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Establishment of ectomycorrhizal fungal community on isolated *Nothofagus cunninghamii* seedlings regenerating on dead wood in Australian wet temperate forests: does fruit-body type matter?

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Abstract Decaying wood provides an important habitat for animals and forms a seed bed for many shade-intolerant, small-seeded plants, particularly *Nothofagus*. Using morphotyping and rDNA sequence analysis, we compared the ectomycorrhizal fungal community of isolated *N. cunninghamii* seedlings regenerating in decayed wood against that of mature tree roots in the forest floor soil. The /cortinarius, /russula-lactarius, and /laccaria were the most species-rich and abundant lineages in forest floor soil in Australian sites at Yarra, Victoria and Warra, Tasmania. On root tips of seedlings in dead wood, a subset of the forest floor taxa were prevalent among them species of /laccaria, /tomentella-thelephora, and /descolea, but other forest floor dominants were rare. Statistical analyses suggested that the fungal community differs between forest floor soil and dead wood at the level of both species and phylogenetic lineage. The fungal species colonizing isolated seedlings on decayed wood in austral forests were taxonomically dissimilar to the species dominating in similar habitats in Europe. We conclude that formation of a resupinate fruit body type on the underside of decayed wood is not necessarily related to preferential root colonization in decayed wood. Rather, biogeographic factors as well as differential dispersal and competitive abilities of fungal taxa are likely to play a key role in structuring the ectomycorrhizal fungal community on isolated seedlings in decaying wood.

Keywords Coarse woody debris (CWD) ·
Forest regeneration · Southern beech (*Nothofagus*) ·
Sporocarp types · Temperate rain forest ·
Wet sclerophyll forest

Introduction

Decaying tree trunks and larger branches (termed coarse woody debris (CWD)) are substantial components in mature and old-growth forest ecosystems (Harmon et al. 1986). In addition to functioning as a carbon store and providing habitat and food source for many vertebrate and invertebrate species (Harmon et al. 1986; Yee et al. 2001), CWD forms an important microsite for seedling establishment in many mesic and humid ecosystems of the world (e.g., Howard 1973; Lawton and Putz 1988; Hofgaard 1993; McGee and

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Birmingham 1997). Seedling establishment and survival on CWD is particularly important in some ectomycorrhizal (EcM) and/or small-seeded plants (Hofgaard 1993; Lusk and Kelly 2003). The significance of CWD in seedling establishment is often ascribed to reduced litter accumulation, lower root and shoot competition, greater light availability, soil moisture, and wind-spread EcM inoculum (Harvey et al. 1978; Christy and Mack 1984; Harmon and Franklin 1989; Lusk 1995; McKenny and Kirkpatrick 1999). The relatively shade-intolerant *Nothofagus* species form EcM (Mejstrik 1971) and produce small seeds (Veblen et al. 1996). These features render the establishment of *Nothofagus* dependent on the presence of CWD or large canopy gaps throughout most of their geographical range (Howard 1973; Lusk 1995; Read and Brown 1996; McKenny and Kirkpatrick 1999; Lusk and Kelly 2003; Christie and Armesto 2003).

The EcM symbiosis is an important nutritional strategy particularly in the utilization of organic sources in nutrient-poor soils (Read et al. 2004). EcM plants may, thus, rely on their fungal symbionts for nutrient capture from strongly decayed CWD and/or its decomposer community (Lindahl et al. 2002; Tedersoo et al. 2003). In boreal forests, conifer and hardwood seedlings become EcM usually within a few months after seed germination in CWD (Christy et al. 1982; L.T. personal observation). Seedlings acquire their EcM symbionts either from spore-derived mycelium or from mycorrhizal mature tree roots that penetrate CWD from soil (Tedersoo et al. 2008b). In boreal forests, these CWD-inhabiting seedlings host a limited number of EcM fungi (Iwanski and Rudawska 2007), depending on the presence of root connections with mature host trees (Tedersoo et al. 2008b). In particular, isolated seedlings harbor only a few EcM fungal species that belong to the orders Atheliales and Thelephorales and form predominately resupinate fruit bodies (Tedersoo et al. 2008b). These taxa have a broad succession and host range and also dominate the root systems of mature trees both in the forest floor and CWD in boreal forest ecosystems (Goodman and Trofymow 1998; Elliott et al. 2007; Tedersoo et al. 2003, 2008b).

Tedersoo et al. (2003) hypothesized that fungal taxa belonging to Thelephorales, Atheliales, and Sebacinales have an evolutionary adaptation colonizing EcM root tips in CWD that is related to their resupinate fruiting habit on the underside of dead wood and potentially elevated saprotrophic abilities. Subsequently, it was suggested that certain pioneer members of the /tomentella-thelephora (Thelephorales) and /amphinema-tylospora (Atheliales) lineages may rather have enhanced dispersal or germination abilities or competitive advantages in young, carbon-starved seedlings (Tedersoo et al. 2008b). This study was undertaken to determine the richness of EcM fungi colonizing isolated seedlings of *Nothofagus cunninghamii* (Hook.) Oerst in an unrelated southern hemisphere ecosystem. We hypothesized that the fungi on CWD are closely related to these European members of Atheliales and Thelephorales. Using anatomotyping and rDNA sequence analysis, we studied the EcM fungal community on seedlings in CWD and on mature *N. cunninghamii* roots in forest floor soil, demonstrating substantial differences in frequency of EcM fungi at the species and lineage level.

Materials and methods

Site description

Root tips of *N. cunninghamii* seedlings and mature trees were collected from a 1-ha site at Acheron Gap in the Yarra Ranges National Park, Victoria, Australia and a 600-m^2 site at Warra LTER near Tahune, Geeveston, Tasmania, Australia. The Yarra site (37°41′ S 145°44′ E) harbored a cool temperate rain forest (Peel 1999) dominated by *N. cunninghamii* that formed a monodominant stand along a creek bank. A few *Acacia dealbata* (Link) F.Muell. trees were present, and there were some large trees of *Eucalyptus regnans* F.Muell. in wet sclerophyll forest around the margins of the site, but other potential EcM hosts were virtually absent from the study site. The understorey was dominated by the tree fern, *Dicksonia antarctica* Labill. The forest floor was strongly disturbed by lyrebird (*Menura novaehollandiae* Latham) activities. Small shrubs and grasses were sparse. The site lies around 770 m a. s. l. The mean annual rainfall is approximately 1,400 mm. The soil is a deep, humus-rich loam over a bedrock of silica-rich Devonian rhyodacite (Geological Survey of Victoria 1977; Peel 1999).

The Warra site (43°04′ S; 146°40′ E) comprised wet sclerophyll vegetation dominated by *Eucalyptus obliqua* L'Hér. with a subdominant layer comprising *N. cunninghamii*, *Eucryphia lucida* (Labill.) Baill., *Atherosperma moschatum* Labill., and *Phyllocladus aspleniifolius* (Labill.) Rich. Ex Hook.f. The understorey was made up of ferns *Anopterus glandulosus* Labill., *D. antarctica*, and *Polystichum proliferum* (R.Br.) C.Presl. Large boles of *E. obliqua* were covered with bryophytes and supported numerous seedlings and saplings of *N. cunninghamii* as well as various shrubs and ferns. The site lies approximately 155 m a.s.l. The mean annual rainfall is 1,080 mm. The soils are dark-brown clay loam of "Kermandie" class on quartzite bedrock with a dolerite talus (Alcorn et al. 2001; Laffan 2001).

Sampling and DNA analysis

At Warra and Yarra, respectively, 45 and 32 seedlings (1–5 years old) of *N. cunninghamii* were sampled by carefully pulling and digging the root systems out from decaying logs of medium decay classes (III and IV *sensu* Christy and Mack 1984). Up to two *Nothofagus* seedlings were sampled from the same log at least 5 m distance. To include the seedlings colonized by spores, not via root contacts, we confirmed the absence of mature tree roots in their rooting zone by digging the wood with a sharp knife. At Warra and Yarra, respectively, 24 and 16 root samples of mature *N. cunninghamii* (15×15 cm diam. to 5 cm depth) were collected from the forest floor soil. Small seedlings were virtually absent from the forest floor, whereas mature *Nothofagus* roots rarely penetrated CWD of medium decay classes. Therefore, our sampling did not include these age and substrate combinations. Roots were further cleaned from adhering debris and placed into Petri dishes with tap water, where *N. cunninghamii* roots were separated from other plant roots based on morphological differences. Only 22 forest-floor root samples included root tips of living *Nothofagus* at Warra, while all 16 forest-floor root samples supported living *Nothofagus* root tips at Yarra. In CWD, 42 and ten seedlings were colonized by EcM fungi at Warra and Yarra, respectively. Samples lacking *Nothofagus* root tips were removed from further analyses. All colonized root tips of *Nothofagus* were assigned to EcM morphotypes based on color, surface texture, presence or absence of cystidia, emanating hyphae, and rhizomorphs. Several root tips of each morphotype per root sample were stored in 1% CTAB DNA extraction buffer (100 mM Tris-HCl (pH8.0), 1.4 M NaCl, 20 mM EDTA, 1% cetyltrimethylammonium bromide) for anatomical studies and molecular analyses. Subsequently, several root tips from each morphotype per sample were subjected to anatomotyping as outlined in Agerer (1991). The DNA of one to five root tips of each anatomotype per site and substrate combination (270 root tips in total) was extracted using a High Pure PCR Template Preparation Kit for Isolation of Nucleic Acids from Mammalian Tissue (Roche Applied Science, Indianapolis, IN, USA) as described in Tedersoo (2007). Using a primer ITS1F (5′ cttggtcatttagaggaagtaa 3′) in combination with LB-W (5′ cttttcatctttccctcacgg 3′) or LA-W (5′ cttttcatctttcgatcactc 3′), we selectively amplified the internal transcribed spacer (ITS) region of basidiomycetes and ascomycetes, respectively. In addition, the rDNA large subunit (LSU) gene was amplified using primers LR0R (5′ acccgctgaacttaagc 3′) and LB-Z (5′ aaaaatggcccactagaaact 3′), LR3-Asc (5′ cacytactcaaatccaagcg 3′), or LB-W (Tedersoo 2007). Using a primer pair ML5 (5′ ctcggaaattatcctcataag 3′)–ML6 (5′ cagtagaagctgcatagggtc 3′), mitochondrial rDNA LSU (mtLSU) was amplified in morphotypes corresponding to Boletaceae sp4 and sp5 and *Tulasnella* sp3 because amplification of nuclear ITS and LSU regions failed consistently. *N. cunninghamii* was confirmed as a host species by recording length differences of plastid trnL region as described in Tedersoo (2007). The presence and length of PCR amplicons was checked on 1% agarose gels under UV light. Single PCR products were purified using Exo-Sap enzymes (Sigma, St. Louis, MO, USA). For sequencing, primers ITS5 (5′ ggaagtaaaagtcgtaacaagg 3′), ITS4 (5′ tcctccgcttattgatatgc 3′), ctb6 (5′ gcatatcaataagcggagg 3′), and ML6 were used. Sequence reads were checked against possible machine errors, edited, and assembled into contigs using Sequencher ver. 4.7 software (GeneCodes Corp., Ann Arbor, MI, USA). A value of 97.0% ITS region identity was used as a molecular species criterion (Tedersoo et al. 2003). For *Cortinarius* and *Laccaria*, 98.0% criterion was used because the ITS region is relatively conserved in these genera (L.T. pers. obs.). All unique sequences were submitted to the UNITE (Kõljalg et al. 2005) and EMBL (http://www.ebi.ac.uk/) public sequence databases. BlastN searches were performed against sequence databases INSD and UNITE to provide as precise identification for the EcM fungi as possible.

Statistical analyses

To compare the species richness between forest floor soil and CWD samples at both study sites, species accumulation curves with standard deviations were computed using EstimateS ver. 8 (Colwell 2006). In addition, samples from isolated 1–2-year-old *Betula pendula* L. (Betulaceae) and 1–5-year-old *Picea abies* (L.) H.Karst. (Pinaceae) seedlings inhabiting CWD in Estonian boreal forests (Tedersoo et al. 2008b) were included to uncover potential differences between the two contrasting ecosystems. In these analyses, 1,000 permutations were performed, fungal species were sampled randomly without replacement, and soil samples or individual seedlings were used as sampling units. Statistical differences between the substrates, two Australian sites and seedlings inhabiting CWD from the Southern vs. Northern Hemisphere were inferred using *Z* tests. For each pairwise comparison, the number of root samples was rarefied to the greatest number of shared samples.

Two-way ANOVA was performed to study the effects of site and substrate on species density (i.e., richness per sample) of EcM fungi. To meet the assumptions of homoscedasticity, species density was square-root transformed. Mann–Whitney *U* tests and Fisher's exact tests were calculated to address the differences in relative abundance of EcM fungal lineages and frequency of species, respectively, between forest floor soil and CWD. To control false discovery rate and reduce familywise error rate arising with multiple statistical testing, the obtained *P* values were subjected to a sharpening procedure of Benjamini–Hochberg correction (a less conservative analog of Bonferroni correction) as implemented in Verhoeven et al. (2005).

Using a computer program CAP (Anderson and Willis 2003), a canonical multivariate analysis was performed to evaluate differences in fungal communities between forest floor soil and CWD and between study sites. Samples containing at least three species were used as individual entities, whereas samples containing 1 to 2 species were randomly pooled within factor combinations to include at least three fungal species each. Because of pooling, binary-transformed species data were used in the analysis. Singletons were excluded from the analysis. Statistical significance of multivariate results was evaluated using 9,999 permutations with randomly assigned species data and calculating 95% confidence intervals for the two main canonical axes.

Using a chi-square test, we tested the null hypothesis that EcM of fungal taxa with resupinate and nonresupinate fruit bodies occur at similar frequency in CWD. Fungal lineages of /tomentella-thelephora, /tomentellopsis, /sebacina, /piloderma, and /tulasnella were considered producing resupinate fruit bodies. Although a minority of /sebacina and /tomentella-thelephora spp. form stipitate fruit bodies, no strong ITS sequence matches to stipitate fruiting taxa were evident and only resupinate members of /tomentella-thelephora and /sebacina fruited near the study site in the sampling period (U. Kõljalg and coworkers, unpublished), further supporting our classification. For this analysis, the frequencies of all molecularly identified species were summed by fruit-body type and substrate combinations. Another chi-square analysis was performed at the level of lineages, classified by fruiting habit and frequency of species in CWD or forest floor soil relative to that of the whole community mean. To further address potential phylogenetic biases among the EcM fungal community among substrates, *G* tests using species frequency data were performed for nine most common EcM fungal lineages (total frequency > 15; Fig. 1) as implemented in the computer program Unifrac (Lozupone et al. 2006). For this analysis, available LSU sequences from all species and ITS sequences from /cortinarius and /laccaria spp. (because of lack of resolution and incomplete coverage by LSU) were aligned using Mafft 5.861 (Katoh et al. 2005) and corrected manually. Eight species lacking >250 bp LSU sequence and *Tulasnella* sp3 were removed from the analysis because of the presence of long, unstable branches and unstable position. A parsimony analysis using 100 random start generations and TBR branch swapping was run in PAUP* 4.0d81 (Swofford 2002). One of the 21,834 most parsimonious trees was randomly selected as an input to Unifrac. Other trees differed mainly by the relative position of species of /cortinarius, /laccaria, and /descolea. The *P* values of *G* tests were subjected to the Benjamini–Hochberg correction as described above.

Results

At Warra and Yarra, respectively, *N. cunninghamii* supported 86 and 25 species of EcM fungi. CWD harbored 32 species at Warra and only six species at Yarra. When rarefied to ten seedlings, marginally significantly more EcM fungi were recovered from CWD at Warra compared to Yarra ($Z=2.05$; $P=0.040$; Fig. 2). Similarly, the number of rarefied species was significantly higher in forest floor soil at Warra compared to Yarra ($Z=4.78$; $P<0.001$). CWD harbored significantly less species compared to forest floor soil both at Warra ($Z=6.93$; $P<0.001$; Fig. 2) and Yarra ($Z=3.04$; $P=0.002$). There were no statistically significant differences in the rarefied richness of EcM fungi among Australian and Estonian sites. Species density (i.e., the number of species per sample) of EcM fungi was significantly higher at Warra than Yarra ($F_{1,86}=27.9$; $P<0.001$) and in forest floor soil compared to CWD ($F_{1,86}=215.4$; $P<0.001$). There was a significant site x substrate interaction ($F_{1,86}=6.4$; $P=0.013$) indicating that substrate differences were more pronounced at Warra.

All site and substrate combinations differed by the most frequent species (see Appendix). *Cenococcum* sp. and *Tomentella* sp2, respectively, were the most frequent in forest floor soil and CWD at Warra. *Russula* sp9 and *Laccaria* sp7 were, respectively, the most frequent in forest floor soil and CWD at Yarra. In terms of species richness, the lineage of /cortinarius dominated both sites, followed by /clavulina and /russula-lactarius at Warra and /descolea and /tomentella-thelephora at Yarra (Fig. 3a). Generally, the same lineages as well as /laccaria were the most abundant on root tips (Fig. 3b).

EcM of the fungal taxa expected to produce resupinate fruit bodies were found on 39.4% occasions ($n=66$) in CWD, whereas taxa with no or other types of fruit bodies ($n=263$) occurred on 24.3% occasions in CWD. Based on chi-square tests, the differences in these ratios were statistically significant ($\chi^2=6.02$; $df=1$; $P=0.014$). Further, lineage-level tests, however, provided no support for preferential association of resupinate fruit-body type with dead wood (Fisher's exact test: $P=1.0$). Nevertheless, there were statistically significant differences in abundance in several fungal lineages between forest floor and CWD (Fig. 3b). In particular, /cortinarius ($Z=6.72$; $P<0.001$), /russula-lactarius ($Z=6.01$; $P<0.001$), and /sordariales ($Z=3.69$; $P=0.009$) were more abundant in forest floor soil. *G* tests confirmed these findings for frequency of /cortinarius ($P<0.001$) and /russula-lactarius ($P=0.003$; Fig. 1). In addition, /laccaria (forming stipitate fruit bodies) was relatively more frequent in CWD than expected ($P=0.004$). Canonical multivariate analysis effectively separated the EcM fungal community by sites and substrates based on species frequency data ($P<0.001$;

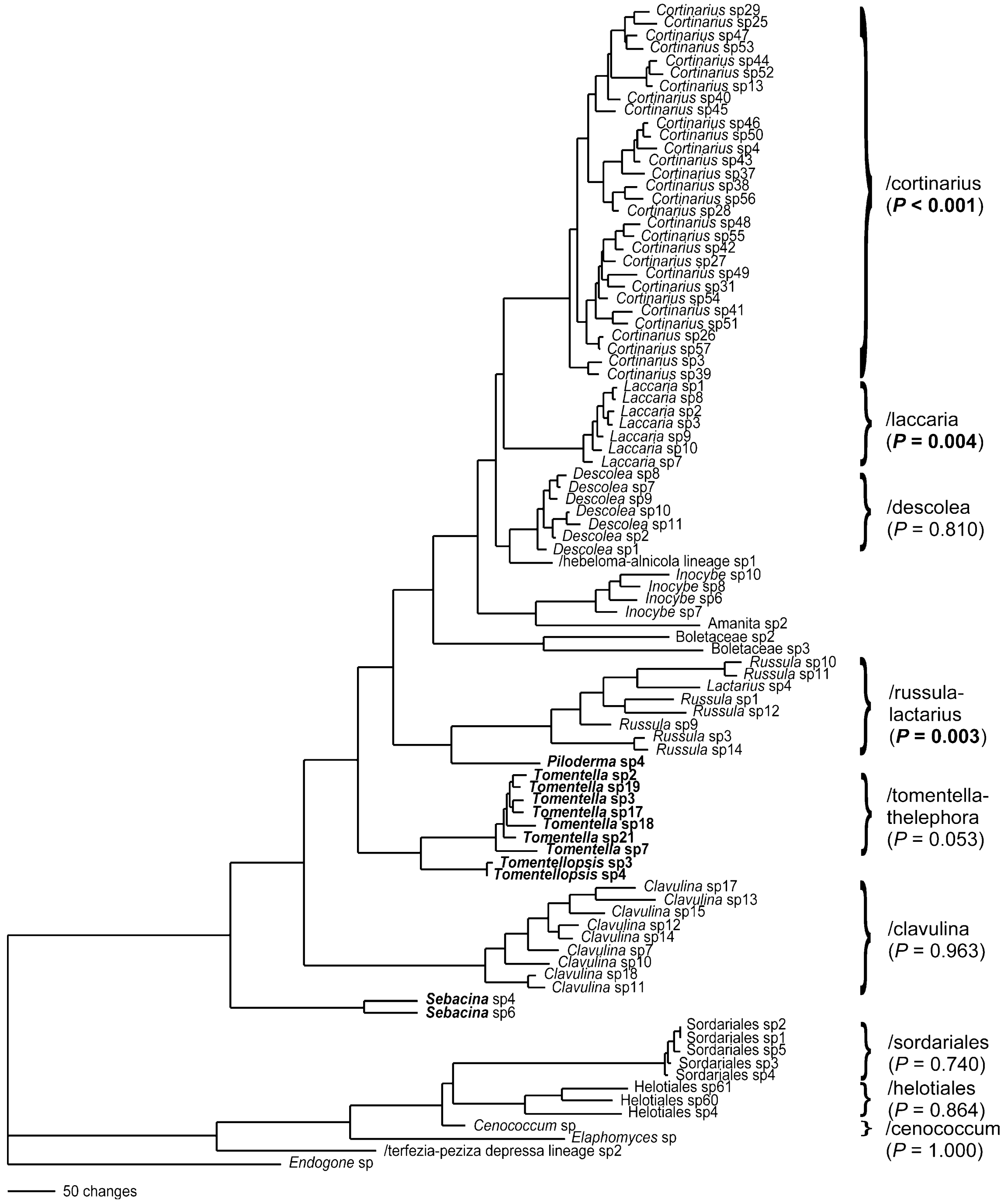

Fig. 1 Phylogram of ectomycorrhizal fungal species based on partial rDNA LSU sequences and ITS sequences (in /laccaria and /cortinarius). Species with putatively resupinate fruit bodies are in *bold*. *P* values of *G* tests for the differences in relative frequency of each fungal lineage among forest-floor soil and CWD are indicated. *Values in bold* denote statistically significant differences following Benjamini–Hochberg correction for familywise error of multiple testing (see Materials and methods)

Fig. 4). At species level, 14 of the 21 (66.7%) statistically compared taxa displayed significant differences for substrate (see Appendix). In particular, /cortinarius (3 spp.), /descolea, /russula-lactarius, and /tomentella-thelephora (2 spp. each) comprised species that were more frequent in forest floor soil than in CWD. Species occurring most commonly in CWD (*Tomentella* sp12, *Laccaria* sp7, *Cenococcum* sp., *Helotiales* sp4) were among the most frequent EcM symbionts in forest floor soil.

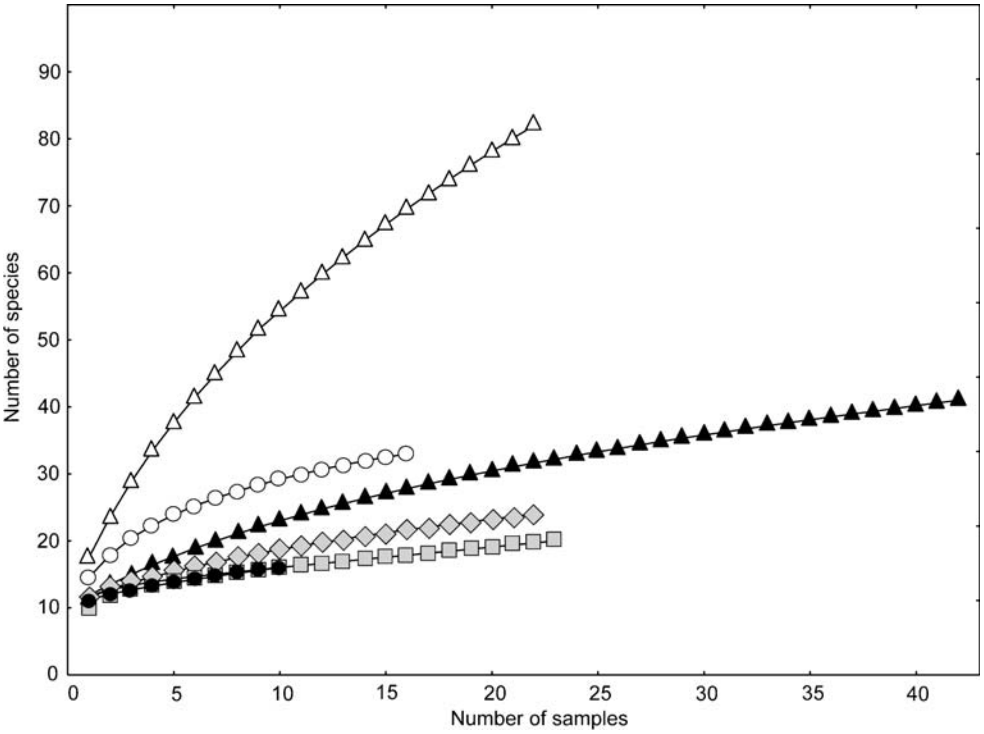

Fig. 2 Rarefaction curves demonstrating the accumulating ectomycorrhizal fungal species richness by root samples in different sites and habitats. *Open triangles* mature *N. cunninghamii* in forest-floor soil, Warra; *open circles N. cunninghamii* seedlings in CWD at Warra; *closed triangles* mature *N. cunninghamii* in forest-floor soil at Yarra; *closed circles N. cunninghamii* seedlings in CWD at Yarra; *shaded diamonds* 1–2-year-old *B. pendula* seedlings in CWD, Estonia (Tedersoo et al. 2008b); *shaded squares P. abies* seedlings in CWD, Estonia

Discussion

Compared to other Fagales-dominated ecosystems in the world, the diversity of EcM fungi was relatively low in the forest floor soil in *N. cunninghamii* forest patch at Yarra. This can be attributed to monospecificity (DeBellis et al. 2006; Tedersoo et al. 2006; Ishida et al. 2007) or bottlenecks in host population sizes during aridification of the Australian climate and increase in fire frequency, leading to fragmentation of *Nothofagus* populations, particularly in Victoria (Peel 1999; Hill 2004). Stressed plants tend to associate with less diverse communities of EcM fungi (Swaty et al. 2004; McHugh and Gehring 2006; Peter et al. 2008). Nevertheless, the EcM fungal community composition was similar at Yarra, Warra, and another Tasmanian site in Mt. Field National Park (Tedersoo et al. 2008a), where the lineages of /cortinarius, /tomentella-thelephora, /laccaria, and /descolea were among the most species-rich and abundant members. As a major difference to Tasmanian sites, we detected neither *Cenococcum* sp. nor *Clavulina* spp. on *Nothofagus* at Yarra. At Mt. Field, most of the dominant species displayed strong host preference for *Pomaderris*, *Eucalyptus*, or *Nothofagus*, which was suggested to contribute to the high EcM fungal richness at this site (Tedersoo et al. 2008a).

Seedlings on CWD at both study sites had fewer species of EcM fungi compared to mature tree roots in the forest floor that is comparable to sites in the northern hemisphere (Christy et al. 1982; Kropp 1982; Iwanski and Rudawska 2007; Tedersoo et al. 2008b). These differences in diversity between seedlings and mature trees and among substrates may be related to the stressful environment created by extreme substrate chemistry, low photosynthetic carbon availability and differential spore dispersal and germination abilities that filter out most taxa. We cannot rule out that differences in fungal richness arise partly from differential root density and abundance between seedling and adult tree root systems (L. Tedersoo, pers. obs.). In this study, we cannot separate host age and substrate effects on fungal richness and community structure due to the paucity of seedlings establishing on the forest floor and scarcity of mature tree roots in CWD of comparable decay classes. Nevertheless, our observations confirm the suggestions that seedling regeneration may be restricted to elevated microsites and occasional large-scale disturbance events in Australian *Nothofagus* ecosystems (Read and Brown 1996; McKenny and Kirkpatrick 1999). Previous studies indicate that seedlings connected to root systems of mature trees share most of their fungal symbionts with their conspecific adults in natural forests of various ecosystems (Simard et al. 1997; Jonsson et al. 1999; Matsuda and Hijii 2004) including CWD (Tedersoo et al. 2008b) that renders the host's age per se relatively unimportant.

The suite of fungi associated with Austral seedlings on CWD differs substantially from those in CWD in the northern hemisphere. In boreal forests, *Tomentella sublilacina* (Ellis and Holw.) Wakef., *Amphinema byssoides* complex, *Tylospora fibrillosa* Donk, or *Suillus* spp. are the most frequent species of EcM fungi on roots in CWD (Iwanski

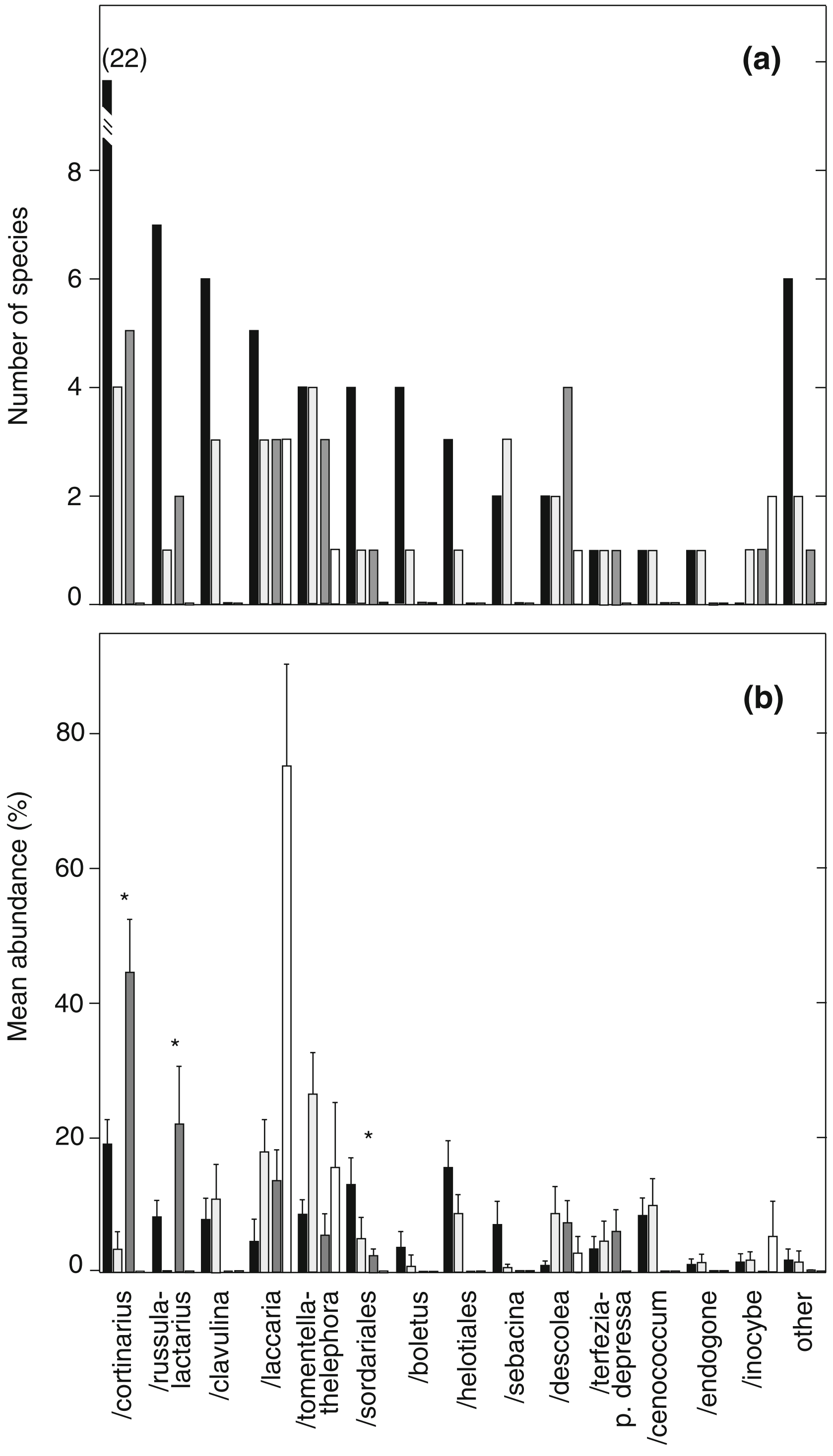

Fig. 3 Importance of EcM fungal lineages as symbionts of N. cunninghamii in forest-floor soil and CWD in two Australian wet temperate forests in terms of **a** species richness and **b** relative abundance (mean ± 95% CI). *Closed columns*, forest floor soil at Warra; *slightly shaded columns*, CWD at Warra; *heavily shaded columns*, forest floor soil at Yarra; *open columns*, CWD at Yarra. *Asterisks* denote statistically significant differences between CWD and forest floor soil using Mann–Whitney *U* tests followed by the sharpening procedure of the Benjamini–Hochberg correction (see "Materials and methods")

and Rudawska 2007; Tedersoo et al. 2008b). The genus *Suillus* is highly host-specific with Pinaceae (native species of which are lacking in Australia). The other three host-promiscuous species fruit on the underside of CWD and are among the most abundant members of EcM fungal communities in boreal mixed forests (Tedersoo et al. 2008b). These three species (or close relatives) are not known to associate with Australian indigenous host trees and were not detected below ground. In Australia, however, the EcM lineages of /laccaria, /descolea (Agaricales), and /tomentella-thelephora (Thelephorales) prevailed on isolated seedlings in CWD. Among these taxa, resupinate fruit bodies are formed only by

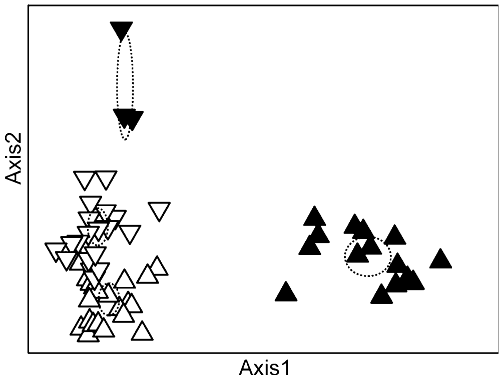

Fig. 4 Canonical multivariate analysis distinguishing fungal communities by species occurrence; to prevent statistical artifacts, CWD samples with <3 species were randomly pooled to comprise at least three species. *Open triangles* Warra site; *closed triangles* Yarra site; *upright triangles*, mature tree roots in forest floor soil; *inverted triangles*, seedlings in CWD. *Dotted ellipses* denote 95% CI. Permutation test ($P<0.001$) and leave-one-out cross-validation test (misclassification error 1.64%) revealed significant structuring in the fungal community

the majority of species in the /tomentella-thelephora lineage. In contrast to Australia, *Laccaria* spp. rarely colonize seedlings on CWD in European boreal forests, although they inhabit root tips and fruit abundantly in other disturbed forest microsites in such forests (Tedersoo et al. 2008b; unpublished). The /descolea lineage has just a few species outside Australia and none is known from natural forests of Europe (Horak 1971). Despite the different fungal assemblages in the two hemispheres and in agreement with the situation in Europe, the fungi colonizing isolated seedlings on CWD are among the dominant or subdominant taxa in the forest floor soil (Tedersoo et al. 2008b). However, not all forest-floor fungi occur in CWD, and the /russula-lactarius and /cortinarius lineages were among the most species rich and abundant lineages in the forest floor soil. These lineages were virtually lacking on seedlings in CWD in this study, which is also in agreement with observations in the northern hemisphere (Iwanski and Rudawska 2007; Tedersoo et al. 2008b). Members of the /russula-lactarius and /cortinarius lineages are considered late successional colonizers that may be excluded from isolated seedlings due to high resource requirements (Last et al. 1987; Gibson and Deacon 1990; Newton 1992; Hutchison and Piché 1995) or poor infectivity from spores (Ishida et al. 2008).

Overall frequency-based statistical analyses suggest that EcM fungi forming resupinate fruit bodies occur more frequently than nonresupinate fungi on EcM root tips in CWD in the Australian sites. However, this relationship collapsed when all EcM lineages were equally weighted or when one of the three most species-rich lineages was removed from the analysis, indicating that common taxa may bias comparative phylogenetic analyses. Nevertheless, the lineage-level analysis indicates that certain dominant non-resupinate fungal taxa such as /cortinarius and /russula-lactarius generally fail to colonize seedlings on CWD. Species-level analysis suggests that certain species from only a few frequently occurring lineages are able to colonize isolated seedlings in CWD, whereas such species with pioneer capacities are lacking or uncommon in other lineages. This highlights an urgent need to address the autecology of individual species and emphasizes that caution is required when interpreting the ecology of higher taxa based on a few examined species. Thus, we can speculate that biogeographic patterns of the distribution of a pioneer strategy in fungal lineages probably contribute to the observed differences in fungal communities between isolated seedlings and mature trees. The means by which pioneer species colonize dead wood is another aspect of autecology that needs investigating. Natural disturbance on the forest floor, created through tree fall or animal burrowing and scratching, could provide niches for colonization by pioneer fungi, whose fruit bodies then provide abundant and easily dispersed spores for inoculation of CWD and other disturbed microsites. Differential spore germination properties have already been shown for early and late successional ECM fungi (Ishida et al. 2008), but germinability in relation to CWD has not been tested.

In conclusion, seedlings of *Nothofagus* associate with EcM fungi during their establishment on CWD in Australian wet temperate forests. The associated fungi are among the generalist dominants of the forest-floor soil EcM fungal community, which is a phenomenon similar to boreal forests of the northern hemisphere. Several fungal lineages irrespective of fruit-body type included species with pioneer capacities that were able to colonize isolated seedlings on CWD presumably by air- or microfauna-dispersed spores. The significant association of resupinate fruit bodies with seedlings in CWD is attributable to an artefactual phylogenetic effect solely based on the ecological traits in a few dominant lineages. The direct contribution of EcM fungi to seedling establishment on CWD remains to be determined in future studies.

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Appendix

Identification, site, and substrate preference of EcM fungi; *P* values of Fisher's exact test for biased frequency towards sites and substrates are indicated. Statistically significant differences following sharpening procedure of the Benhamini–Hochberg corrections (see "Materials and methods") are shown in bold.

Species identified	UNITE accession	Identification of species: best BLASTn match(es) in INSD and/or UNITE		Species relative frequency / abundance (%)				*P* value for differences between substrates
				Yarra		Warra		
Species		Specimen accession number	ID (%)^c	Soil (n=16)	CWD (n=10)	Soil (n=22)	CWD (n=42)	
Cortinarius sp3	UDB004000	*Thaxterogaster albocanus* AF325599	93.1	56.3/16.3	0.0/0.0	0.0/0.0	0.0/0.0	**<0.001**
Russula sp9	UDB004001	*Russula crustosa* EU598194	89.2	50.0/21.8	0.0/0.0	0.0/0.0	0.0/0.0	**0.001**
Laccaria sp7	UDB004002	*Laccaria laccata* AJ699075	94.2	43.8/9.3	60.0/60.2	0.0/0.0	7.1/3.3	1.000
7terfezia-peziza depressa lineage sp2	UDB004003	*Peziza badia* DQ384574	77.7	37.5/5.6	0.0/0.0	18.2/3.0	7.1/4.3	**0.013**
Sordariales sp2	UDB004004	*Vateriopsis* EcM fungus AM412279	89.6	37.5/2.3	0.0/0.0	0.0/0.0	0.0/0.0	0.160
		Rhodoveronaea varioseptata EU041813	86.0					
Descolea sp10	UDB004005	*Setchelliogaster australiensis* AF325628	97.5	31.3/5.4	0.0/0.0	0.0/0.0	0.0/0.0	**0.011**
Cortinarius sp26	UDB004006	*Cortinarius amoenus* UDB001007	93.7	31.3/13.3	0.0/0.0	18.2/0.5	0.0/0.0	**<0.001**
Cortinarius sp13	UDB004007	*Cortinarius cystidiocatenatus* AY669651	94.7	25.0/9.1	0.0/0.0	0.0/0.0	0.0/0.0	**0.029**
Tomentella sp2	UDB004008	*Tomentella lateritia* UDB000268	93.9	18.8/1.1	20.0/14.8	31.8/3.7	28.6/17.3	1.000
Tomentella sp18	UDB004009	*Tomentella lateritia* UDB000963	89.5	12.5/3.2	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Cortinarius sp28	UDB004010	*Cortinarius walkeri* AY669632	96.5	12.5/2.8	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Cortinarius sp25	UDB004011	*Quadrispora tubercularis* DQ328113	94.4	12.5/1.6	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Tomentella sp17	UDB004012	*Tomentella lilacinogrisea* UDB000272	92.8	12.5/0.8	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Cortinarius sp31	UDB004013	*Cortinarius collariatus* AY033115	94.4	12.5/0.6	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Cortinarius sp27	UDB004014	*Thaxterogaster albocanus* AF325599	94.7	12.5/0.2	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Laccaria sp8	UDB004015	*Laccaria laccata* AJ699075	95.0	6.3/3.6	10.0/9.7	0.0/0.0	0.0/0.0	n.d.
Descolea sp8	UDB004016	*Descomyces albus* DQ328168	94.6	6.3/1.3	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Cortinarius sp29	UDB004017	*Thaxterogaster levisporus* DQ328105	93.5	6.3/0.6	0.0/0.0	9.1/0.8	0.0/0.0	n.d.
Russula sp10	UDB004018	*Russula clelandii* DQ328136	93.9	6.3/0.4	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Laccaria sp9	UDB004019	*Laccaria laccata* AJ699075	98.2	6.3/0.3	10.0/7.8	0.0/0.0	0.0/0.0	n.d.
Descolea sp7	UDB004020	*Descomyces albus* DQ328168	93.9	6.3/0.2	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Descolea sp9	UDB004021	*Descomyces* sp. DQ328062	99.0	6.3/0.1	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Elaphomyces sp	UDB004022	*Elaphomyces muricatus* DQ974740	87.7	6.3/0.1	0.0/0.0	0.0/0.0	0.0/0.0	n.d.
Inocybe sp6	UDB004023	*Inocybe subnudipes* AM882809	85.7	0.0/0.0	10.0/5.0	0.0/0.0	0.0/0.0	n.d.
Descolea sp11	UDB004024	*Setchelliogaster australiensis* AF325628	99.2	0.0/0.0	10.0/2.5	0.0/0.0	0.0/0.0	n.d.

Species identified	UNITE accession	Identification of species: best BLASTn match(es) in INSD and/or UNITE		Species relative frequency / abundance (%)				P value for differences between substrates
				Yarra		Warra		
Species		Specimen accession number	ID (%)[c]	Soil (n=16)	CWD (n=10)	Soil (n=22)	CWD (n=42)	
Cenococcum sp	UDB004025	*Pinus* EcM root tip (Japan) AB251837	99.6	0.0/0.0	0.0/0.0	72.7/7.8	16.7/9.4	0.001
		Cenococcum geophilum AB089816	99.1					
Clavulina sp12	UDB004026	*Clavulina cinerea* AY456339	83.7	0.0/0.0	0.0/0.0	36.4/4.8	0.0/0.0	<0.001
Helotiales sp61	UDB004027	*Bryoglossum gracile* AY789421	85.9	0.0/0.0	0.0/0.0	31.8/7.5	0.0/0.0	0.001
Helotiales sp4	UDB004028	*Roesleria subterranea* EF060309	86.5	0.0/0.0	0.0/0.0	31.8/4.7	16.7/6.9	1.000
Tomentella sp7	UDB004029	*Tomentella badia* UDB001656	92.7	0.0/0.0	0.0/0.0	31.8/2.5	4.8/1.1	0.033
Sordariales sp1	UDB004030	*Vateriopsis* EcM fungus AM412279	88.6	0.0/0.0	0.0/0.0	27.3/9.6	7.1/4.7	n.d.
		Rhodoveronaea varioseptata EU041813	86.3					
Tomentella sp21	UDB004031	*Tomentella lateritia* UDB000268	91.5	0.0/0.0	0.0/0.0	27.3/1.8	0.0/0.0	0.004
Russula sp1	UDB004032	*Russula persanguinea* EU019916	99.2	0.0/0.0	0.0/0.0	22.7/3.4	0.0/0.0	0.011
Endogone sp	UDB004033	*Endogone pisiformis* AF006511	Partial[d]	0.0/0.0	0.0/0.0	22.7/1.1	2.4/2.4	n.d.
Sebacina sp4	UDB004034	*Sebacina helvelloides* AJ966750	90.4	0.0/0.0	0.0/0.0	18.2/5.9	0.0/0.0	0.029
Boletaceae sp2	UDB004035	*Xerocomus chrysonemus* DQ066383	84.3	0.0/0.0	0.0/0.0	18.2/0.6	0.0/0.0	0.029
Russula sp11	UDB004036	*Russula clelandii* DQ328136	93.4	0.0/0.0	0.0/0.0	13.6/0.7	0.0/0.0	n.d.
Laccaria sp1	UDB004037	*Laccaria laccata* AJ699075	95.9	0.0/0.0	0.0/0.0	9.1/3.5	7.1/5.1	1.000
Cortinarius sp44	UDB004038	*Cortinarius cystidiocatenatus* AY669651	95.9	0.0/0.0	0.0/0.0	9.1/2.7	0.0/0.0	n.d.
Sordariales sp5	UDB004039	*Vateriopsis* EcM fungus AM412279	89.2	0.0/0.0	0.0/0.0	9.1/2.5	0.0/0.0	n.d.
		Rhodoveronaea varioseptata EU041813	86.0					
Russula sp12	UDB004040	*Russula pallidospora* DQ422032	84.1	0.0/0.0	0.0/0.0	9.1/2.3	0.0/0.0	n.d.
Clavulina sp10	UDB004041	*Clavulina cinerea* AY456339	79.1	0.0/0.0	0.0/0.0	9.1/1.5	0.0/0.0	n.d.
Cortinarius sp41	UDB004042	*Cortinarius subcastanellus* AY669623	93.0	0.0/0.0	0.0/0.0	9.1/1.5	0.0/0.0	n.d.
Sebacina sp6	UDB004043	*Tremellodendron pallidum* AF384862	92.4	0.0/0.0	0.0/0.0	9.1/1.4	2.4/0.2	n.d.
Inocybe sp8	UDB004044	*Inocybe hystrix* AM882810	79.3	0.0/0.0	0.0/0.0	9.1/1.4	0.0/0.0	n.d.
Piloderma sp4	UDB004045	*Piloderma olivaceum* DQ469291	85.6	0.0/0.0	0.0/0.0	9.1/1.1	0.0/0.0	n.d.
Cortinarius sp56	UDB004046	*Cortinarius teraturgus* AF389151	91.4	0.0/0.0	0.0/0.0	9.1/1.0	2.4/0.7	n.d.
Descolea sp1	UDB004047	*Descolea phlebophora* AF325656	100.0	0.0/0.0	0.0/0.0	9.1/0.7	2.4/1.2	n.d.
Laccaria sp10	UDB004048	*Laccaria laccata* AJ699075	97.0	0.0/0.0	0.0/0.0	9.1/0.4	0.0/0.0	n.d.
Cortinarius sp47	UDB004049	*Thaxterogaster levisporus* DQ328105	93.8	0.0/0.0	0.0/0.0	9.1/0.2	0.0/0.0	n.d.
Tomentellopsis sp4	UDB004050	*Tomentellopsis zygodesmoides* AJ410761	93.0	0.0/0.0	0.0/0.0	9.1/0.1	0.0/0.0	n.d.
Helotiales sp60	UDB004051	*Leptodontidium elatius* AY787713	86.4	0.0/0.0	0.0/0.0	4.5/3.0	0.0/0.0	n.d.
Cortinarius sp39	UDB004052	*Cortinarius teraturgus* AF389151	93.7	0.0/0.0	0.0/0.0	4.5/2.5	0.0/0.0	n.d.
Boletaceae sp3	UDB004053	*Chamonixia caespitosa* DQ534565	81.3	0.0/0.0	0.0/0.0	4.5/2.3	0.0/0.0	n.d.

Cortinarius sp46	UDB004054	*Cortinarius ochrophyllus* UDB000675	95.3	0.0/0.0	0.0/0.0	4.5/1.6	0.0/0.0	n.d.
Cortinarius sp53	UDB004055	*Thaxterogaster levisporus* DQ328105	94.1	0.0/0.0	0.0/0.0	4.5/1.5	0.0/0.0	n.d.
Cortinarius sp42	UDB004056	*Cortinarius huronensis* UDB002251	95.9	0.0/0.0	0.0/0.0	4.5/1.0	0.0/0.0	n.d.
Russula sp14	UDB004057[b]	*Russula* sp. MEL2238395 EU019920	100.0	0.0/0.0	0.0/0.0	4.5/0.9	0.0/0.0	n.d.
Cortinarius sp50	UDB004058	*Cortinarius walkeri* AY669632	96.1	0.0/0.0	0.0/0.0	4.5/0.7	0.0/0.0	n.d.
Cortinarius sp54	UDB004059	*Cortinarius collariatus* AY033115	94.2	0.0/0.0	0.0/0.0	4.5/0.7	0.0/0.0	n.d.
Cortinarius sp45	UDB004060	*Cortinarius amoenus* AF389160	92.7	0.0/0.0	0.0/0.0	4.5/0.6	0.0/0.0	n.d.
Boletaceae sp4	UDB004061[a]	*Boletellus projectellus* DQ534582	99.4	0.0/0.0	0.0/0.0	4.5/0.5	2.4/1.7	n.d.
Clavulina sp14	UDB004062	*Clavulina cinerea* AY456339	85.2	0.0/0.0	0.0/0.0	4.5/0.5	0.0/0.0	n.d.
Cortinarius sp4	UDB004063	*Cortinarius badiovinaceus* UDB002221	94.4	0.0/0.0	0.0/0.0	4.5/0.5	0.0/0.0	n.d.
Cortinarius sp43	UDB004064	*Cortinarius badiovinaceus* UDB002221	96.0	0.0/0.0	0.0/0.0	4.5/0.5	0.0/0.0	n.d.
Cortinarius sp49	UDB004065	*Cortinarius delibutus* AY669587	92.5	0.0/0.0	0.0/0.0	4.5/0.5	0.0/0.0	n.d.
Cortinarius sp51	UDB004066	*Cortinarius cephalixus* AY174784	93.7	0.0/0.0	0.0/0.0	4.5/0.5	0.0/0.0	n.d.
Amanita sp2	UDB004067[b]	*Amanita rubescens* AF097383	98.0	0.0/0.0	0.0/0.0	4.5/0.3	0.0/0.0	n.d.
Cortinarius sp48	UDB004068	*Thaxterogaster* sp DQ328183	94.4	0.0/0.0	0.0/0.0	4.5/0.2	0.0/0.0	n.d.
Russula sp3	UDB004069	*Russula ingwa* EU019919	94.5	0.0/0.0	0.0/0.0	4.5/0.2	0.0/0.0	n.d.
Sordariales sp3	UDB004070	*Vateriopsis* EcM fungus AM412279	88.6	0.0/0.0	0.0/0.0	4.5/0.2	0.0/0.0	n.d.
		Rhodoveronaea varioseptata EU041813	86.2					
Unidentified sp7	n.d.	n.d.	n.d.	0.0/0.0	0.0/0.0	4.5/0.2	0.0/0.0	n.d.
Laccaria sp2	UDB004071	*Laccaria laccata* AJ699075	97.4	0.0/0.0	0.0/0.0	4.5/0.1	9.5/9.4	0.392
Tulasnella sp3	UDB004072	*Stelis* orchid mycorrhizal fungus DQ178118	76.4	0.0/0.0	0.0/0.0	4.5/0.1	4.8/1.2	n.d.
		Tulasnella tomaculum AY373296	partial					
Clavulina sp13	UDB004073	*Clavulina cinerea* AY456339	80.8	0.0/0.0	0.0/0.0	4.5/0.1	2.4/1.7	n.d.
Tomentella sp3	UDB004074	*Tomentella lilacinogrisea* UDB000953	93.7	0.0/0.0	0.0/0.0	4.5/0.1	2.4/0.7	n.d.
/hebeloma-alnicola lineage sp1	UDB004075	*Alnicola escharoides* AF124714	90.4	0.0/0.0	0.0/0.0	4.5/0.1	2.4/0.5	n.d.
Russula sp13	UDB004076	*Russula clelandii* DQ328136	93.9	0.0/0.0	0.0/0.0	4.5/0.1	2.4/0.1	n.d.
Descolea sp2	UDB004077	*Descolea recedens* AF325650	99.2	0.0/0.0	0.0/0.0	4.5/0.1	11.9/7.1	0.395
Boletaceae sp5	UDB004078[a]	*Leccinum duriusculum* AF484444	99.7	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Clavulina sp11	UDB004079	*Clavulina* sp DQ822798	78.2	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Clavulina sp16	UDB004080	*Clavulina* sp. DQ822798	78.2	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Cortinarius sp38	UDB004081	*Cortinarius walkeri* AY669632	94.1	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Cortinarius sp40	UDB004082	*Thaxterogaster* sp. DQ328121	94.3	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Cortinarius sp52	UDB004083	*Cortinarius cystidiocatenatus* AY669651	94.3	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Cortinarius sp55	UDB004084	*Thaxterogaster* sp. DQ328183	97.9	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Cortinarius sp57	UDB004085	*Cortinarius amoenus* AF389160	95.3	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Laccaria sp3	UDB004086	*Laccaria laccata* AJ699075	99.0	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Lactarius sp4	UDB004087	*Lactarius gerardii* EF560688	82.4	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Sordariales sp4	UDB004088	Vateriopsis EcM fungus AM412279	89.4	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
		Rhodoveronaea varioseptata EU041813	86.0					

Species identified	UNITE accession	Identification of species: best BLASTn match(es) in		Species relative frequency / abundance (%)				*P* value for differences between substrates
		INSD and/or UNITE		Yarra		Warra		
Species		Specimen accession number	ID (%)[c]	Soil (n=16)	CWD (n=10)	Soil (n=22)	CWD (n=42)	
Tomentellopsis sp3	UDB004089[2]	*Tomentellopsis bresadoliana* EU118674	99.0	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Unidentified sp6	n.d.	n.d.	n.d.	0.0/0.0	0.0/0.0	4.5/0.1	0.0/0.0	n.d.
Tomentella sp19	UDB004090	*Tomentella botryoides* UDB000258	94.9	0.0/0.0	0.0/0.0	0.0/0.0	9.5/7.1	n.d.
Clavulina sp7	UDB004091	*Clavulina cristata* EF559274	84.3	0.0/0.0	0.0/0.0	0.0/0.0	7.1/6.7	n.d.
Inocybe sp10	UDB004092	*Inocybe lanuginosa* DQ367905	86.6	0.0/0.0	0.0/0.0	0.0/0.0	4.8/2.5	n.d.
Cortinarius sp36	UDB004093	*Cortinarius walkeri* AY669632	95.8	0.0/0.0	0.0/0.0	0.0/0.0	2.4/2.4	n.d.
Sebacina sp7	n.d.	n.d.	n.d.	0.0/0.0	0.0/0.0	0.0/0.0	2.4/2.3	n.d.
Clavulina sp17	UDB004094	*Clavulina cinerea* AY456339	83.8	0.0/0.0	0.0/0.0	0.0/0.0	2.4/1.3	n.d.
Clavulina sp18	UDB004095	*Clavulina cristata* EF559274	partial	0.0/0.0	0.0/0.0	0.0/0.0	2.4/0.5	n.d.
Inocybe sp7	UDB004096	*Inocybe ambigua* AM882800	85.2	0.0/0.0	0.0/0.0	0.0/0.0	2.4/0.5	n.d.
Clavulina sp15	UDB004097	*Clavulina cristata* EF559274	93.8	0.0/0.0	0.0/0.0	0.0/0.0	2.4/0.2	n.d.
Cortinarius sp37	UDB004098	*Cortinarius amoenus* AF389160	94.5	0.0/0.0	0.0/0.0	0.0/0.0	2.4/0.1	n.d.
Sebacina sp5	UDB004099	*Tremellodendron* sp. DQ411526	89.5	0.0/0.0	0.0/0.0	0.0/0.0	2.4/0.1	n.d.

n.d. not determined

[a] Identification based on mtLSU sequence

[b] Identification based on nuLSU sequence

[c] Identical nucleotides (%) spanning the whole length of the ITS region (comprising ITS1, 5.8S rRNA gene and ITS2)

[d] Sequences only partially alignable

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