa* spp. (30% average midpoint of Daubenmire cover classes based on visual estimate of cover), *Bromus* spp. (25%), *Panicum* sp. (10%), *Ambrosia* sp. (4%), and *Equisetum* sp. (2%), while Field 57 was dominated by *Poa* spp. (52%), as well as *Ambrosia* sp. (7%) and *Agropyron* sp. (3%). Other species individually contributed less than 2% cover. *Helianthemum bicknellii*, an herbaceous plant known to share ectomycorrhizal fungi with *Quercus* (Dickie *et al*. 2004), was present in both fields at < 1% cover. With the notable exception of one transect (see Results), *H. bicknellii* was generally restricted to within 12 m of the forest edge. Both fields have remained largely uncolonized by oak trees, although Field 57 did have a number of oak seedlings and oak sprouts near its northern edge. The fields are separated by about 420 m distance.

EXPERIMENTAL DESIGN

In each of the two fields we established four transects running from the forest edge into the field with plots at 0, 4, 8, 12, 16 and 20 m from a point approximately 1 m from the base of an established *Quercus ellipsoidalis* tree (focal tree). Focal trees were 17–26 cm diameter at breast height (d.b.h.) in Field 56, and 28–46 cm d.b.h. in Field 57. Based on tree diameter-age correlations for oak woodlands at Cedar Creek (P. B. Reich, unpublished data), these trees were likely to be between 40 and 60 and 60–100 years old, respectively, and therefore likely to have established at or before the time when the fields were abandoned from agriculture. The 0-m plot was within the drip line, but none of the subsequent plots were under canopy cover. All transects ran approximately due south from the north edge of fields, so that only the 0-m plot had experienced significant shading from the forest. In hindsight, it would have been interesting to extend transects further into the forest, but this was not necessary for our objectives.

PLANTING FOR 2001

Each plot was planted with four bur oak (*Q. macrocarpa*) acorns. Using con-generic (rather than con-specific) seedling/focal tree combinations reduces the possibility of root grafting, which is common in oaks (Lyford 1980), while host specificity of fungi tends to be at the genus,

not species level (Molina *et al.* 1992). Acorns were collected from local seed sources in the autumn of 2000, stored under refrigeration, and planted on 9 July 2001. A 30 cm diameter, 60 cm tall cage of 1.25 cm mesh galvanized 'hardware cloth' was placed over seedlings to protect them from mammalian herbivory. Seedlings were watered during early establishment one to two times per week, and on a few occasions during the dry summer of 2001 to avoid seedling mortality. In September 2001, all surviving oak seedlings were harvested. Due to mortality during the study, only 40 of the initial 48 plots had at least one seedling for harvest in 2001. Only one seedling from each plot was measured for ectomycorrhizal infection (data on seedling mortality, biomass and nutrient uptake were recorded, but will be presented in a later paper after third-year growth data are obtained).

PLANTING FOR 2002

Q. macrocarpa seeds normally disperse, germinate and begin to establish a root system in the autumn. We were concerned that planting in spring (as in 2001) might have artificially reduced the ability of seedlings to encounter fungi, as many fungal spores disperse in the autumn. We therefore planted acorns for 2002 analyses in the autumn of 2001 rather than in spring 2002. Acorns were collected from the same sources as in 2001, stored for approximately 3 weeks under refrigeration, and planted in late September. Plots for 2002 were placed as close as possible to, but not overlapping, the plot locations from 2001 to avoid the area disturbed during the 2001 harvest. In September 2002 one seedling was harvested from each of a total of 40 plots (four plots had no surviving seedlings, and an additional four had only one seedling, which was left for future measurement of growth and nutrient uptake). Seedlings were harvested from each position on each transect in at least one of the two years.

HARVESTING AND IDENTIFICATION OF ECTOMYCORRHIZAL FUNGI

Although the extremely sandy soils made it possible to harvest root systems in excellent condition, we did not obtain 100% of the seedling roots. The extremely long tap root of many seedlings was not harvested; in some cases this root was followed as far as 1 m into the soil without reaching a natural termination. Harvested roots were washed under running water over a wire screen and then stored in water under refrigeration for no more than 21 days before measurement of mycorrhizal infection. No visible degradation of roots occurred during storage. Fine roots were cut into 1–3 cm sections and a random sample of at least 100 root tips per seedling was examined. On each root fragment we counted all ectomycorrhiza as number of root tips infected, regardless of whether tips were individual monopoid mycorrhizas or part of a larger cluster of ectomycorrhizal roots (tuberculate mycorrhizas were not observed). In

total, 30 815 root tips were counted (average 385 root tips per seedling); only five seedlings had fewer than 100 root tips counted. Very few root tips were encountered that appeared dead, but any necrotic root tips or root tips that compressed in forceps were disregarded.

On each seedling we visually categorized all ectomycorrhizal root tips into *ad hoc* morphological groups. Here we are using the term 'morphological group' to indicate a less detailed examination than a true 'morphotype' in the sense defined by Agerer (1987), as RFLP analysis rather than morphology was used to combine morphological groups across seedlings. The morphotype *Cenococcum geophilum* was recognized on the basis of characteristic radiate mantle morphology (LoBuglio 1999). Recent work suggests that the *C. geophilum* morphotype encompasses significant genetic variation even within individual stands and soil cores (Jany *et al.* 2002), and we now know that there are at least two distinct RFLP patterns obtainable from *C. geophilum* morphotypes in other studies at nearby locations (Avis *et al.* 2003). Our identification of *C. geophilum* based on morphology may therefore underestimate diversity. From all other morphological groups on each seedling we saved three (or as many as available up to three) samples for DNA analysis, freezing samples in liquid N for later RFLP analysis. All frozen root tips were lyophilized, then stored at −20 °C until DNA analysis. For each morphological group from each seedling we performed two or more replicate RFLP analyses. DNA was extracted and amplified from root tips using the kit-based protocol of Avis *et al.* (2003), with internal transcribed spacer rDNA amplified using ITS1F and ITS4 primers (Gardes & Bruns 1993), and RFLP patterns obtained for two enzymes, HinfI and DpnII (NEB, Beverly, MA, USA). Morphological groups from different seedlings were combined on the basis of RFLP matching using the GERM program (Dickie *et al.* 2003).

We attempted to sequence all RFLP-species found at least five times, although one species (RFLP matching to an *Entoloma* sp. sporocarp collection) was never successfully sequenced, probably due to the presence of more than one fungal DNA sequence in root tip samples. Sequences were obtained using the ITS primers ITS1F and ITS4 or the LSU primers Ctb6 and TW13, following the same procedures as in Avis *et al.* (2003). Sequenced samples were compared with known species in GenBank using BLAST searching. Matching sequences were obtained, aligned, and phylogenies examined using PAUP 4.0b (Swofford 2001) to corroborate RFLP and BLAST identifications. In no case did the conclusions of phylogenetic analyses substantially differ from the best matching known species from BLAST searches.

STATISTICS

Analysis of presence of ectomycorrhiza

The effect of distance on the presence of mycorrhiza was analysed with a generalized linear mixed effects

model using the glmmPQL function in the MASS (Venables & Ripley 2002) package of R (RAqua 1.8.1, R Foundation for Statistical Computing, Vienna, Austria). We designated transect (grouped within field and year) as a random effect, and used a binomial family with a logit link to test the effects of distance as a first order polynomial on mycorrhizal presence. Where the quadratic term was not significant, it was removed and a linear model tested. The significance of field and year effects was tested by removing each random effect and comparing alternative models using likelihood ratio tests (Pinheiro & Bates 2000). *P*-values for distance and the square of distance and correlation coefficients are reported for the full model (including all random effects).

Analysis of extent of ectomycorrhizal infection

We used a maximum likelihood approach to find the most parsimonious model to describe the decline in ectomycorrhizal infection as a function of distance. Our initial (full) model of ectomycorrhizal infection as a function of distance from trees was a four-parameter logistic model:

$$E = \alpha + \frac{\beta - \alpha}{1 + e^{[(D-\lambda)/\theta]}} \qquad \text{eqn 1}$$

where E = ectomycorrhizal infection (0–100%), D = distance from trees (0–20 m in our data), α = infection distant from trees (the right asymptote), β = infection near trees (the left asymptote), λ = distance where ectomycorrhizal infection is halfway between α and β, and θ determines the slope (Venables & Ripley 2002). Terms were removed (set equal to 0 or, in the case of θ, 1) and Akaike information criterion (AIC) used to select the most parsimonious model for the data (Burnham & Anderson 1998). Random terms for field and year were tested by the same procedure. Because of over-parameterization, a random effect of transect was not included.

Community level analyses (richness, composition, relative abundance)

Given that mycorrhizal fungi have an extended life span, the detection of a particular species in a given plot in two consecutive years cannot be considered independent observations. We therefore aggregated data from the 2 years for the analysis of community variables, including analyses of richness, community composition and relative abundance. Because of the potential for confounding year effects with actual distance effects (particularly due to changes in methods between years), we also performed analyses on only the 2002 subset of the data to confirm results. There were 38 plots with ectomycorrhizal fungi present in 2002 (lowering replication from 41 in the combined data).

For all community analyses, including the calculation of species richness, we excluded plots where ectomycorrhizal fungi were absent. There does not appear to be consensus on whether such plots should be included in community analyses and we wanted to avoid confounding the previously analysed effects of distance on the presence of mycorrhiza with the effect of distance on ectomycorrhizal communities *per se*. Excluding plots with no ectomycorrhizas reduced replication from 48 (all plots had data for at least one of the two years) to 41.

Analysis of species richness

Richness was calculated as the number of species detected in a plot across both years, and analysed using the same procedure as for the initial analysis of extent of mycorrhizal infection (first-order polynomial mixed effects model). Random terms were included for transect within field, with field effects tested using likelihood ratio tests.

### *Analysis of community composition*

All species found in five or more plots were analysed in univariate tests for frequency and relative abundance. Frequencies of each species were analysed following the same generalized mixed effects first-order polynomial model (with a binomial family and logit link) as used for presence of ectomycorrhiza. Relative abundances of individual species were analysed using the approach described for the analysis of species richness.

To avoid potential concerns over experiment-wise error rates, we also performed MANOVAs on frequencies and rank transformed abundances of all species found five or more times, with distance as a factor and including transect within field effects (although because of software limitations these were fit as fixed, not random effects) using JMP (5.0.1a, SAS Institute, Cary, NC, USA). Rank transformation was used to control for unequal variances (Conover 1999). Pillai's trace was used to evaluate the significance of MANOVA results, as recommended for correlated variables (Zar 1999); this gave the most conservative estimate of significance compared with other standard tests.

Results

PRESENCE OF ECTOMYCORRHIZA

All seedlings 0, 4 and 8 m from the forest edge had mycorrhizal infection in both years, while there was a substantial decline in the presence of ectomycorrhizas at 12–20 m in 2001, where they were absent from 65% of seedlings (Fig. 1a,b). In 2002, only three seedlings had no ectomycorrhizas (one each at 12, 16 and 20 m, representing 16% of seedlings at these distances). In a complete model, distance from trees had significant linear and quadratic effects on the presence of ecto-mycorrhizas ($P = 0.0146$ and $P = 0.0330$, respectively) and there was a significant effect of Year ($P < 0.0001$). Although the quadratic distance term was statistically significant, the fitted model implied that the frequency
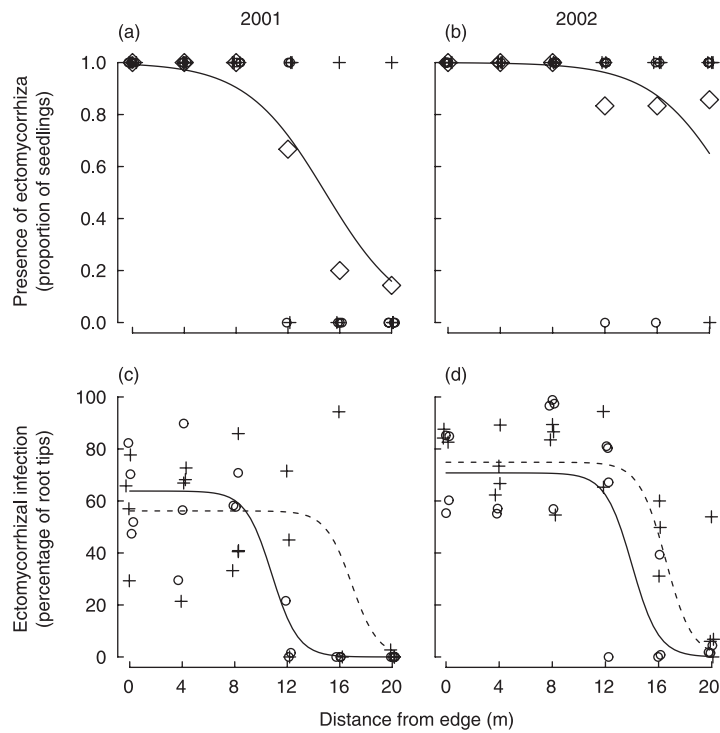
Fig. 1 Presence (a, b) and extent (c, d) of ectomycorrhizal infection as a function of distance from the forest edge for 2001 (a, c) and 2002 (b, d). Circles indicate data points from Field 56, crosses indicate data points from Field 57. A small amount of 'noise' has been added to distance to separate overlapping data points, all data points actually fell at the distances labelled on the *x*-axes. For the presence of ectomycorrhiza (a, b), lines indicate the linear fixed effect of distance on the probability of seedling infection for each year, back-transformed from logistic analysis (i.e. curvature is due to back-transformation), diamonds indicate mean values for each distance. Year and distance were both significant, $P < 0.0001$ for both, $r^2 = 0.41$. For extent of mycorrhizal infection (c, d), lines indicate the most parsimonious model (two parameter logistic model, equation 2, $r^2 = 0.46$). Including terms for both year and field resulted in lower AIC values than models without terms for year or field, solid line indicates fit for Field 56, dashed line indicates fit for Field 57.

of ectomycorrhizal infection increased back to 100% at some distance beyond 20 m. We therefore opted for a more biologically reasonable model with the linear effect of distance as the only fixed effect ($P < 0.0001$, $r^2 = 0.41$, Fig. 1). Note that the curvature of the fitted line in Fig. 1(a,b) is a result of back-transformation of the binomial model, not due to a non-linear term in the model.
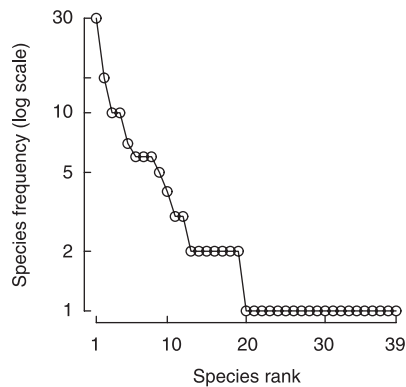
EXTENT OF ECTOMYCORRHIZAL INFECTION

The decline in ectomycorrhizal infection as a function of distance was highly non-linear, with uniformly high infection at 0, 4 and 8 m and near zero infection by 20 m (Fig. 1c,d). Although there was variation between years and fields, the overall pattern was highly robust. The most parsimonious (lowest AIC) fit to the data in non-linear model fitting was a two-parameter logistic model (a special case of equation 1):

$$E = \frac{\beta}{1 + e^{(D-\lambda)}} \qquad \text{eqn 2}$$

Ectomycorrhizal fungal infection was high near trees ($\beta = 66\% \pm 5$; mean and SE) and declined rapidly (θ from equation 1 effectively 1) to near 0 (α from equation 1 effectively 0), with 50% infection at around 15.5 m

from the base of trees ($\lambda = 14.5 \text{ m} \pm 1.4$, +1 m to account for the offset of the 0-m plot from the base of trees). Including terms for Year and Field significantly improved the model. Differences between years were largely due to differences in infection near trees (β), with greater infection near trees in 2002 than in 2001 in both fields, while differences between fields were largely due to differences in the distance at which infection declined (λ). The differences between years may reflect the substantial change in methods (2001 seedlings were planted 4 months before harvest, 2002 seedlings were planted 12 months before harvest), or somewhat moister climatic conditions in 2002 than in 2001. Differences between fields may reflect the difference in edge character. Focal trees were larger in Field 57 than Field 56 (39 cm vs. 26 cm average d.b.h.). Field 57 was also abandoned from agriculture 15 years earlier than Field 56 and had a greater number of oak grubs at the forest edge, while the edge of Field 56 was more abrupt. Excluding a random term for field from models tended to result in inflation of θ and the impression of a more gradual decline in ectomycorrhizal infection than was actually observed.

Only one of the most distant (20 m) plots had any ectomycorrhizal fungi present in 2001 (infecting 2.7% of root tips) and the same plot was a substantial outlier

Fig. 2 Rank abundance diagram for the fungal community, with data averaged across years before calculation of abundances. Abundances are out of a total of 48 plots with seedlings, 41 of which had ectomycorrhizal infection present.

for ectomycorrhizal infection in 2002 (with 54% of root tips infected). Intriguingly, this plot was the only plot beyond 12 m from the forest edge that was near established *Helianthemum bicknellii*, a herbaceous perennial that we have since learned shares ectomycorrhizal fungi with *Quercus* and creates patches of elevated ecto-mycorrhizal inoculum potential for *Quercus* seedlings (Dickie *et al*. 2004). The presence of *H. bicknellii* in this plot may therefore have facilitated the ectomycorrhizal infection of *Quercus* seedlings distant from established trees.

SPECIES RICHNESS OF ECTOMYCORRHIZAL FUNGI

We found a total of 39 RFLP species (hereafter 'species') of fungi, with the majority of species occurring only one or two times (Fig. 2). Species richness of fungi declined markedly with distance from trees, from an average of 3.4 ectomycorrhizal fungi per plot at 0 m to an average 1.8 species per plot at 20 m (Fig. 3a, $r^2 = 0.43$, $P = 0.0015$). Including plots without ectomycorrhizal fungi results in a similar but steeper decline from 3.4 species at 0 m to 0.9 species at 20 m (not shown; $r^2 = 0.41$, $P < 0.0001$). Differences between fields were not significant ($P = 0.68$).

COMMUNITY COMPOSITION

The ectomycorrhizal fungal community was dominated by *Cenococcum geophilum*, *Russula* aff. *amoenolens*, *R.* aff. *pectinatoides* and *Astraeus* sp. (Table 1). The identified *Russula* aff. *amoenolens* was the same species as in Avis *et al*. (2003) and is believed to be related to, but distinct from, *Russula amoenolens*. *Russula* aff. *pecti-natoides* matched in RFLP patterns with Avis *et al*.'s (2003) '*Russula* sp. RFLP18' group. Two Pezizalean fungi were found (Pezizalean I and Pezizalean II here-after). Phylogenetic analysis of LSU sequences placed Pezizalean I in clade VI of Hansen *et al*. (2001) with *Ruhlandiella*, several *Peza* and *Tirmania*; this clade
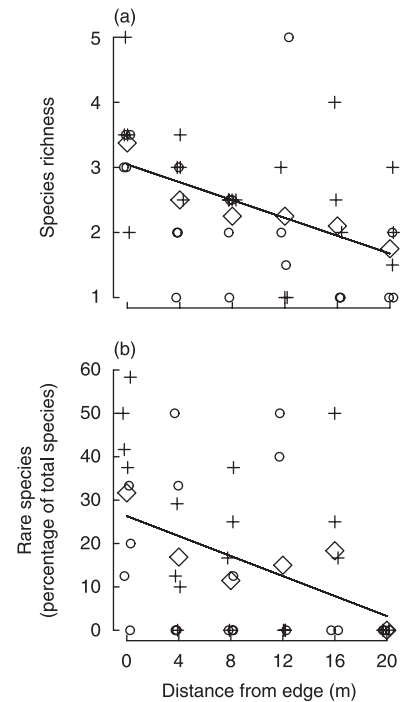
Fig. 3 Species richness (a) and the frequency of rare species (b) as functions of distance. Richness is calculated excluding plots without ectomycorrhiza. Frequency of rare species (species found two or fewer times) is calculated as a percentage of total species in plot. Line indicates the effect of the distance term in the full model. Symbols are as in Fig. 1.

also includes *Terfezia* (K. Hansen, personal communica-tion). Pezizalean II was placed in Clade III of Hansen *et al*. (2001) with *Amylascus*, *Glischroderma* and *Scabropezia* (which form a well-supported subclade within Hansen's Clade III).

There were marked shifts in community composi-tion with distance. Nine species occurred five or more times and were analysed for changes in frequency as a function of distance. Of these, five showed significant changes in frequency with distance (Fig. 4). *Cenococcum geophilum* was more frequent near trees and distant from trees, with lower frequency at intermediate dis-tances. The linear distance term was significant ($P = 0.0432$) and the quadratic term marginally significant ($P = 0.0577$). Both *Russula* species were more frequent at intermediate distances, with significant linear and quadratic terms ($P = 0.0369$ and 0.0213 for *R.* aff. *amoenolens*, and $P = 0.0268$ and 0.0176 for *R.* aff. *pectinatoides*). *Astraeus* sp. increased in frequency as a linear function of distance ($P = 0.0050$). Pezizalean II was also more abundant distant from trees, with a significant linear and quadratic term ($P = 0.0025$ and 0.0035). There was never a significant effect of Field ($P > 0.1$ for all). Quite remarkably, all of the 11 most common species were found in both fields, and the patterns of frequency as a function of distance were similar between fields for the nine species analysed.

Apart from *C. geophilum*, none of the species found five or more times were found more frequently at 0 m than at greater distances, despite the observation that

Table 1 All species in order of number of occurrences (N) giving basis of identification, and best match to a known species in BLAST search of LSU and/or ITS sequence data

			Best BLAST match to known species	
Identity	*n*	Identification basis*	LSU	ITS
Cenococcum geophilum	30	Morphotype		
Russula aff. *amoenolens*	15	Morphotype + RFLP + ITS sequence		AF418615 – Russula amoenolens
Astraeus sp.	10	LSU sequence	AF336238 – *Astraeus hygrometricus*	
Russula aff. *pectinatoides*	10	Morphotype + ITS sequence		AY061732 – *Russula pectinatoides*
Pezizalean I	7	ITS sequence + LSU sequence	AF335175 – *Ruhlandiella berolinensis*	(AF501260 – *Terfezia spinosa*)†
Entoloma sp.	6	RFLP		
Scleroderma sp.	6	LSU sequence	AF336263 – *Scleroderma areolatum*	
Unknown Agaricalean	6	ITS sequence		ER0510276 – (Agaricales)‡
Pezizalean II	5	ITS sequence + LSU sequence	AF335113 – *Amylascus tasmanicus*	AY299231 – (Pezizaceae)‡
Russula aff. *puelaris*	4	Morphotype + RFLP		
Laccaria laccatta	3	RFLP		
Lactarius cf. *argicillifoleus*	3	RFLP		
Tomentella bryophyla	2	RFLP		
Six unidentified species	2			
Amanita sp.	1	Morphotype + ITS sequence		AB015678 – *Amanita longistriata*
Cortinarius sp.	1	RFLP		
Lactarius maculatipes	1	RFLP		
17 unidentified species	1			

*RFLP indicates a match between RFLP pattern and a known RFLP pattern from sporocarp DNA collected within the Cedar Creek Natural History Area. All DNA collections were first sorted by RFLP into 'types'.
†Relatively poor match, perhaps reflective of low numbers of Pezizalean ITS sequences in GenBank.
‡No good matches to identified fungi in BLAST, but good matches to the indicated 'uncultured ectomycorrhiza' sequence data.

species richness within plots was highest at 0 m. In post hoc data analysis to determine what species were responsible for the high species richness at 0 m, we examined the 27 species found only one or two times in the data. These 'rare' species were particularly common at 0 m, declined with distance and were entirely absent at 20 m from the forest edge (Fig. 3b, $P = 0.0071$, $r^2 = 0.24$, field effect not significant). This is independent of the effect of distance on species richness, as the percentage rare species was calculated for each seedling as the number of rare species divided by species richness.

Only the two most abundant species, *Cenococcum geophilum* and *Russula* aff. *amoenolens*, showed significant changes in relative abundance as a function of distance (not shown), which were largely similar to the patterns for frequency. *Cenococcum geophilum* showed the interesting pattern of having the highest relative abundance near the forest edge and distant from the edge ($P < 0.0001$ for linear and quadratic distance terms, field not significant, $r^2 = 0.48$). This was due to the absolute abundance (percentage of root tips) of *C. geophilum* being high near trees, initially falling off more quickly than total ectomycorrhizal infection at intermediate distances, but levelling off at a higher asymptote than most other fungi at 20 m. The relative abundance of *Russula* aff. *amoenolens* was highest at intermediate distances ($P = 0.0459$ and 0.0154 for linear and quadratic distance terms, field not significant, $r^2 = 0.18$), showing the same pattern as for frequency. MANOVA analysis confirmed the presence of overall significant differences between distance classes for species frequencies and rank-transformed relative abundances

($P = 0.017$ and $P = 0.014$, respectively) and the absence of significant field effects for either species frequencies or abundances ($P = 0.28$ and $P = 0.40$).

REANALYSIS OF 2002 SUBSET OF DATA

In the reanalysis of only the 2002 subset of the data to check for artifacts due to aggregation of data, there were only very minor changes in significance or qualitative patterns of results. There were no changes in observed patterns or overall significance (all *P*-values remained < 0.05) of species richness, the proportion of rare species, the frequency of *Astraeus* sp., *Russula* aff. *amoenolens* or *Pezizalean* II, or the relative abundance of *C. geophilum*. The quadratic term in the frequency of *C. geophilum* became significant at $P < 0.05$, while the frequency of *R.* aff. *pectinatoides* and the relative abundance of *R.* aff. *amoenolens* showed only marginal significance ($P < 0.1$ rather than $P < 0.05$). The only qualitative change in the results was that the decline in *R.* aff. *amoenolens* and *R.* aff. *pectinatoides* frequencies at 0 m was less apparent in the 2002 data set than in the aggregated data, as a result of fewer occurrences at intermediate distances in the 2002 data. The overall lack of qualitative changes in the outcomes suggests that the community analysis results based on the aggregated data are valid.

Discussion

The ectomycorrhizal fungal community varied dramatically with distance from the forest edge, with responses
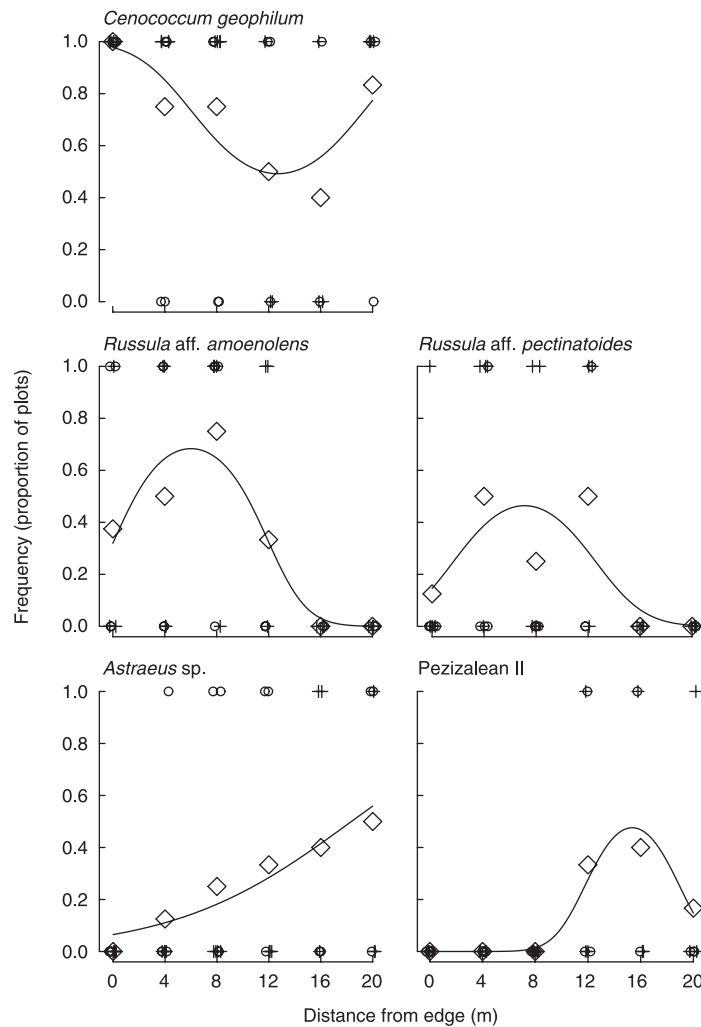
Fig. 4 Significant relationships between distance and the frequency of different species. Lines indicate the linear and quadratic fixed effects of distance on the probability of a species being present, back-transformed from logistic analysis. Symbols are as in Fig. 1.

of each component of the community (total infection, richness and community composition) responding in different ways to distance. These patterns represent a complex spatial mosaic, with implications for both plant succession and fungal community ecology. A lack of ectomycorrhizal infection distant from trees may represent an important limitation on seedling establishment in areas with low densities of established ectomycorrhizal vegetation. This may result in multiple steady states, such that areas without ectomycorrhizal vegetation can persist for long periods without woody encroachment (Perry *et al*. 1989; Terwilliger & Pastor 1999). In this system, seedlings germinating at intermediate distances from trees may have adequate mycorrhizal infection, but are likely to be infected primarily by *Russula* species and to have a lower overall diversity of fungi than seedlings germinating closer to trees. Lower diversity of infection (Baxter & Dighton 2001; Jonsson *et al*. 2001) and shifts in the community composition of fungi (Lilleskov *et al*. 2002) with distance may influence seedling nutrient uptake and growth, although further work is needed in this area.

PRESENCE AND EXTENT OF ECTOMYCORRHIZAL INFECTION

The uniformly high infection near forest edges followed by a rapid decline suggests that seedling mycorrhizal infection is influenced by established tree roots (Newman 1988). Lyford (1980) found roots of mature *Q. rubra* trees terminated approximately 15 m from the base of the tree, while Weaver & Kramer (1932) describe lateral roots of *Q. macrocarpa* extending more than 18.3 m; these distances are similar to the value found for λ for mycorrhizal infection (15.5 m). Our results suggest that seedlings establishing in forest gaps, or in old fields beyond some minimum distance from adult trees, are likely to be limited by a lack of ectomycorrhizal infection. The minimum distance is likely to be a function of both tree size and between-species differences in the lateral extent of root distributions, as well as the age of the gap (Parsons *et al*. 1994). Infection may also be limiting in forests with low densities of ectomycorrhizal trees, including savanna and mixed ectomycorrhizal/arbuscular mycorrhizal forests dominated by

the latter species. The presence of ectomycorrhizal shrubs, established seedlings and stump sprouts also needs to be considered, as they may provide additional sources of infection (Horton *et al.* 1999; Hagerman *et al.* 2001; Dickie *et al.* 2004; Hagerman & Durall 2004).

The virtual absence of mycorrhizas at 16–20 m from the forest edge in one of the two years of the study is somewhat remarkable, given that ectomycorrhizal fungi produce copious sporocarps and spores. Although sporocarps were not frequently observed within the fields, there were abundant sporocarps in the adjacent woodlands and spores of fungi should be wind dispersed considerable distances. Nonetheless, a similar absence of ectomycorrhizas has been found in other studies on disturbed sites (Boerner *et al.* 1996; Brundrett *et al.* 1996), in beaver meadows (Terwilliger & Pastor 1999) and in plots dominated by arbuscular mycorrhizal vegetation (Horton *et al.* 1999). The presence of mycorrhizas is distinct from the extent of infection of seedlings. A seedling that obtains even very low levels of initial mycorrhizal infection can, at least in principle, provide the fungus with sufficient resources to grow and eventually increase in abundance (Dickie *et al.* 2002a). In contrast, a seedling with no mycorrhizal infection can only wait until mycorrhizal propagules arrive via some stochastic dispersal process.

SPECIES RICHNESS

The high species richness of fungi (39 RFLP species) found in the present study is typical of the diversity of mycorrhizal communities described in other studies (Allen *et al.* 1995; Bruns 1995). The high number of rare species (found one or two times) suggests that many other species may have not been encountered due to limited sampling. In other studies in oak-grassland ecosystems equal or even higher species richness of ectomycorrhizal fungi has been reported (79 in Lindahl 2002; 72 in Avis *et al.* 2003; 39 in Valentine *et al.* 2004), although it is difficult to directly compare studies with different sampling methods and intensities. Although 79% of the fungi in our root tip collections were identified, our failure to match most (23 of 39) of the RFLP types we found to a known sporocarp species is also indicative of a high total diversity. Our present data base of sporocarps is also limited, with only 69 unique RFLP patterns from locally collected epigeous sporocarps and no hypogeous sporocarps (D. McLaughlin *et al.*, unpublished data).

The low species richness of fungi distant from trees is similar to the low richness found on seedlings planted distant from trees in other studies (Kranabetter & Wylie 1998; Hagerman *et al.* 1999; Kranabetter 1999; Dickie *et al.* 2002a; Lindahl 2002). The decline in species richness was largest between 0 and 4 m, with a continued decline out to 20 m. The 0-m plot was 1 m from the base of a tree, and was therefore effectively inside the forest, on soil that was unlikely to have been tilled during agricultural use of the field (given that many

focal trees were present during agricultural use), while at 4 m and beyond plots were within the area affected by agricultural use. This may have resulted in greater heterogeneity of soil substrates at 0 m, creating more potential niches for resource partitioning along vertical (Dickie *et al.* 2002b; Rosling *et al.* 2003) or other axes. There are also persistent shifts in pH (5.09 ± 0.09 at 0 m, 5.53 ± 0.03 at 12–20 m, means and SE), base cations, nutrients and other factors with agricultural use in these sites (Grigal *et al.* 1974), declining quantity of oak litter with distance from the forest edge (declining asymptotically but present in low quantities even at 20 m; data not shown), increased N under trees as compared with grass dominated areas (Reich *et al.* 2001), and likely shifts in other nutrients and soil moisture under oak canopies (Ko & Reich 1993), all of which may also have influenced species richness and community composition of fungi (Last *et al.* 1987).

The lack of a significant field effect on the frequency or abundance of individual species was unexpected, given the high diversity of fungi overall and the marked effect of distance from the forest edge on community composition. This suggests that the contribution of between-site differences (β diversity) in dominant species to total diversity may be relatively small compared with the contribution of more local patterns of niche differentiation (α diversity), as found here for distance from the forest edge, or between-site differences in less dominant species. This finding is similar to patterns of diversity of trees in tropical forests, which are dominated by a few common species across wide areas and a much higher diversity of rare species (Pitman *et al.* 2001).

COMMUNITY COMPOSITION

There appear to be three distinct patterns of niche differentiation by ectomycorrhizal species along horizontal gradients from trees: species found primarily near trees (rare species), species found further from the edge but within the presumptive root zone of trees (*Russula* species), and species found more abundantly distant from trees (*Astraeus* sp. and Pezizalean II), although other species may be found both near and distant from trees (*Cenococcum geophilum*). Although only three patterns of horizontal niche differentiation were found, if these are multiplied by other axes of resource partitioning, such as vertical soil profiles (Dickie *et al.* 2002b; Rosling *et al.* 2003) and successional changes through time (Dighton *et al.* 1986), niche differentiation provides a viable hypothesis for explaining fungal diversity (Bruns 1995). Species found at different distances from trees may have distinct ecological strategies. Species found primarily very near trees (rare species in this study) may require soil conditions created by overstorey trees, particularly the development of forest litter or a high density of ectomycorrhizal roots (Baar & deVries 1995). Kranabetter & Friesen (2002) found that seedlings transplanted from mature forests into openings had a reduction in the richness and a shift in the community

of fungi infecting seedlings, suggesting that these species may require the presence of overstorey roots or forest soils to persist.

Species found exclusively within the root zone of the forest edge, but beyond the forest edge (*Russula* species in this study), may be unable to infect seedlings in the absence of overstorey roots, as suggested by shifts in the community of fungi infecting seedlings following trenching (Fleming 1983; Fleming 1984; Simard *et al.* 1997). These species may be incapable of infecting seedlings as opposed to mature roots directly from spores (Fox 1983; Munyanziza & Kuyper 1995), which may explain their absence distant from trees.

The abundance of *C. geophilum*, *Astraeus* sp. and Pezizalean II at 20 m suggests that these species may have a distinct strategy for infecting seedlings distant from trees that other ectomycorrhizal species do not. Ascomycetes (including *C. geophilum* and Pezizalean II) may be particularly adapted to disturbed sites, including glacial forefronts (Alfredsen & Hoiland 2001; Trowbridge & Jumpponen 2004). *Astraeus* species (which are Basidiomycetes) have also been found more frequently in disturbed sites (Danielson 1984), as have related *Geastrum*, *Scleroderma* (Ingleby *et al.* 1998) and *Pisolithus* (Chambers & Cairney 1999) species. Species found distant from trees may be highly stress tolerant, persisting as saprotrophs, as has been suggested for *Scleroderma* (Jeffries 1999), infecting typically non-ectomycorrhizal hosts, as has been suggested for *C. geophilum* (LoBuglio 1999), or more effectively using the few and potentially low resource quality overstorey roots that might extend unusually far from trees (Gibson & Deacon 1988; Gibson & Deacon 1990). Alternatively, these species may be dispersing through space or time through long rhizomorphs in the soil, persistent sclerotia or spores (Shaw & Sidle 1983; Horton *et al.* 1998; Jones *et al.* 2003) or effective dispersal via wind and/or animals (Horton *et al.* 1998; Terwilliger & Pastor 1999). As suggested by Smith & Read (1997), it is probably premature to classify these species into ecological groups ('early stage' vs. 'late stage') given the range of possible strategies from stress tolerance to ruderal.

Astraeus sp. and Pezizalean II also were observed infrequently close to the forest edge, suggesting that these species may be poor competitors for seedling roots in the presence of other ectomycorrhizal species or may be actively excluded by seedlings when other ectomycorrhizal fungi are present, as has been suggested for *Rhizopogon* species in pine dominated systems (Horton *et al.* 1998). This is consistent with the concept of trade-offs between traits that allow species to be competitive under stressful conditions (s-selected) or able to rapidly colonize following disturbance (r-selected) and traits that allow species to be competitive under higher resource levels in undisturbed sites (Grime 1977).

Interestingly, in both the present study and others, *C. geophilum* appears to be an ultra-generalist found near and distant from trees (Kranabetter & Wylie 1998,

present study), as a generalist across all layers of soil profiles (Dickie *et al.* 2002b; Tedersoo *et al.* 2003), in both early and late succession (Visser 1995), on all continents, and across a broad range of host species, including some not generally ectomycorrhizal with other fungi (LoBuglio 1999). Nonetheless, the concept of trade-offs may apply to *C. geophilum* as well, as it appears to reach peak abundances where other fungi are absent or in stressful environments (LoBuglio 1999). It should be noted that the *C. geophilum* morphotype encompasses considerable genetic variation, which may also contribute to its wide distribution (LoBuglio 1999; Jany *et al.* 2002).

IMPLICATIONS

The spatial complexity of ectomycorrhizal fungal communities may have important implications for seedling establishment and, by extension, forest succession, dynamics and expansion. A lack of ectomycorrhizal infection distant from trees may reduce the ability of tree seedlings to establish in old fields and other sites with low densities of established ectomycorrhizal plants. Similar processes may occur where ectomycorrhizal trees are present in low densities in a matrix of arbuscular mycorrhizal vegetation. In addition to the effects of a lack of total infection, a low diversity of ectomycorrhizal infection distant from trees may further influence seedling establishment (Baxter & Dighton 2001; Jonsson *et al.* 2001). Whether the shifts in community composition *per se* (independent of the extent of infection and diversity) are beneficial, negative or neutral from the plant perspective is unclear from the present study, but might be a valuable area for further research.

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