

Fungus diversity in revegetated paddocks compared with remnant woodland in a south-eastern Australian agricultural landscape

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Summary Despite the importance of fungi for restoration, their presence in revegetated sites has received little attention. We compared the diversity and composition of macrofungi (i.e. those that form fleshy mushrooms and truffles) in 12 sites where 3-to-6-year-old native trees and shrubs had been planted (woodland restoration sites), with that in six woodland remnants. All sites were within an agricultural landscape near Holbrook in New South Wales. Of 58 fungal genera recorded, 57% occurred in woodland restoration sites and 83% in nearby patches of remnant woodland. Of the genera found in restoration sites, 70% were also found in the woodland remnants. The dominance of early successional genera such as *Laccaria* and *Scleroderma* in restoration sites suggests windblown colonisation by fungi. The reduced proportion of hypogeous genera (truffles) that rely on mammal vectors, which are less likely to occur in the restoration sites, also supports the view that most fungi occurred in restoration through colonisation rather than being generated from soil spores. Greatest overall fungal diversity occurred in large remnants that had greater structural complexity. Across all sites, epigeous genera (mushrooms) were most common (78% of all taxa collected across 46 genera) and of the nutritional modes, mycorrhizal genera (forming symbiotic associations with plants) were the most common (206 collections, 71%, 25 genera). Both hypogeous and mycorrhizal fungi were positively associated with the diversity of native forb species (wildflowers), suggesting that lower fungal diversity in restoration sites is likely to be a consequence of long-term agricultural practices, particularly fertilizer use.

Key words: agricultural impacts, mycorrhizae, restoration, structural complexity, tree planting, woodlands.

Introduction

There is growing evidence that planting native trees and shrubs is an effective way to restore biodiversity in Australian agricultural landscapes. This new resource can be used by birds (Ryan 1999; Kinross 2004), mammals (Hobbs *et al.* 2003; Cunningham *et al.* 2007) and reptiles (Kavanagh *et al.* 2005; Cunningham *et al.* 2007). Faunal responses to revegetation in agricultural landscapes are reviewed by Munro *et al.* (2007). Colonisation of destocked and revegetated agricultural sites by native plants has also been studied (Spooner *et al.* 2002). But what of fungi in these woodland restoration sites? Recent work in south-eastern Australia has shown that the abundance and diversity of mycorrhizal fungi declines rapidly with distance from remnant eucalypts into adjacent farm paddocks (Stol & Trappe 2006), and we know that soil fungal diversity is reduced by live-stock grazing (Fleischner 1994), cultivation

(Tisdall & Oades 1980), fertilizer use (Hossain *et al.* 1995; Pampolina *et al.* 2002), fire (Chen & Cairney 2002) and tree clearing (Jones *et al.* 2003). The roles of fungi and importance of conserving their diversity are reviewed by May (1997), who points out that Australian macrofungi are understudied.

Successful restoration of planted trees and subsequent colonisation by mycorrhizal fungi is related to the proximity of surrounding remnant vegetation (West & Jones 2000; Enkhtuya *et al.* 2005), but little is known about the ability of fungi to colonise and persist in restoration (planted) sites in Australian agricultural landscapes (see Tommerup & Bougher 2000).

Fungi are likely to be important in restoration processes because they maintain soil structure, are vital to the establishment of trees (Oades 1984; West & Jones 2000), and have a broad role in maintaining nutrient cycles and other ecosystem services

within forest and woodland habitats (Claridge & Trappe 2005; Maser *et al.* 2008). Fungi are also important food resources for wildlife such as bettongs and potoroos (Trappe & Claridge 2005).

We hypothesized that macrofungi found in restoration sites, based on sampling of macrofungal fruit-bodies, would be a subset of those in nearby remnant woodland, and that the diversity and composition of fungal genera will be greater in large restoration sites that are less isolated. We also expected increased fungal diversity in sites where the vegetation has greater structural and floristic diversity.

Methods

Study site

The town of Holbrook is located in the Upper Billabong Catchment (watershed) of southern New South Wales. The catchment has an average rainfall ranging from

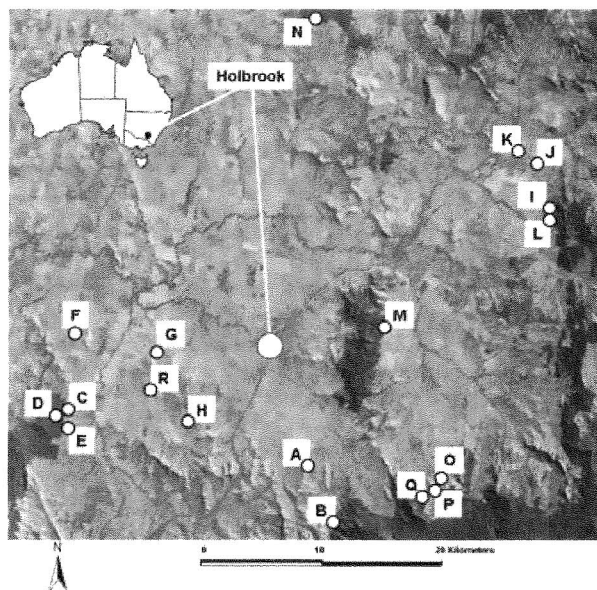


Figure 1. Location of the 12 restoration (planted) sites (A, C, E, F, G, H, J, K, L, O, P, R) and six sites in large patches of remnant woodland (sites B, D, I, M, N, Q). Remnant woodland is identified by the darker patches.

600–800 mm and elevations of 300–450 m (Fig. 1; 35° 42' 54" S, 147° 19' 04" E.). Less than 20% of the original tree cover remains. On more fertile lower slopes this figure is < 1%, usually existing as small, isolated remnants and scattered trees. Current land use is dominated by livestock grazing (58% of the catchment) and cereal cropping (7%, Lawson 2001). Since 1990, revegetation works by Holbrook Landcare Group have restored native trees and shrubs to around 2% of the catchment, with approximately 80% of farms undertaking some Landcare initiated planting.

Site histories

At the start of the study (2004), the average age of the woodland restoration plantings ranged between three and four years old. All occurred on cleared pastures dominated by exotic grass and legume species, or in areas that had been cultivated for cereals (primarily wheat). These restoration sites were typically associated with long-term superphosphate use, usually applied annually or biennially at a standard rate of 125 kg/ha. Woodland restoration sites were established around existing mature trees (paddock trees). Locally indigenous tree and shrub species were planted, including: Blakely's Red Gum (*Eucalyptus*

blakelyi), White Box (*E. albens*), Apple Box (*E. bridgesiana*). Yellow Box (*E. melliodora*), Silver Wattle (*Acacia dealbata*), Varnish Wattle (*A. uernictfua*), White-wood (*A. implexa*) and Teatree (*Melaleuca* sp.). Apart from short intensive grazing events, livestock were excluded from all restoration sites.

Six remnant woodland sites were located within the lower slopes of two national parks: Benambra (site D, Fig. 1) and Woomargama (sites B and Q), also Nest Hill Nature Reserve (site N) and within two privately owned properties (Sites M and I). The national parks and nature reserve tended to be on agriculturally marginal country, subject to logging, the removal of fallen trees and regular grazing to reduce fuel loads, until conversion from State Forests in 2001. As such, these remnant woodland sites only approximate the original pre-agricultural woodland. The most common tree species were Blakely's Red Gum, White Box, Red Box (*E. polyantbemos* ssp. *vestita*) and Red Stringybark (*E. macrorhynchay* and the most common shrub species were Silver Wattle, Daphne Heath (*Bracbytoma daphnoides*), Peach Heath (*Lissanthe strigosa*) and Prickly Teatree (*Leptospermum continentale*).

Study design

Woodland restoration (planted) sites ($n = 12$) were stratified with respect to their size and proximity to large patches of remnant woodland (> 10 ha). There were three small (< 3 ha) and three large (> 10 ha) woodland restoration sites that were relatively isolated (> 3 km to nearest patch of remnant woodland > 10 ha), as well as three small and three large restoration sites that were proximal < 1.5 km to nearest patch of remnant woodland > 10 ha, Figs 1,2). The average size of small sites was 2.0 ha (SE $\pm$ 0.3 ha) and large sites was 13 ha ($\pm$ 0.8 ha). The average distance to the nearest patch of woodland at least 10 ha in size was 4 km ($\pm$ 0.5 km) for isolated restoration sites and 0.5 km ($\pm$ 0.3 km) for proximal restoration sites. Six remnant woodland sites were selected as reference sites, occurring within extensive remnant woodland cover (> 400 ha, Figs 1,2). All sites included gullies and one or more mature trees. For more details of the study site and survey design, see Barrett *et al.* (2008).

Fungus surveys

Mushrooms and truffles were recorded in two 50x20 m (0.1 ha) plots that were placed at least 200 m apart in each of the 12 restoration sites and the six remnant woodland sites. In each of the restoration sites, the two fungus survey plots were stratified with respect to the presence or absence of isolated mature trees (one plot with and one without trees), but otherwise

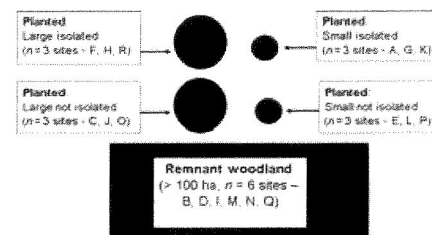


Figure 2. Description of 12 restoration (planted) sites defined as small (≤ 3 ha), large (> 10 ha), isolated (> 3 km to nearest patch of remnant woodland > 10 ha) or not isolated (< 1.5 km to nearest patch of remnant woodland > 10 ha). There were also six sites in extensive patches of remnant woodland (> 400 ha).

RESEARCH REPORT

randomly placed. In the remnant woodland sites, both fungus survey plots contained mature trees that formed a near complete canopy cover.

The fungus surveys were conducted once during autumn - winter of 2005 and autumn - winter 2006, sampled by active searches for 50 person-minutes, as described by Claridge *et al.* (2001b). In each fungus survey plot, litter and soil

were raked with a four-tined garden cultivator to depths of about 4 cm, proven to be effective for macrofungi but dependent on rainfall patterns. Fungi were identified to genus by use of standard references such as Grgurinovic (1997) and classified as epigeous (above-ground fruit-bodies, e.g. mushrooms, see Table 1 for definitions) or hypogeous (below ground, e.g. truffles) and by functional groups:

mycorrhizal (symbiotic with plants) or nonmycorrhizal: terrestrial decomposers (soil saprotrophs), wood decomposers, basidiolichens, dung inhabitants and insect pathogens (Dighton *et al.* 2005). Identification to species was possible for only a few taxa, as many collections were of undescribed species. However, Claridge *et al.* (2001a) has demonstrated that analysis of fungus collections at the genus level can yield meaningful inferences (Claridge *et al.* 2009).

Table 1. Definition of fungal terms

Fungal term	Definition
Macrofungi	Those that form fleshy mushrooms and truffles
Mycorrhizal	Form symbiotic associations with plant root systems
Epigeous	Above-ground fruit, i.e. mushrooms such as <i>Agaricus</i> , <i>Amanita</i> and <i>Russula</i> spp.
Hypogeous	Below ground fruit, i.e. truffles such as <i>Descomyces</i> , <i>Gymnomyces</i> , and <i>Hydnangium</i> spp.
Soil saprotrophs	Feed on decaying organic matter
Basidiolichens	Lichenized fungi (Basidiomycota)
Mycophagy	Feed on fungi

Table 2. Area, isolation and structural complexity scores, in restoration (planted) sites and remnant woodland sites. Results expressed as means ($\pm$ SE). * $P < 0.05$; ** $P < 0.01$; *** $P > 0.05$ (Kruskal-Wallis, $df = 1$). †Identifies variables included in modelling analysis

Explanatory variable	Remnant woodland ($n = 6$)	Planted sites ($n = 12$)
Remnant woodland vs planted site†	—	—
Mature trees present or absent†	—	—
Area of patch (ha) ***,†	> 100	7.4 $\pm$ 1.7
km to nearest woodland > 10 ha†	—	2.2 $\pm$ 0.6
Structural complexity score **,†	6.4 $\pm$ 0.5	4.1 $\pm$ 0.3
% Canopy cover (>4 m) **,†	33.9 $\pm$ 7.4	7.4 $\pm$ 1.3
% Tall shrub cover (2–4 m)*,†	16.3 $\pm$ 3.3	5.6 $\pm$ 1.2
% Low shrub cover (0.5–2 m)***,†	17.2 $\pm$ 3.6	15.2 $\pm$ 2.6
% Ground cover**	45.4 $\pm$ 8.2	78.5 $\pm$ 3.3
% Cover fallen timber***,†	12.7 $\pm$ 5.4	3.0 $\pm$ 1.4
% Cover leaf litter**	27.0 $\pm$ 5.9	6.9 $\pm$ 1.2

Vegetation structure and diversity

Structural complexity was estimated by visual assessments of the percent cover of the different vegetation strata, in three 50x50 m vegetation plots per restoration site, and three 25 m x 25 m vegetation plots in remnant woodland sites (Table 2). The percent cover of each stratum was visually scored (0 = <10%, 1 = 10–20%, 2 = 20–50%, 3 = >50%) and the scores summed for each plot, following the methods of Watson *et al.* (2001). Overall structural complexity scores, as well as scores for the separate components of vegetation complexity, were averaged across the three vegetation plots and two years (2005/6).

At each site, tree and shrub volume and density were measured, along three 45 m point-centred quarter (PCQ) transect lines (Krebs 1998), in spring over three years (2004 to 2006, Table 3). The species composition of forbs and grasses (including sedges) was sampled within four 1-m² ground cover plots, placed at four points (10 m, 20 m, 30 m and 40 m) along each of the 45 m transect lines. Species composition data from the PCQ transects and ground cover plots were combined to measure total floristic diversity. The 1-m² ground cover plots were also used to assess the percent cover of leaf litter, bare earth and the cover of native and exotic plant species. The ground cover plots and PCQ transects were randomly placed, and in the same location as the fungal survey plots (for more details see Barrett *et al.* 2008).

Analysis

General Linear Mixed Models were used to describe the response of fungal diversity

Table 3. Plant species diversity, volume and density in remnant woodland and restoration (planted) sites. Results expressed as the means ($\pm$ SE). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$, **** $P > 0.05$ (Kruskal-Wallis, $df = 1$). †Identifies variables included in modelling analysis

Explanatory variable	Remnant woodland ($n = 6$)	Planted sites ($n = 12$)
No. tree species**	3.0 $\pm$ 0.5	4.7 $\pm$ 0.3
No. shrub species****	4.5 $\pm$ 1.0	5.5 $\pm$ 0.6
No. native vegetation species **	33.8 $\pm$ 4.4	21.3 $\pm$ 1.4
No. native grass species****,†	6.7 $\pm$ 1.8	5.7 $\pm$ 0.9
No. native forb species***,†	14.7 $\pm$ 1.9	3.9 $\pm$ 0.6
Tree and shrub volume (m ³)***,†	4206 $\pm$ 1675	4055 $\pm$ 1376
Tree and shrub density (stems/ha) *,†	1507 $\pm$ 378	383 $\pm$ 59
% Ground cover – native forbs **,†	36.3 $\pm$ 7.8	9.7 $\pm$ 2.0
% Ground cover – exotic forbs****,†	39.9 $\pm$ 12.8	65.9 $\pm$ 9.3
% Bare ground*,†	9.2 $\pm$ 2.7	3.9 $\pm$ 0.8
% Leaf litter cover*,†	33.5 $\pm$ 9.0	13.1 $\pm$ 1.7

Table 4. Number of fungal genera in remnant woodland ($n = 12$ plots) and woodland restoration sites (planted, $n = 24$ plots). Percent values (in parentheses) are relative to the total number of genera (58). Note fungal categories are not mutually exclusive. All hypogeous genera were mycorrhizal

Location	Epigeous genera	Hypogeous genera (all mycorrhizal)	Mycorrhizal genera	Epigeous/mycorrhizal	Epigeous/non-mycorrhizal (non-mycorrhizal genera)	All fungal genera
Remnant woodland sites	37	11	21	9	27	48 (83)
Planted sites	27	6	15	4	18	33 (57)
Both remnants and planted sites	18	5	11	5	12	23 (40)
Exclusive to remnant woodland	19	6	10	4	15	25 (43)
Exclusive to planted sites	9	1	4	4	6	10 (17)
Total (% of 58 genera)	46 (79)	12 (21)	25 (43)	13 (22)	33 (57)	58

Table 5. Number of epigeous, hypogeous and mycorrhizal fungal genera in remnant woodland and woodland (planted) sites (mean $\pm$ SE, n = number of plots). ** $P < 0.01$ (Kruskal-Wallis, $df = 1$)

Nos. of genera in different groups	Remnant woodland ($n = 12$)	Planted sites ($n = 24$)
All genera**	9.4 $\pm$ 1.1	3.6 $\pm$ 0.4
Epigeous genera**	6.8 $\pm$ 0.9	3.0 $\pm$ 0.4
Hypogeous genera**	2.5 $\pm$ 0.5	0.6 $\pm$ 0.2
Mycorrhizal genera**	4.7 $\pm$ 0.6	2.3 $\pm$ 0.2

(based on the number of genera recorded in each plot) to 17 explanatory variables (fixed effects, Tables 2,3). Using the GENSTAT5.4.1 statistical package (Payne *et al.* 2006), a Poisson error was assumed and a logarithmic link function used, with an estimated dispersion parameter (Collett 1991). Fungal Survey Plot was included as a random variable (two plots in each of 18 Sites, $n = 36$). An iterative, interactive model building process was used (Henderson & Velleman 1981) with estimates of variance, significance levels, and the contribution towards a maximised Wald statistic as criteria for selecting explanatory variables (Payne *et al.* 2006). All variables (Table 2) were tested across all sites

($n = 36$ plots), within restoration sites ($n = 24$) and within remnant woodland sites ($n = 12$). Only significant predictors are presented (Table 6). Spearman rank correlations between explanatory variables are presented in Appendix 1. Standardization of generic richness using rarefaction to account for different sample sizes produced results qualitatively similar to the unstandardized results reported in the study (EcoSim version 7; Gotelli & Entsminger 2006).

Results

The 291 fungal collections across all 36 plots represent 58 fungal genera (Table 4

and Appendix 2). Species identifications are still underway, but we estimate about 90 species, of which five to ten are new to science. Epigeous (mushroom) fungi (Figs 3-6) were more common (226 collections, 78% of all collections, 46 genera) than hypogeous (truffle) fungi (65 collections, 22%, 12 genera, Figs 7-8). All hypogeous fungi were mycorrhizal species. Of the nutritional modes, mycorrhizal fungi were the most common (206 collections,

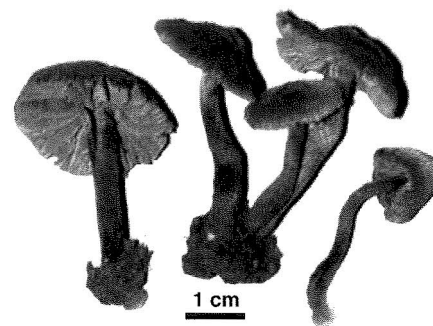


Figure 3. *Laccaria fraterna* (Hydnangiaceae), an epigeous, ectomycorrhizal fungus.

Table 6. GLMM models showing the relationship between fungal diversity and habitat variables. All variables were tested across all sites ($n = 36$ plots) as well as within restoration (planted, $n = 24$) and remnant woodland sites ($n = 12$). Only significant variables are presented. See Appendix 1 for fungal genera

Nos. of genera in different groups	(Estimates) Fitted term (standard errors)	Wald statistic	d.f.	Chi-sq prob.
Total genera	(0.007) Area of patch (0.002)	47	1	<0.001
	(0.15) Structural complexity (0.07)	5	1	0.035
Epigeous genera	(0.014) % cover native forb spp. (0.005)	33	1	<0.001
	(0.15) Structural complexity (0.06)	6	1	0.017
Epigeous genera in remnant sites only ($n = 12$)	(0.015) % cover native forb spp. (0.005)	10	1	0.02
Hypogeous genera	(0.075) % cover tall shrubs (0.018)	29	1	<0.001
	(0.06) No. native forb species (0.02)	6	1	0.013
Hypogeous genera in planted sites only ($n = 24$)	(0.51) Structural complexity (0.27)	4	1	0.054
Mycorrhizal genera	(0.04) % cover tall shrubs (0.009)	32	1	<0.001
	(0.028) No. native forb species (0.013)	3	1	0.031
Mycorrhizal genera in remnant sites only ($n = 12$)	(0.04) % cover tall shrubs (0.017)	7	1	0.01
				0.031

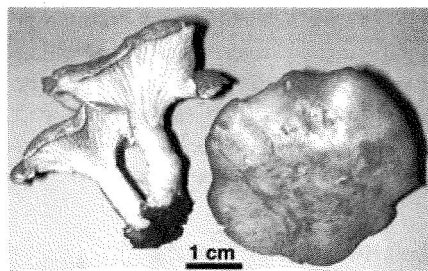


Figure 4. *Clitocybe* sp (Tricholomataceae), an epigeous, terrestrial decomposer fungus.

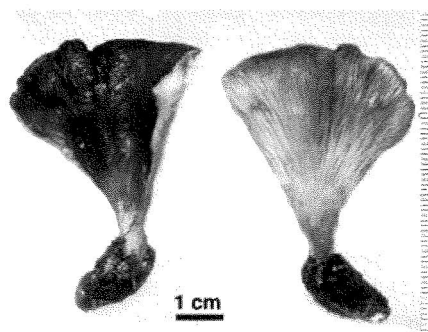


Figure 5. *Hohenbuehelia karrara* (Pleurotaceae), an epigeous wood decomposer.

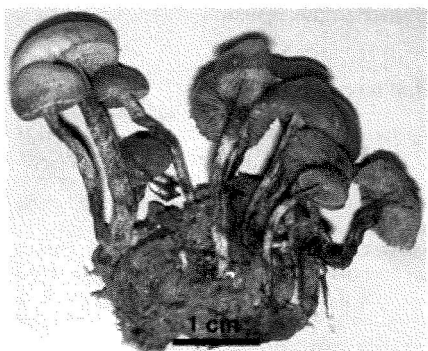


Figure 6. *Psilocybe argentina* (Strophariaceae), an epigeous dung decomposer on wombat dung.

71%, 25 genera, Figs 3, 7, 8) followed by soil saprotrophs (68 collections, 23%, 27 genera, Fig. 4, Appendix 2). Wood decomposers (Fig. 5), basidiolichens, dung inhabitants (Fig. 6) and insect pathogens together accounted for only 25 collections (9%, 13 genera).

Remnant woodland vs restoration sites

Of the 58 fungal genera recorded, 33 (57%) occurred in restoration sites,

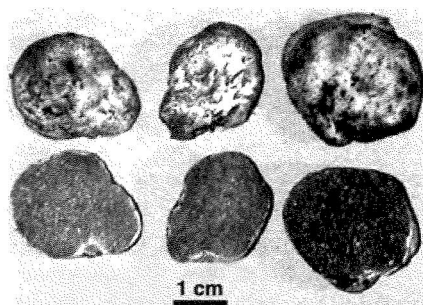


Figure 7. *Descomyces varians* (Bolbitaceae), a hypogeous, ectomycorrhizal fungus.

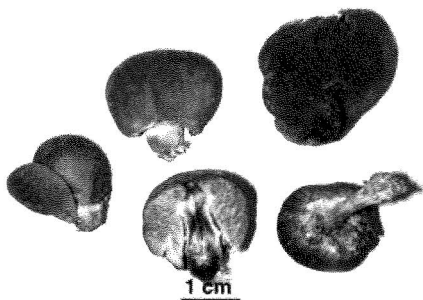


Figure 8. *Setchelliogaster tenuis* (Bolbitiaceae), a hypogeous, ectomycorrhizal fungus.

compared with 48 genera (83%) in remnant woodland sites (Table 4). The average number of genera per site and the total number of collections were also greater in remnant woodland sites (Table 5, Appendix 2). The 33 genera that occurred in restoration sites were largely a subset of those found in remnants, with 23 of these genera (70%) also occurring in remnant sites (Table 4). The proportion of epigeous:hypogeous:mycorrhizal fungi in remnant woodland (54%:16%:30%, that is, 37:11:21 genera respectively) was similar to that found in restoration sites (56%:13%:31%, that is, 27:6:15 genera, Table 4). However, the proportion of hypogeous genera recorded only in remnants was greater (6 out of the 35 epigeous, hypogeous and mycorrhizal genera exclusive to remnants, 17%) than the proportion of hypogeous genera recorded only in restoration sites (1 out of the 14 epigeous, hypogeous and mycorrhizal genera exclusive to restoration sites, 7%, Table 4).

The mycorrhizal genera differed between restoration and remnant woodland

sites. *Laccaria* (Hydnangiaceae, Fig. 3) strongly dominated restoration sites (45 collections, 16% of all collections, Appendix 2) but was less than half as common in remnants (20 collections, 7%). By contrast, *Cortinarius* (Cortinariaceae) dominated remnants, with 36 collections (12%) but was infrequent in restoration sites (5 collections, 2%). The relatively common *Scleroderma* (Sclerodermataceae) was found only in restoration sites, whereas *Gymnomyces* (Russulaceae) occurred four times as often in remnants than in restoration sites.

Fungi and environmental variables

The total number of fungal genera recorded in each site was most strongly correlated with area of woodland patch and structural complexity of the vegetation (GLMM, Table 6, Appendix 1 - Table 1, $P < 0.05$). Area of patch as an explanatory variable (Tables 2, 6) was stronger than the categorical distinction between remnant woodland and restoration sites. Larger patches, in addition to being associated with remnant woodland, were also positively correlated with the number of native vegetation species, leaf litter and structural complexity (Appendix 1 - Table 2).

The diversity of epigeous fungi was associated with the percent cover of native forb species (wildflowers) and structural complexity (Table 6, Appendix 1 - Table 1, $P < 0.05$). The diversity of both hypogeous and mycorrhizal fungi was greater in sites with a greater percent cover of tall shrubs (2–4 m) and a greater diversity of native forb species (Table 6, Appendix 1). The model results for all sites ($n = 36$) were broadly consistent with models generated within remnant woodland ($n = 12$) or within restoration sites ($n = 24$, Table 6).

Discussion

Our results indicate that even in landscapes with a long history of agriculture, many fungal genera can occur in 3- to 6-year-old woodland restoration sites. Of the 58 fungal genera recorded, nearly two thirds occurred in restoration sites (57%). Below average rainfall in south-eastern

Australia (<http://www.bom.gov.au>) is likely to have reduced the number of fruiting fungi in all sites. Yet the diversity of genera recorded resembled those found outside of drought periods in other woodland restoration sites in the region O. M. Trappe, unpubl. data, 2005). The prominence of epigeous fungi across all sites is linked to the visibility of above-ground fruiting bodies.

Fungal diversity in restoration sites

Whether fungi in the restoration sites came from windblown spores or those that were in the soil prior to planting is hard to determine. Even if surveys show fungi to be absent in paddock sites, as was demonstrated by Stol and Trappe (2006), this may be due to conditions being unsuitable for spores to germinate, one reason for untreated paddock controls not being included in our study. There is potential here for further research involving DNA sampling of soil samples to determine which spores have persisted in the agricultural matrix.

The dominance of *Laccaria* in and restriction of *Scleroderma* to restoration Sites, suggests introduction by wind-blown spores (or introduction from the nursery where the host plants were grown). Both these genera are common colonising species in eucalypt plantations (pampolina *et al.* 2002; see Appendix 2 for other genera that include pioneering species). The reduced proportion of hypogeous genera that were exclusive to restoration sites (7% compared with 17% hypogeous fungi that were exclusive to remnant woodland sites) may be related to minimal visitation by mycophagous mammals, ie. those that eat hypogeous fungi and disperse the unharmed spores in their scats (Claridge & Trappe 2005). That is, it is likely that suitable ground-foraging mammal vectors such as Swamp Wallabies (*Wallabia bicolor*) and Bush-rats (*Rattus fuscipes*) are less likely to visit and inoculate the relatively small, isolated restoration sites than the remnant woodland Sites, which also had greater habitat complexity (Goldney & Bowie 1990). This provides further support for the idea that colonisation is a primary

source of fungi in restoration sites (Enkhtuya *et al.* 2005).

Mature trees

It could be expected that the presence of mature trees in the woodland restoration sites would be associated with greater diversity of fungal fruit-bodies, because they provide a host plant for mycorrhizal species. That no such association was evident (Appendix 1 - Table 1) may have been due to much of the rainfall required for the fruiting of mushrooms and truffles being intercepted by the mature tree crowns. Impacts from previous livestock use, such as reduced leaf litter and soil compaction may have also reduced the fungal diversity under mature trees (Fleischner 1994).

Patch area and isolation

Our finding that overall fungal diversity was greater in larger patches is consistent with a study by Peay *et al.* (2007) that describes fewer microbes on smaller, isolated 'tree islands'. Patch area was a stronger predictor of fungal diversity than site type (remnant vs restoration site) or site isolation (Table 6). Although, it should be noted that vegetation structural complexity and forb species diversity, both of which were associated with fungal diversity, were greater in larger patches (Appendix 1 - Table 2).

Fungi and native forbs

It appears that the lower diversity of fungal genera in woodland restoration sites compared with remnants is correlated with lower native forb species diversity (wildflowers). The fact that forb species often rely on symbiotic fungal associations (J. Trappe, pers. comm., 2008) suggests a causal link may also exist between the absence of fungi and a lack of forb diversity. Both a decline in fungal and forb diversity are linked to intensive agriculture and the associated fertilizer use (Hossain *et al.* 1995; Dorrrough *et al.* 2006). Reduced native forb diversity has implications for the diversity of invertebrates (Atkinson *et al.* 2005) as well as ground-foraging, insectivorous woodland birds (Barrett *et al.* 2008) in woodland restoration sites. Options for reducing nutrient loads prior

to woodland restoration include removal of the upper soil layer, repeated harvesting of vegetation to run down nutrient levels, or experimenting with spring burns and carbon supplements, to manipulate the nitrogen cycle (Prober *et al.* 2005).

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Appendix 1

Correlation matrices for response and explanatory variables

Table 1. Correlation matrix for fungal groups and explanatory variables used in modelling analysis ($n = 36$).

	No. fungal genera	No. epigeous genera	No. hypogeous genera	No. mycorrhizal genera
No. fungal genera	1			
No. epigeous genera	0.946*	1		
No. hypogeous genera	0.645*	0.426*	1	
No. Mycorrhizal genera	0.827*	0.671*	0.81*	1

Table 1. (continued)

	No. fungal genera	No. epigeous genera	No. hypogeous genera	No. mycorrhizal genera
Mature trees present	-0.126	-0.117	-0.003	-0.147
Area of patch (ha)	0.711*	0.635*	0.554*	0.515*
km to nearest woodland (>10 ha)	-0.581*	-0.488*	-0.569*	-0.43*
No. tree species	-0.436*	-0.442*	-0.267	-0.276
No. shrub species	-0.097	0.033	-0.344*	-0.303
No. native vegetation species	0.533*	0.508*	0.326*	0.344*
No. native grass species	0.055	0.116	-0.111	-0.041
No. native forb species	0.673*	0.623*	0.496*	0.491*
Tree and shrub volume (m ³)	0.199	0.087	0.426*	0.321*
Tree and shrub density (stems/ha)	0.354*	0.272	0.411*	0.273
% Ground cover – native forbs	0.538*	0.535*	0.349*	0.38*
% Ground cover – exotic forbs	-0.214	-0.14	-0.259	-0.233
% Bare ground	0.227	0.108	0.399*	0.248
% Leaf litter cover	0.488*	0.379*	0.528*	0.504*
Structural complexity Score	0.612*	0.5*	0.624*	0.533*
% Canopy cover (>4 m)	0.53*	0.455*	0.484*	0.498*
% Tall shrub cover (2–4 m)	0.533*	0.406*	0.632*	0.58*
% Low shrub cover (0.5–2 m)	0.12	0.136	0.072	-0.016
% Cover fallen timber	0.283	0.255	0.197	0.254

* $P < 0.05$ (Kruskal–Wallis, $df = 1$, $n = 36$).**Table 2.** Correlation matrix for explanatory variables used in modelling analysis ($n = 18$).

Explanatory variable	Mature trees present	Area of patch (ha)	Km to nearest woodland (>10 ha)	No. tree species	No. shrub species	No. native vegetation species	No. native grass species	No. native forb species	Tree and shrub volume (m ³)
Mature trees present	1.00								
Area of patch (ha)	0.09	1.00							
Km to nearest woodland (>10 ha)	0.00	-0.69*	1.00						
No. tree species	0.06	-0.59*	0.59*	1.00					
No. shrub species	0.11	-0.03	0.08	-0.08	1.00				
No. native vegetation species	0.06	0.80*	-0.55*	-0.70*	0.16	1.00			
No. native grass species	-0.01	0.19	0.02	-0.47*	0.14	0.68*	1.00		
No. native forb species	0.25	0.87*	-0.70*	-0.78*	-0.05	0.86*	0.46*	1.00	
Tree and shrub volume (m ³)	-0.12	-0.10	-0.23	0.16	-0.52*	-0.21	-0.26	-0.13	1.00
Tree and shrub density (stems/ha)	0.05	0.20	-0.56*	-0.29	-0.04	0.06*	-0.16	0.24	0.37
% Ground cover – native forbs	0.12	0.59*	-0.57*	-0.848	0.09	0.62*	0.37	0.80*	-0.09
% Ground cover – exotic forbs	0.12	-0.18	0.39	0.32	0.04	-0.20	-0.15	-0.31	-0.19
% Bare ground	-0.29	0.29	-0.48*	-0.23	-0.34	0.02	-0.24	0.24	0.09
% Leaf litter cover	0.25	0.46*	-0.39	-0.19	-0.38	0.13	-0.20	0.49*	0.29
Structural complexity Score	0.15	0.59*	-0.45*	-0.23	-0.36	0.27	-0.16	0.47*	0.42*
% Canopy cover (>4 m)	0.23	0.51*	-0.46*	-0.52*	-0.21	0.27	-0.09	0.58*	0.03
% Tall shrub cover (2–4 m)	-0.20	0.45*	-0.61*	-0.30	-0.53*	0.24	-0.17	0.36	0.68*
% Low shrub cover (0.5–2 m)	-0.23	0.11	-0.02	0.17	0.10	-0.10	-0.17	-0.13	0.15
% Cover fallen timber	0.18	0.40*	-0.13	-0.21	0.11	0.24	-0.07	0.28	0.02

* $P < 0.05$ (Kruskal–Wallis, $df = 1$, $n = 18$).

Table 2. (continued)

Explanatory variable	Tree and shrub density (stems/ha)	% Ground cover – native forbs	% Ground cover – exotic forbs	% Bare ground	% Leaf litter cover	Structural complexity Score	% Canopy cover (>4 m)	% Tall shrub cover (2–4 m)	% Low shrub cover (0.5–2 m)	% Cover fallen timber
Tree and shrub density (stems/ha)	1.00									
% Ground cover – native forbs	0.26	1.00								
% Ground cover – exotic forbs	–0.51*	–0.25	1.00							
% Bare ground	0.53*	0.26	–0.48*	1.00						
% Leaf litter cover	0.31	0.48*	–0.42	0.51*	1.00					
Structural complexity Score	0.53*	0.35	–0.06	0.42*	0.66*	1.00				
% Canopy cover (>4 m)	0.55*	0.61*	–0.21	0.57*	0.69*	0.77*	1.00			
% Tall shrub cover (2–4 m)	0.39	0.25	–0.29	0.36	0.47*	0.64*	0.45*	1.00		
% Low shrub cover (0.5–2 m)	0.34	–0.08	0.24	0.19	–0.14	0.42*	0.12	0.06	1.00	
% Cover fallen timber	0.34	0.09	–0.10	0.06	0.31	0.60*	0.53*	0.19	0.20	1.00

* $P < 0.05$ (Kruskal–Wallis, $df = 1, n = 1$).**Appendix 2**

Fungal genera in remnant woodland and restoration (planted) sites, identified as epigeous (46 genera), hypogeous (12 genera), mycorrhizal (25 genera) and non-mycorrhizal: p, genera that include pioneering species; s, soil saprotrophs (68 collections, 27 genera); w, wood decomposers (17 collections, 11 genera); b, basidiolichens (6 collections, 1 genus); d, dung inhabitants (1 collection, 1 genus) and i, insect pathogens (1 collection, 1 genus).

Fungal genus	Remnant woodland	Planted sites	Epigeous genera	Hypogeous/mycorrhizal	Mycorrhizal genera	Epigeous/mycorrhizal	Epigeous/non-Mycorrhizal (non-mycorrhizal)
<i>Agaricus</i> ^s	1	1	2				2
<i>Agrocybe</i> ^{p,s}	1	0	1				1
<i>Amanita</i>	1	1	2		2	2	
<i>Arcangeliella</i>	5	0		5	5		
<i>Bisporella</i> ^s	1	0	1				1
<i>Bovista</i> ^{p,s}	5	3	8				8
<i>Calocera</i> ^{s,w}	2	1	3				3
<i>Calvatia</i> ^s	0	2	2				2
<i>Clitocybe</i> ^{p,s}	7	4	11				11
<i>Collybia</i> ^{p,s,w}	2	1	3				3
<i>Coltricia</i>	1	0	1		1	1	
<i>Coprinus</i> ^{p,s}	1	0	1				1
<i>Cordyceps</i> ⁱ	1	0	1				1
<i>Cortinarius</i>	36	5	41		41	41	
<i>Crepidotus</i> ^{s,w}	2	1	3				3
<i>Crucibulum</i> ^s	1	0	1				1
<i>Cystangium</i>	2	0		2	2		
<i>Dermocybe</i>	1	1	2		2	2	
<i>Descomyces</i> ^p	7	4		11	11		
<i>Dingleya</i>	1	0		1	1		
<i>Discinella</i> ^s	1	0	1				1
<i>Galerina</i> ^{p,s,w}	6	4	10		10	10	
<i>Gautieria</i>	1	0		1	1		
<i>Geastrum</i> ^s	1	0	1				1
<i>Glomus</i> ^p	0	1	1		1	1	
<i>Gymnomyces</i>	17	4		21	21		
<i>Gymnopilus</i> ^s	2	0	2				2
<i>Hohenbuehelia</i> ^w	3	0	3				3
<i>Humaria</i>	1	0	1		1	1	
<i>Hydnangium</i> ^p	3	1		4	4		
<i>Hydnoplicata</i>	0	1	1		1	1	

Appendix 2 (continued)

Fungal genus	Remnant woodland	Planted sites	Epigeous genera	Hypogeous/mycorrhizal	Mycorrhizal genera	Epigeous/mycorrhizal	Epigeous/non-Mycorrhizal (non-mycorrhizal)
<i>Hygrocybe</i> ^s	1	0	1				1
<i>Hymenogaster</i>	5	7		12	12		
<i>Hypholoma</i> ^w	1	0	1				1
<i>Hysterangium</i> ^p	0	3		3	3		
<i>Inocybe</i> ^p	1	0	1		1	1	
<i>Laccaria</i> ^{p,s}	20	45	65		65	65	
<i>Lichenomphalia</i> ^b	5	1	6				6
<i>Marasmius</i> ^s	0	1	1				1
<i>Mycena</i> ^s	3	2	5				5
<i>Nolanea</i> ^s	2	1	3				3
<i>Omphalina</i> ^{p,s}	1	1	2				2
<i>Paneolus</i> ^s	0	1	1				1
<i>Peziza</i>	1	0	1		1	1	
<i>Pisolithus</i> ^p	0	2	2		2	2	
<i>Pleurotus</i> ^w	0	1	1				1
<i>Pluteus</i> ^s	0	1	1				1
<i>Pogisperma</i>	1	0		1	1		
<i>Polyporus</i> ^w	1	0	1				1
<i>Psathyrella</i> ^s	1	2	3				3
<i>Psilocybe</i> ^{p,s,w,d}	2	5	7				7
<i>Scleroderma</i> ^p	0	13	13		13	13	
<i>Setchelliogaster</i>	2	1		3	3		
<i>Stereum</i> ^w	1	0	1				1
<i>Thaxterogaster</i>	1	0		1	1		
<i>Tubaria</i> ^{p,s}	4	0	4				4
<i>Xerula</i> ^{s,w}	2	0	2				2
Unknown	0	1	1				1
Total no. collections (% of total – 291)	168 (58)	123 (42)	226 (78)	65 (22)	206 (71)	141 (48)	85 (29)