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Ectomycorrhizal fungal communities of oak savanna are distinct from forest communities

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Abstract: Oak savanna is one of the most endangered ecosystems of North America, with less than 0.02% of its original area remaining. Here we test whether oak savanna supports a unique community of ectomycorrhizal fungi, a higher diversity of ectomycorrhizal fungi or a greater proportional abundance of ascomycete fungi compared with adjacent areas where the absence of fire has resulted in oak savanna conversion to oak forest. The overall fungal community was highly diverse and dominated by *Cenococcum geophilum* and other ascomycetes, *Cortinarius*, *Russula*, *Lactarius* and Thelephoraceae. Oak savanna mycorrhizal communities were distinct from oak forest communities both aboveground (sporocarp surveys) and belowground (RFLP identification of ectomycorrhizal root tips); however total diversity was not higher in oak savanna than oak forests and there was no evidence of a greater abundance of ascomycetes. Despite not having a higher local diversity than oak forests, the presence of a unique fungal community indicates that oak savanna plays an important role in maintaining regional ectomycorrhizal diversity.

Key words: Ascomycota, conservation, diversity, fire, habitat loss, mycorrhizal ecology

INTRODUCTION

Oak savanna once dominated large areas of North America, but less than 0.02% of its original area remains (Nuzzo 1985). The oak savanna landscape (areas with continuous herbaceous cover dominated by grass and with discontinuous tree coverage) is fire dependent; frequent fires prevent woody dominance and maintain an open park-like setting (Tester 1996, Peterson and Reich 2001). While most of the loss of oak savanna has been due to agricultural conversion, other large areas have been lost due to the suppression of fire. In the absence of fire oak savanna converts first to oak forests (here defined as closed-canopy oak-dominated forest) and eventually to more shade-tolerant tree species (Peterson and Reich 2001). The conversion of oak savanna to oak forest is accompanied by a number of changes in soil fertility (White 1983, Reich et al 2001, McGill et al 2007).

Although the loss of any habitat is of conservation concern, there is limited evidence of any negative effects of savanna conversion on particular taxonomic groups. A number of plant species, particularly shrubs and forbs, reach their peak abundances in savanna, but most of these also are present in prairie (Bray 1960). Individual plant functional groups tend to show lower or higher species richness in savannas than adjacent grasslands or forests but are not restricted to the savanna (Peterson and Reich 2001). Similar limited fidelity for savanna habitat has been found for birds and arthropods, for which savanna communities are a mixture of forest and grassland communities (Siemann et al 1997, Grundel and Pavlovic 2007).

The effect of savanna loss on fungal communities is unclear. Oak forests in general are dependent on and support a diverse community of ectomycorrhizal fungi (Walker et al 2005, Bergemann and Garbelotto 2006, Avis et al 2008), but whether any ectomycorrhizal fungal species specifically require oak savanna is unclear. Fire, the driving ecological force in the persistence of savanna, has a number of known effects on fungal communities. Severe wildfire can kill fungal ectomycorrhizal genets, reduce diversity (Bruns et al 2002) and result in an increased dominance by fungi with resistant propagules (Taylor and Bruns 1999) and facultatively mycorrhizal species capable of surviving as saprotrophs (Egger 1986). Fires in savanna however are typically low intensity; the stems of trees smaller than 5 cm diam are almost always killed but the individual almost always survives and resprouts while the stems of most larger trees

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generally survive. Similarly grasses quickly resprout from roots and crowns and soil heating is minimal over large areas. Further the three major ectomycorrhizal hosts in Minnesota oak savanna are *Quercus macrocarpa*, *Q. ellipsoidalis* and *Corylus americana*, all three of which are also present in oak forests, while the one host plant that does decline with fire suppression, *Helianthemum bicknelii*, is relatively rare and appears to support largely the same fungal community as *Quercus* (Tester 1996, Dickie et al 2004). Oak savannas are highly spatially heterogeneous, creating a potentially greater number of spatial niches for fungi that preferentially occur distant from trees (Last et al 1984, Dickie and Reich 2005); this might be expected to increase fungal diversity in savanna. On the other hand oak savannas harbor fewer trees (i.e. smaller islands of habitat) compared with forests and because ectomycorrhizal species richness is known to relate to the size of habitat (Peay et al 2007) this might be expected to result in fewer species in savanna than in forest.

Higher level taxonomic groups might also be expected to occur preferentially in oak savanna. One fungal group that might be particularly expected preferentially to occur in oak savannas is the ectomycorrhizal ascomycetes (Tedersoo et al 2006). In extreme pinyon pine scrub ascomycetes are abundant (Gehring et al 1998), perhaps suggesting a higher abundance in savanna, which also can be more extreme than oak forests. We also have found ascomycetes to be more common on seedlings planted in abandoned agricultural fields than in adjacent oak forests (Dickie and Reich 2005), which suggests they might prefer open, disturbed habitat. Warcup (1991) found that ascomycetes dominated ectomycorrhizal communities on *Eucalyptus* after fire and Fujimura et al (2005) showed 15% of ectomycorrhiza on roots of *Pinus ponderosa* in a postfire site were ascomycetes but in neither case was a direct comparison made with sites without fire. Ectomycorrhizal ascomycetes may be facultatively saprotrophic, permitting survival in the absence of host roots (Egger 1986). Increased soil pH in savanna might also favor ascomycetes because it has been suggested that higher soil pH favors sporocarp production of the ascomycetous genus *Tuber* (Hall et al 2008).

We studied the belowground and aboveground fungal community at the Cedar Creek Science Ecosystem Reserve, where prescribed burning has been performed for more than 30 y to maintain oak savanna. We tested three hypotheses: (i) oak savanna and oak forests would support distinct communities of ectomycorrhizal fungi; (ii) the diversity of ectomycorrhizal fungi would be greater in oak savanna than in oak forest; and (iii) ascomycete ectomycorrhizal fungi would comprise a greater proportion of ectomycorrhizal fungi in oak savanna compared with oak forest.

MATERIALS AND METHODS

We used a combined belowground and aboveground sampling of fungal structures. Sampling ectomycorrhizal roots belowground, with a combined morphotyping and RFLP approach, let us detect species such as hypogeous ascomycetes that were not detectable aboveground (Smith et al 2007). Including aboveground sporocarp collections let us build a species list based on robust identifications with sporocarp vouchers, and permitted a much greater total area to be sampled.

Sites.—Cedar Creek Science Ecosystem Reserve and Long Term Ecological Research site in Minnesota, including burn history, has been described by Tester (1996) and Reich et al (2001). We sampled ectomycorrhizal fungi from six sites, three of which have had frequent controlled burns (plots 103, 104, 106), which we will refer to as savanna, and three of which have had no fire history during that period (plots 109, 110, 309), which we will refer to as forests. The sites differed markedly in soil parameters, including pH and nitrogen availability (TABLE I), both of which are likely to influence fungal communities (Lilleskov et al 2002, Avis et al 2008). A portion of plot 106 had been included in a nutrient addition experiment; we avoided this portion of the plot in belowground sampling (TABLE II).

Belowground sampling.—We sampled ectomycorrhizal fungi from individual roots collected in soil cores 2.8 cm diam and 20 cm deep. A common approach to sampling ectomycorrhizal communities is to use tree-centered soil cores (Gehring et al 1998, Avis et al 2003). This technique would be inappropriate for our sites because distance from trees is an important factor structuring fungal communities (Dickie and Reich 2005) and savanna sites have a highly heterogeneous spatial pattern of tree occurrence. We therefore established six, parallel, 100 m transects spaced 10 m apart in each plot, with 20 sampling points identified by flags every 5 m along these transects, and sampled cores randomly from these 120 points (80 in plot 106) until a minimum of 30 cores were obtained that contained ectomycorrhizal roots. In forests 100% of cores taken contained ectomycorrhizal roots; the final sample size was 31–32 cores (TABLE II). In savanna 61% of cores contained ectomycorrhizal roots, requiring 40–64 cores to obtain the desired sample size of a minimum of 30 cores with roots; the final sample size was 30–33 cores (TABLE II). Sampling was spread over 3 y (2002–2004), with sampling conducted in August each year and approximately 10 samples with roots per plot processed each year.

From each core we examined all root tips and sampled all unique morphological types, using PCR with ITS1F and ITS4 primers and RFLP to identify species. In comparing diversity estimates it is important to have equal sampling effort (Taylor 2002). For this study we equalized our sampling at the level of soil cores with ectomycorrhizal roots, sampling until approx-

TABLE I. Site characters of research plots (data from Reich et al 2001)

Plot number	Burn frequency (burns/years) since 1964	Basal area[a] (m^2 ha^{-1})	Soil pH	Nitrogen availability (g m^{-2} yr^{-1})
103	9/10	12.4	5.8	3.0
104	9/10	7.8	5.7	1.9
106	2/3	17.4	5.5	4.2
109	0	15.5	4.8	10.9
110	0	19.3	4.6	15.7
309	0	28.3	4.1	15.9

[a] Basal area is for tree-dominated areas within sites.

imately the same number of cores with ectomycorrhizal roots were obtained from each site and identifying all morphological species within those cores. An alternative approach would have been to treat individual root tips as the basis for equalizing sampling effort, counting the number of root tips of each unique morphological type and using rarefaction analysis to subsample equal numbers of root tips from all samples (Taylor 2002). We opted against this alternative for two reasons. First, treating individual root tips as independent sampling units assumes that each individual root tip in a soil core represents a fungal individual, which is clearly incorrect (Taylor 2002). Second, by sampling all morphological types within a set volume of soil, our results can be scaled on the same basis as sporocarp measurements, which are on a set area basis, or compared to studies of other taxonomic groups, which are almost universally taken on a set area or set volume basis. Nonetheless it is worth noting that sampling equal numbers of root tips would have increased the relative diversity of savanna samples, which had lower root densities compared to woodland samples (Taylor 2002).

Individual root tips were frozen in liquid N, lyophilized and stored at −20 C until DNA analysis. DNA was extracted and amplified following the kit-based protocol of Avis et al (2003). RFLP patterns were obtained with HinfI and DpnII enzymes (NEB, Beverly, Massachusetts), run in 2% agarose gels, with RFLP patterns matched with the GERM (good-enough RFLP matcher) spreadsheet program (Dickie et al 2003). Our overall success rate for PCR amplification was 64% of total

TABLE II. Species diversity in sporocarp collections and on root tips by plot and fire frequency (plot 106 not included for sporocarps due to unequal sampling)

Plot	n	Species	Chao[a]	Jackknife[b]	H′[c]
Sporocarps					
103	120	25	53.1 (20.9)	50.0	2.8
104	120	37	49 (9.2)	54.7	2.1
Burned	240	46	66.6 (13.5)	72.7	2.3
109	120	40	52.8 (8.4)	61.8	2.5
110	120	60	86.9 (15.4)	94.7	2.8
309	120	31	87.3 (49.8)	58.6	2.2
Unburned	360	72	105.3 (21.0)	105.9	2.8
All	600	88	117 (16.6)	124.9	3.1
Root tips					
103	33	33	51.1 (10.9)	60.2	2.8
104	30	31	79.4 (30.7)	68.3	2.9
106	33	37	67.3 (17.6)	71.7	3.0
Burned	96	67	97.0 (14.2)	112.5	3.3
109	31	43	75.0 (17.8)	80.5	3.3
110	32	61	127.7 (29.7)	126.4	3.6
309	32	35	101.1 (43.8)	75.2	3.1
Unburned	95	92	134 (17.1)	154.3	3.7
All	191	123	160.1 (14.0)	187.7	3.8

[a] Chao estimator of total species richness and standard error.
[b] Second-order jackknife estimator of total species richness.
[c] Shannon diversity.
n, number of sample points (sporocarps) or cores (mycorrhizal root tips).

reactions producing sufficient product for visualization. Of the 64% of samples producing PCR products, 13% had multiple bands and were therefore not analyzed with RFLP.

Sporocarp sampling.—For aboveground data we sampled 4 m^2 circles around the same 120 points established for belowground sampling, totaling 480 m^2 of area sampled per plot (1440 m^2 for each forest type). The total area sampled for each forest type falls within the suggested minimum of 1000–1600 m^2 required to evaluate species diversity and abundance (Bills et al 1986). Plots were visited every 7–10 d or more or less frequently according to patterns of precipitation, late Jun through mid-Oct 1998, 1999 and 2002. At each sample point we recorded the species identity and number of sporocarps for groups of taxa containing probable ectomycorrhizal species within each 4 m^2. Collections were made when the identity of the taxon could not be verified in the field. Each species recorded from the plots is represented by at least one voucher collection deposited at the University of Minnesota Herbarium. Ectomycorrhizal taxa outside the sample points also were recorded or collected to completely document species diversity in the plots, but these were not included in our analysis.

At five of our six sites (forests 109, 110 and 309 and savannas 103 and 104) we collected sporocarp data based on the plot design described above. At the sixth site (savanna 106) sporocarp collections were available from only three collection events from 80 sample points instead of 120 as at other sites; however we were able to supplement the species lists from collections by Avis et al (2003) that were immediately adjacent to the belowground sampling plot. Avis et al (2003) included fertilized plots, but we included supplementary collection information from unfertilized plots only.

Statistics.—For belowground collections we considered all occurrences of a species within a soil core as a single occurrence. Replication (three replicates of each site type) was insufficient to determine whether any particular species showed a habitat preference. We therefore developed a Monte Carlo model to test whether overall more species were exclusive to savanna or forest than would be expected based on random chance. We considered only the numbers of species that were both exclusive to a site type and occurred in all three replicates of that site type. We then approximated the probability of this number of ''exclusive'' species arising by chance given the total number of species found in exactly three sites by rerandomizing species occurrences 100 000 times with both equal and species-richness-adjusted probabilities of occurrences. This method assumes that species occurrences are independent (i.e. that the presence of one fungal species does not determine whether a second species is present or absent). All statistics were carried out in R (R Development Core Team 2008) with estimates of species richness calculated with the package vegan (Oksanen et al 2008).

RESULTS

Belowground sampling.—From 191 soil cores with ectomycorrhizal roots a total of 123 RFLP types were detected belowground (FIG. 1), of which 48 were found only once and 31 only twice. We identified 42 of the 123 types, including all but one of the species found more than five times; in total we identified 73% of RFLP pattern occurrences. Three RFLP patterns matching *Cenococcum geophilum* were treated as independent ''species''; of these one pattern (*C. geophilum* A) accounted for 93% of *C. geophilum* collections. Species found in 10 or more soil cores were *C. geophilum* A, *Cortinarius* sp 4 (matching *C.* cf. *iliopodius*), *Russula* aff. *seperina*, *Lactarius* sp 1 (matching *L. camphoratus*), *Russula* aff. *amoenolens*, Pezizales sp 1 (matching *Humaria hemisphaerica*) and *Genea* sp 1 (matching *G. hispidula*, TABLE III). Of the 42 identified species, seven were ascomycetes (182 occurrences), 15 were Russulaceae (123 occurrences), six were Cortinariaceae (45 occurrences), four were Amanitaceae (all *Amanita* spp., 10 occurrences), three were Thelephoraceae (16 occurrences), with the remaining families having only two (Atheliaceae, Sebacinaceae) or one (Boletaceae, Leotiaceae, Tricholomataceae) species found belowground. The estimates of total richness were 160 ± 14 (Chao1 ± SE) and 188 RFLP species (second-order jackknife).

Sixteen species occurred belowground in exactly three sites; of these five were exclusive to either savanna or forest. Two species occurred exclusively in all three savannas, Pezizales 2 (matching *Hydnotrya tulasnei*) and IDMT00825. Three species occurred exclusively in all three forests, *Piloderma* sp 1 (matching *P. lanatum*), IDMT00280a and Pezizales sp 1. The probability of this level of exclusivity (five out of 16) occurring by chance is $P = 0.017\%$ or $P = 0.020\%$ based on 100 000 randomizations of species with equal and species-richness-adjusted probabilities of species occurrences respectively.

There was no evidence of higher diversity in burned than unburned plots from belowground data. Estimated species richness (Chao, second-order jackknife) and Shannon Diversity (H′) were generally higher in forests than savannas (TABLE I). The rate of species accumulation with sampling effort for savannas also was lower for savannas than for forests (FIG. 1).

Two families, the Russulaceae and Thelephoraceae, were more frequent in forests than savannas ($P = 0.036$ and $P = 0.044$ respectively). The frequency of ascomycetes was not significantly affected by site type, regardless of whether *Cenococcum geophilum* was included.

Sporocarp collections.—A total of 3631 individual sporocarps were observed, representing 110 identified taxa (TABLE IV). In the forest plots the most frequently encountered taxa throughout the warmer

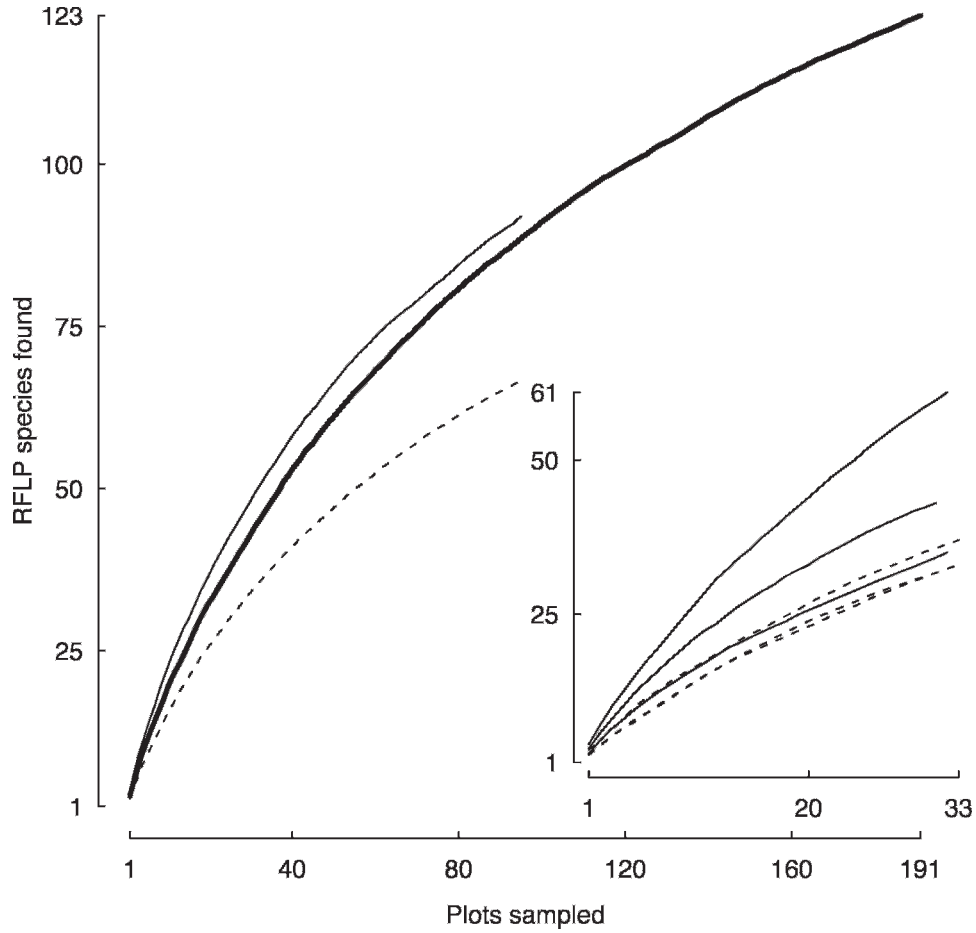

FIG. 1. Belowground sampling effort curve for all sites (thick line), all forests (thin line) and savannas (dashed line); curves for individual plots inset (forests as thin lines, savannas as dashed lines).

months were Russulaceae (especially *Lactarius camphoratus*, *Russula seperina* and other *Russula* spp.), Cortinariaceae (*Cortinarius* subgen. Telamonia), Amanitaceae (especially *Amanita* sect. Vaginatae), *Laccaria* spp. and *Tremellodendron pallidum*, whereas *Tricholoma* spp. became the most abundant in the later, cooler months. Sporocarp records in savanna plots were made almost exclusively after recent rains whereas sporocarps were recorded in forest plots on most visits, although sporocarp abundance was largely dependent on recent rains. There was substantial interannual variation in sporocarp numbers in forest plots (178 min, 813 max, sum across all plots) with lower numbers but also less inter-annual variability in savannas (150 min to 208 max across all plots). Sporocarps in savanna plots were rarely recorded distant from trees, creating a noticeable clumping effect not observed in the forest plots.

The lowest diversity encountered was in savanna plot 103, with 25 fungal species collected (estimated total 50–53 species), with the highest diversity in forest plot 110 with 60 fungal taxa (estimated total 87–95 species, TABLE II). There was no evidence that diversity was higher in savanna than forests.

Fourteen species occurred as sporocarps in exactly three sites; of these six were exclusive to either savanna or forest. Two species occurred exclusively in all three savannas, *Boletus nobilissimus* and *Russula brevipes*. Four species occurred exclusively in all three forests, *Amanita brunnescens*, *Laccaria amethystina*, *L. ochropurpurea* and *Lactarius subserifluus* (TABLE IV). This level of exclusivity (six or more of 14 species) was observed in 0.0014% and 0.0011% of 100 000 randomizations of species with equal and species-richness-adjusted probabilities of species occurrences respectively.

DISCUSSION

The community was broadly similar to those described for *Quercus*-associated ectomycorrhizal fungi elsewhere, with a relatively high dominance by ascomycetes (*Cenococcum*, Pezizales, *Genea*), *Cortinarius*, *Russula*, *Lactarius* and Thelephoraceae and a

TABLE III. Collection numbers of RFLP species by site for all species collected at least three times, including GenBank accession numbers for sequence based identifications and best matching named sequence in GenBank

Species	Savannas			Forests			Total	Accession	Basis for identity[a]
	103	104	106	109	110	309			
Cenococcum geophilum A	27	20	27	23	26	17	140	—	RFLP match to sclerotia
Cortinarius sp 4	7	3	5	7	6	5	33	EU880222	AJ889948.1 *Cortinarius* cf. *iliopodius*; 584/607
Russula aff. *seperina*	2	1	6	7	6	8	30	—	RFLP match to sporocarp djm1099
Lactarius sp 1	1	2	7	4	4	1	19	EU880219	AJ889960.1 *Lactarius camphoratus*; 686/723
Russula aff. *amoenolens*	1	4	2	1	0	6	14	—	RFLP match to sporocarp djm1192
Pezizales sp 1	0	0	0	2	5	4	11	FJ147328	DQ200832.1 *Humaria hemisphaerica*; 693/699
Genea sp 1	0	1	2	2	1	4	10	EU880217	AJ969622.2 *Genea hispidula*; 629/634
Russula aff. *pectinatoides*	2	3	0	0	1	3	9	AY640411	EU598185.1 *Russula pectinatoides* 624/625
Thelephoraceae sp 1	1	0	1	4	1	1	8	EU880218	DQ150117.1 Uncultured Thelephoraceae; 632/661
Russula sp 2	0	1	2	2	2	1	8	EU880215	DQ777996.1 *Russula* sp; 659/665
Pezizalean I	2	2	2	1	0	0	7	EU588985	EU427549.1 *Pachyphloeus* sp; 302/354
Cortinarius violaceus	1	0	1	3	2	0	7	—	RFLP match to sporocarp kh137
Russulaceae sp 3	1	1	1	0	0	4	7	EU880224	AY239349.1 *Gymnomyces fallax*; 673/691
Lactarius maculatipes	1	1	0	2	1	1	6	—	RFLP match to sporocarp djm1208
Russula sp 4	0	0	2	2	1	1	6	—	RFLP match to sporocarp kh91
PAMT332	1	0	0	0	4	1	6	—	= MT846-2000-RFLP36 in Avis et al (2003)
Cenococcum geophilum B	1	1	3	0	0	1	6	—	RFLP match to morphotype with stellate mantle
IDMT00114a	1	0	3	1	0	0	5	—	(unknown)
Piloderma sp 1	0	0	0	2	1	2	5	EU880221	DQ469288.1 *Piloderma lanatum*; 625/634
IDMT00307	0	0	1	1	3	0	5	—	(unknown)
IDMT00834	3	0	0	1	1	0	5	—	(unknown)
IDMT01806	0	0	2	0	1	2	5	—	(unknown)
Russula sp 5	0	0	2	3	0	0	5	EU880216	AY750164.1 *Russula* sp; 665/684
Amanita cf. *fulva*	0	0	3	0	2	0	5	—	RFLP match to sporocarp kh27
Lactarius camphoratus	0	0	0	4	0	1	5	—	RFLP match to sporocarp kh61
Tomentella cf. *bryophila*	2	0	0	1	2	0	5	—	RFLP match to sporocarp PA380
Byssocorticium sp 1	0	0	1	3	0	1	5	EU880220	AJ889936.1 *Byssocorticium atrovirens*; 610/624
IDMT00280a	0	0	0	2	1	1	4	—	(unknown)
Pezizales sp 2	1	2	1	0	0	0	4	EU880227	AJ969621.1 *Hydnotrya tulasnei*; 702/746
Russula sp 6	3	0	1	0	0	0	4	EU880225	AF418639.1 *Russula* sp; 651/668
Sebacinoid ''RFLP 4''	0	0	0	0	4	0	4	—	= ''RFLP 4'' in Avis et al (2003)
Cenococcum geophilum C	2	0	0	0	1	1	4	—	RFLP match to morphotype with stellate mantle
Amanita pantherina	1	2	0	0	0	0	3	—	RFLP match to sporocarp djm1151 (*A. pantherina* var. *multisquamosa*)
Thelephora terrestris	0	0	0	0	1	2	3	—	RFLP match to sporocarp djm1152
IDMT00106a	0	0	0	2	0	1	3	—	(unknown)
IDMT00305a	0	0	0	3	0	0	3	—	(unknown)
IDMT00307	1	0	0	1	1	0	3	—	(unknown)
IDMT00651a	0	0	0	0	3	0	3	—	(unknown)
IDMT00825	1	1	1	0	0	0	3	—	(unknown)
Sebacinaceae sp 1	0	0	1	1	1	0	3	EU880223	EF372401.1 Sebacinaceae sp; 537/622
IDMT01852	2	0	1	0	0	0	3	—	(unknown)
IDMT02360	1	0	0	1	1	0	3	—	(unknown)
Russula sp 7	2	1	0	0	0	0	3	EU880225	DQ422015 *Russula* cf. *maculata*; 608/673
Russula silvicola	0	0	0	1	2	0	3	—	RFLP match to sporocarp kh83

[a] Species were identified by RFLP matching to sporocarps. For any species found more than five times that failed to match a sporocarp we attempted sequence identification; the best matching named species in GenBank is noted with accession number, species name and matching base pairs.

high level of diversity (Valentine et al 2004, Richard et al 2005, Gebhardt et al 2007, Smith et al 2007).

Community differences.—Our first hypothesis, that oak savanna and oak forests would support distinct communities of ectomycorrhizal fungi, was supported. The analysis of ectomycorrhizal communities has been problematic in studies where the treatment of interest is at a large scale (e.g. stand, plot). The high diversity of ectomycorrhizal fungal communities has meant that the characterization of each replicate comes at a high cost in time and resources; hence replication has been severely limited (e.g. three replicates of two treatments in this study). In this study we have used a novel statistical approach to attempt to surmount the difficulty of low replication of diverse communities. Looking only at the subset of species with exactly three occurrences, we observed a much higher degree of ''exclusivity'' to one of the two plot types than would have been expected by random chance. Although this indicates that the two communities are different, this approach cannot indicate which particular species drive this difference. Thus we can draw a community-level conclusion (the communities are different) but cannot use the result to infer anything about species level properties.

Although sporocarp and belowground data revealed different communities, both datasets showed evidence of greater than expected species exclusivity to savanna or forests. The results were somewhat more significant for sporocarps ($P = 0.0014$) than for belowground data ($P = 0.017$). In part this might reflect the greater sample size and better taxonomic resolution of aboveground sporocarp surveys than is possible belowground (Lilleskov et al 2002). It is also possible that sporocarp production is a more sensitive indicator of species responses than belowground presence. Sporocarp and belowground fungal communities are often only loosely correlated, with many species present belowground not forming sporocarps (Dahlberg et al 1997, Zhou and Hogetsu 2002). Sporocarp production requires a significant ability to capture resources (Dahlberg et al 1997, Zhou and Hogetsu 2002); this might make sporocarp production a more sensitive measure of habitat preference and environmental impact than belowground surveys.

Species diversity.—Our second hypothesis, that the diversity of ectomycorrhizal fungi would be greater in oak savanna than in oak forest, was not supported; if anything the opposite pattern appears to be true. We had expected higher diversity based on oak savanna being the more ''natural'' habitat for the area and the greater spatial heterogeneity of trees creating more fungal niches in terms of distance from trees (Dickie and Reich 2005). Our failure to find higher fungal diversity in oak savanna might reflect a loss of soil horizon complexity because fire removes organic horizons (Dickie et al 2002, Genney et al 2006) and woody debris (Tedersoo et al 2008), which are important ectomycorrhizal fungal niches. Further the reduction in tree density in oak savannas might represent a loss of effective habitat area, which might reduce fungal diversity (Peay et al 2007).

Ascomycete abundance.—Our third hypothesis, that ascomycete ectomycorrhizal fungi would be favored in oak savanna over basidiomycete ectomycorrhizal fungi, was not supported. We found no evidence of increased ascomycete abundance in savannas. Further, while Pezizales sp 2 was exclusively found in savanna, Pezizales sp 1 was found only in forests. Thus, while some studies have suggested that some Pezizaceae might be favored in openings and high stress sites (Dickie and Reich 2005, Smith et al 2006), our results suggest that this result cannot be generalized at the family level. This is consistent with the suggestion of Tedersoo et al (2006) that, while a number of ascomycete species appear to specialize on early successional and disturbed habitats, other ascomycete species are important components of mature forests. It is also possible that the frequent, low-intensity fire of oak savanna has less of an effect on ascomycete abundance than higher-intensity fires in pine or spruce ecosystems because Vrålstad et al (1998) found that ascomycetes increased in abundance only in areas with severe fire and not in areas where fire was less intense.

Ascomycetes in general were important components of the fungal community, with an overwhelming abundance of *Cenococcum geophilum* (140 occurrences compared with 33 occurrences for the second most abundant species belowground), as well as Pezizales sp 1, *Genea* sp 1 and Pezizalean I (TABLE III). The particularly high dominance of *Cenococcum geophilum* in the present study (and many others) might be partially an artifact of presorting root tips by morphotypes; highly distinct morphotypes such as *C. geophilum* (black) or *Byssocorticium* (bright blue) are less likely to be inadvertently omitted than less conspicuous morphotypes. Bulked root sampling (e.g. Smith et al 2007) might avoid this bias. On the other hand our sampling of single root tips reduces the potential for PCR biases to influence results. On the whole our results suggest that Ascomycetes are an important component of *Quercus*-associated ectomycorrhizal communities and that the ecology of this group of fungi remains largely unclear.

Importance of savanna in species conservation.—*Boletus nobilissimus* Both & Riedel was found in all three savanna plots but not in forest plots. This commer-

TABLE IV. Sporocarp collections (presence/absence) by site

Species	Savannas			Forests			Number of sites
	103	104	106	109	110	309	
Amanita bisporigera	0	0	1	0	0	0	1
Amanita brunnescens	0	0	0	1	1	1	3
Amanita cf. *porphyria*	0	1	0	0	0	0	1
Amanita cf. *spreta*	0	1	1	0	0	0	2
Amanita citrina	0	1	0	1	1	1	4
Amanita constricta	0	1	0	0	0	0	1
Amanita flavoconia	0	1	1	1	1	1	5
Amanita flavorubescens	0	1	1	0	1	0	3
Amanita fulva	0	0	0	0	1	1	2
Amanita gemmata	0	1	0	0	0	0	1
Amanita muscaria var. *alba*	1	1	0	0	0	0	2
Amanita pantherina	0	1	1	0	0	0	2
Amanita peckiana	0	1	0	0	1	0	2
Amanita rubescens	0	1	1	0	1	0	3
Amanita sect. *lepidella*	0	1	0	0	0	0	1
Amanita sect. *mappae*	0	0	0	1	1	0	2
Amanita sp 1	0	1	0	1	1	0	3
Amanita sp 9	0	0	1	0	0	0	1
Amanita vaginata	0	1	1	1	1	1	5
Amanita verna/virosa/bisporigera	1	1	1	1	1	0	5
Boletus bicolor	0	0	1	0	0	0	1
Boletus fraternus	0	0	0	0	1	0	1
Boletus nobilissimus	1	1	1	0	0	0	3
Boletus pallidus	1	0	1	1	1	1	5
Boletus pulverulentus	0	0	0	0	1	0	1
Boletus sp 1	0	1	0	0	1	0	2
Boletus subtomentosus	0	0	0	0	1	0	1
Cantharellus cibarius	0	0	0	1	1	0	2
Clavulina sp 1	0	0	1	1	0	0	2
Coltricia cinnamomea	0	0	0	1	0	0	1
Coltricia perennis	1	0	1	0	1	0	3
Cortinarius alboviolaceus	0	0	0	0	1	0	1
Cortinarius cf. *violaceus*	0	0	0	0	1	0	1
Cortinarius sect. *Phlegmacium*	1	1	0	0	1	0	3
Cortinarius rapaceus group	0	0	1	0	0	0	1
Cortinarius sect. *Dermocybe*	0	0	0	0	1	1	2
Cortinarius sp 1	0	0	1	0	0	0	1
Cortinarius sp 2	0	0	1	0	0	0	1
Cortinarius sp 3	0	0	0	0	1	1	2
Cortinarius sp 4	0	0	0	0	1	0	1
Cortinarius sect. *Telamonia*	1	1	1	1	1	1	6
Craterellus fallax	0	0	0	1	1	0	2
Elaphomyces sp 1	0	0	0	1	1	0	2
Entoloma abortivum	0	0	1	0	0	0	1
Entoloma cf. *griseus*	1	1	0	1	1	1	5
Entoloma cf. *sinuatum*	0	0	0	0	1	1	2
Entoloma cf. *strictius*	1	1	1	1	0	1	5
Entoloma sp 1	0	0	1	0	0	0	1
Entoloma sp 2	0	0	1	0	0	0	1
Entoloma sp 3	0	0	0	1	1	0	2
Gyroporus castaneus	0	0	0	0	1	0	1
Gyroporus cyanescens	0	0	1	1	0	0	2
Hebeloma cf. *testaceum*	0	0	1	0	0	0	1
Hebeloma mesophaeum group	0	0	1	0	0	0	1

TABLE IV. Continued

Species	Savannas			Forests			Number of sites
	103	104	106	109	110	309	
Hebeloma sinapizans	0	1	0	1	0	0	2
Hebeloma sp 1	0	1	0	0	0	0	1
Hebeloma sp 2	0	0	0	0	0	1	1
Helvella crispa	0	0	1	0	0	0	1
Hydnellum concrescens	1	0	1	0	0	0	2
Hydnellum velutinum var. *spongiosipes*	0	0	0	0	1	0	1
Hygrophorus paludosoides	0	0	1	1	1	0	3
Hygrophorus russula	0	0	0	1	0	0	1
Inocybe sp 1	1	1	0	1	0	1	4
Laccaria aff. *laccata*	0	0	1	0	0	0	1
Laccaria amethystina	0	0	0	1	1	1	3
Laccaria laccata	1	1	1	1	1	1	6
Laccaria ochropurpurea	0	0	0	1	1	1	3
Lactarius argillaceifolius	0	1	1	0	0	0	2
Lactarius atroviridis	0	0	0	0	1	0	1
Lactarius camphoratus	1	1	1	1	1	1	6
Lactarius cf. *fuliginosus*	0	0	1	0	0	0	1
Lactarius mutabilis	0	1	0	0	0	0	1
Lactarius sp 1	0	0	0	0	1	0	1
Lactarius sp 6	0	0	0	0	0	1	1
Lactarius subserifluus	0	0	0	1	1	1	3
Lyophyllum cf. *decastes*	0	0	1	0	0	0	1
Phellodon confluens	0	0	0	0	1	0	1
Phellodon niger	0	0	0	1	0	0	1
Ramaria cf. *abietina*	0	0	0	0	0	1	1
Ramaria cf. *aurea*	1	0	1	1	1	0	4
Ramaria cf. *stricta*	0	0	0	0	1	1	2
Russula aff. *amoenolens*	1	1	1	1	1	1	6
Russula brevipes	1	1	1	0	0	0	3
Russula cf. *amygdaloides*	1	1	0	1	1	1	5
Russula cf. *appalachiensis*	0	0	0	0	1	0	1
Russula cf. *fragilis*	0	1	0	0	1	1	3
Russula cf. *macropoda*	0	0	0	0	1	0	1
Russula cf. *silvicola*	0	0	0	0	1	0	1
Russula laurocerasi	1	0	1	0	1	1	4
Russula seperina	1	1	1	1	1	1	6
Russula sp 1	1	1	1	1	1	1	6
Russula variata	1	0	1	1	1	0	4
Russula xerampelina group	1	0	1	1	1	1	5
Scleroderma areolatum	0	0	0	0	0	1	1
Strobilomyces floccopus	1	0	1	1	1	1	5
Thelephora anthocephala	0	0	1	0	0	0	1
Thelephora cf. *caryophylla*	0	1	0	0	0	0	1
Thelephora terrestris	0	1	0	0	0	0	1
Tomentella cf. *bryophila*	0	0	1	0	0	0	1
Tremellodendron pallidum	1	1	1	1	1	0	5
Tricholoma cf. *flavobrunneum*	1	0	0	0	0	0	1
Tricholoma saponaceum	0	0	0	0	1	0	1
Tricholoma sejunctum	0	0	1	1	1	0	3
Tricholoma sulphurescens	0	0	0	1	1	0	2
Tricholoma venenatum	0	0	0	1	1	0	2
Total species	25	37	47	40	60	31	

cially valuable mushroom in the edible porcini complex was described only recently from oak-pine woods in western New York (Bessette et al 2000) but was probably reported from Minnesota under various incorrect names (e.g. *Boletus edulis* [Bull.] Fr., *B. aestivalis* [Paulet] Fr., *B. variipes* Peck) since at least the late 19th century (Peck 1889). The identity of this species was initially problematic due to incomplete and confusing taxonomic data on this group of mushrooms, but ITS sequences from our collections matched the ITS from the holotype specimen at 100% identity (Dentinger 2007). Although we have collections of this species from forest habitats near our sites, it was never found in our sampled plots and we only encountered it consistently in open savanna habitats. This pattern reveals the importance of savanna habitat to the maintenance of fungal diversity. In addition to highlighting the need for conserving savanna habitats the great abundance of *B. nobilissimus* in oak savanna after periods of warm weather (≥ 33 C) and heavy rains (Dentinger pers obs) illustrates the potential for making conservation of savanna a profitable endeavor through sustainable development of non-timber forest products such as edible mushrooms. Similarly certain truffles (*Hydnotrya*, *Pachyphloeus*) appear to be mainly savanna species. Hypogeous fungi typically go unnoticed but their conservation might depend on savanna preservation.

The fungal community overall was highly diverse but is consistent with other studies of mixed-host-species communities (Dickie 2007, Ishida et al 2007). Despite being the more ''natural'' state of these ecosystems, oak savannas were not more diverse than oak forests. Nonetheless oak savanna might play an important role in supporting regional diversity because some ectomycorrhizal species were savanna specialists, while other species were restricted to forests. Maintaining a heterogeneous mixture of habitats therefore will result in the highest across-site diversity of ectomycorrhizal fungi.

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