Diversity and distribution of saprobic microfungi in leaf litter of an Australian tropical rainforest

Barbara C. PAULUS*, John KANOWSKI, Paul A. GADEK, & Kevin D. HYDE

*Corresponding author: paulusb@landcareresearch.co.nz

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Abstract:

The diversity and distribution of microfungal assemblages in leaf litter of a tropical Australian forest was assessed using two methods: (1) cultures were isolated using a particle filtration protocol (wet season 2001), and (2) fruit bodies were observed directly on leaf surfaces following incubation in humid chambers (wet and dry season of 2002). Four tree species were studied using both methods, namely *Cryptocarya mackinnoniana* (Lauraceae), *Elaeocarpus angustifolius* (Elaeocarpaceae), *Ficus pleurocarpa* (Moraceae), and *Opisthiolepis heterophylla* (Proteaceae). An additional two species, *Darlingia ferruginea* (Proteaceae) and *Ficus destruens* (Moraceae), were studied using direct observations. In total, fruiting bodies of 185 microfungal species were recorded on leaf surfaces (31–81 species per tree species), and 419 morphotypes were detected among isolates obtained by particle filtration (111–203 morphotypes per tree species). Although the observed microfungal diversity was higher with the particle filtration protocol, both methods concurred with respect to microfungal distributions. The overlap of microfungal species in pair wise comparisons of tree species was low (14–30 %), and only 2 and 3 % of microfungal species were observed in leaves of all tree species by particle filtration and by direct observations respectively. Multivariate analysis of data from direct observations confirmed the hypothesis that microfungal assemblages are strongly influenced by host phylogeny and are also affected by seasonal and site factors. The importance of host species in shaping microfungal distributions was also supported by the particle filtration data. Several taxa new to science, as well as some widespread saprotrophs, were detected on only one host. The underlying reasons for this affinity remain unclear, but we hypothesise that a number of factors may be involved such as fungal adaptation to plant secondary metabolites or the presence of a biotrophic phase in the fungus' life cycle.

Keywords: biodiversity, host affinity, multivariate analysis, particle filtration

INTRODUCTION

Fungi are vital contributors to ecosystems, because of their involvement in nutrient cycling (Jordan 1985; Lodge 1992), their mycorrhizal and endophytic associations with plants (Allen 1991; Suryanarayanan & Thennarasan 2004; Iotti et al. 2005), and their interactions with insects (Wilding et al. 1989; Zhou et al. 2004). They also hold a vast unknown genetic potential for pharmaceutical research and other biotechnological applications (Zhigiang 2005). Despite their importance, fungi are understudied, particularly in tropical regions, and are rarely considered in conservation plans (Hyde 2003).

Understanding the factors that influence fungal assemblages is essential for designing efficient sampling strategies for bioprospecting or for inventory and monitoring (Lodge & Cantrell 1995; Fryar et al. 2004). These factors may include competition for resources, the nutrient status of substrata, and climatic and microclimatic conditions (Cooke & Whipps 1993; Fryar et al. 2005). Empirical evidence also suggests that some saprotrophic microfungi are restricted to certain hosts and in some instances to specific tissue types (e.g. Monod 1983). In addition, a number of studies have shown quantitative differences in species abundances between host substrata (Cowley 1970; Heredia 1993; Cornejo et al. 1994; Lodge & Cantrell 1995; Polishook et al. 1996; Lodge 1997; Dulyamamode et al. 2001; Parungao et al. 2002).

The question of host specificity is relevant for estimates of global species numbers, which vary from 100K (Martin 1951) to 9.9M species (Cannon 1997). The widely quoted working estimate of 1.5M fungal species proposed by Hawksworth (1991) is essentially derived from the 6:1 ratio of known fungal species to the number of vascular plants in the British Isles. Extrapolation based on this ratio is sensitive to the degree of fungal host specificity (Lodge 1997). It has been argued that the high diversity of tree species and unpredictable dispersal patterns in tropical rainforests may limit the colonisation of widely spaced hosts (May 1980, 1991). If this hypothesis is valid, global fungal species numbers may be lower than estimated by Hawksworth (1991) due to the selection for insects and fungi with a wide host range. However, measurements of host specificity in tropical regions are still lacking for fungi (Jeewon et al. 2004).

Defining and measuring host specificity is not a simple task. The affinity of a fungus towards a host may be expressed at different levels of the taxonomic hierarchy (e.g. Carroll & Carroll 1978; Carroll 1994). It may range from a complete restriction to one host to quantitative differences of fungal occurrences on different hosts (Lodge 1997). The terms 'host specificity', 'host exclusivity', 'host fidelity', 'host affinity', 'host preference', and 'host recurrence' have been used to describe nutritive plant–fungal associations along this continuum (e.g. Secord & Kareiva 1996; Zhou & Hyde 2001). However, the precise application of these terms is often hampered by a lack of basic knowledge about the biology of many of the fungi encountered. In the present paper, the term 'host affinity' is applied when fungi were observed solely on one host species or genus and when differences in abundances on different hosts were detected.

Fungal diversity assessments are highly dependent on sampling effort and the method used to detect fungi. Methods that rely on the observation of fungal fruiting bodies

(also termed ‘direct methods’; Booth 1971) usually underestimate fungal diversity and may potentially bias results in favour of readily fruiting species. Even life-cycle independent methods (or ‘indirect methods’; Booth 1971), such as cultural methods or molecular diversity estimates, may miss species that do not grow in culture (Bills 1995) or resist DNA amplification. Conversely, these methods may overestimate fungal diversity involved in decomposition by detecting fungi that are not metabolically active. Crosschecks using complementary protocols are, therefore, recommended if a more complete understanding of microfungal diversity is required (Frankland et al. 1990).

In this paper, we used two complementary methods to test the hypothesis that microfungal distribution patterns are strongly influenced by host phylogeny. First, we observed fruiting bodies following incubation in humid chambers, and second, we isolated fungal cultures from leaf particles. We applied multivariate analyses to explore to what extent seasonal variations and spatial heterogeneity, in addition to host-related factors, might modulate microfungal distribution patterns. We also investigated whether leaf attributes affected species richness and considered individual micro-fungal species, which showed an affinity towards specific hosts.

MATERIALS AND METHODS

Study sites and climate

The study was conducted at two sites on the Atherton Tablelands, north-east Queensland, Australia. One site was situated at Topaz (17°24’00’’S, 145°43’30’’E; 710 m elevation) and the other near Millaa Millaa (17°32’30’’S, 145°42’50’’ E, 570 m elevation). Both sites support complex mesophyll rainforest (Tracey 1982) and have high tree species diversity. Past selective logging events have resulted in a mosaic of early and late successional tree species. The sites were approximately matched for altitude, rainfall and rainforest type, but varied to some extent in species composition and canopy height. Both sites have a high mean annual rainfall. At Topaz, the rainfall records for 1954 to 2000 ranged from 2242 to 5916 mm p.a., with an average of 3811 mm (Bureau of Meteorology 2003). At the Millaa Millaa site, rainfall was slightly lower with an average rainfall of approximately 3400 mm. Rainfall in the study area is

strongly seasonal, with a wet season from December to May. Litter fall and accumulation are also seasonal. Typically, maximum leaf litter fall occurs over the first few months of the wet season, but litter accumulation is greatest towards the end of the dry season when low precipitation retards decay. Generally, litter breakdown in these forest types occurs within a year (Brassell 1981; Spain 1984).

Rainfall data for Topaz were obtained from the Bureau of Meteorology (2003), and rainfall records for Millaa Millaa were provided by N. Tucker (personal communication). Temperature and relative humidity were measured in duplicate under each study tree of the four main species during the wet and dry season collections using a Thermo-Hygrometer (RS Components, Smithfield, NSW). Kruskal-Wallis tests were used to test for differences in temperature and relative humidity between sites, season and tree species (SPSS 2001).

Survey design

To examine the effects of host species, seasonality and site on microfungal assemblages, we surveyed four tree species from four common plant families of the region, namely *Cryptocarya mackinnoniana* F. Muell. ('Cm', Lauraceae), *Elaeocarpus angustifolius* Blume ('Ea', Elaeocarpaceae), *Ficus pleurocarpa* F. Muell. ('Fp', Moraceae) and *Opisthiolepis heterophylla* L.S. Smith ('Oh', Proteaceae). Due to the high plant diversity and hence low abundance of most tree species, it was impractical to randomise the selection of trees, and individuals were selected arbitrarily. Three individual trees of each species were located at each of two study sites and tagged. To assess in more detail the effect of host phylogeny on

microfungal assemblages, we also conducted less intensive surveys of two additional tree species using a direct method only. These included a second *Ficus* species, *F. destruens* F. Muell. ex C.T. White ('Fd'), and *Darlingia ferruginea* J.F. Bailey ('Df', Proteaceae). For these species, we sampled leaves of two trees from one site only. This protocol allowed us to examine the leaves of these species within the same time frame as the four main species. Previous research and our experience have shown that sampling during different time periods can provide markedly different estimates of diversity and species composition (Paulus *et al.* 2003b).

For the assessment of microfungal fruiting bodies following incubation, five fallen leaves were collected from a 7 × 7 m quadrat under each study tree on 28 May (late wet season) and 3 September (late dry season) 2002 and returned in plastic bags to the laboratory within 4 hours. Leaves at different stages of decay were selected arbitrarily. Although the age of leaves was unknown, the fast decay rate meant that the collected leaves had probably fallen during the same year.

For the isolation of fungal cultures by particle filtration, ten leaves per tree were collected on 27 February and 30 March 2001 (wet season) from four (*C. mackinnoniana*, *E. angustifolius*, *F. pleurocarpa*, and *O. heterophylla*) rather than six tree species in the wet season, because of labour intensity of the particle filtration protocol.

Assessment of microfungal diversity

For the assessment of fungal fruiting bodies, all leaves were incubated in separate humid chambers containing tissue paper moistened with sterile distilled water. Leaves were observed after 2 d (Hudson 1968) and again after 7–17 d (Hering 1965).

Microfungal fruiting bodies were sampled along the leaf margins, the midrib, and two additional 'microtransects' parallel to the midrib from both adaxial and abaxial leaf surfaces (ten 'microtransects' per leaf). A slide was prepared for one representative fruiting structure of each morphologically distinct fungal entity from each microtransect. Slides were rendered semipermanent by addition of 90% lactic acid. In view of the relative paucity of taxonomic treatise available for microfungi of the tropics in general and of North Queensland in particular, species identifications were deliberately conservative. Herbarium specimens were prepared for each taxon by removing a section of leaf with a scalpel blade and drying it at 37° C for 3 days. Cultures were isolated for selected taxa (Booth 1971). Fungal specimens of taxa recognised as new to science were deposited at the Queensland Plant Pathology Herbarium (BRIP), Department of Primary Industries. Representative specimens of other species are retained at the School of Tropical Biology, James Cook University, Cairns, Australia.

Fungal diversity was also assessed by isolating strains using a particle protocol (Bills & Polishook 1994; Paulus *et al.* 2003b). In brief, leaves were washed, surface treated with 5% hypochlorite, and homogenised in a sterile Waring blender. Leaf particles were washed through a series of polypropylene mesh with distilled water, and particles between 105 and 210 µm in size were plated onto four isolation media (Paulus *et al.* 2003b). Plates were checked daily for three weeks and emerging hyphae were transferred to fresh plates containing potato dextrose agar (Merck). Following incubation, isolates were sorted into morphotypes based on morphological characters, such as colour of colony and medium, growth rate, surface texture, margin characters, aerial mycelium, and spore production and characteristics (Arnold *et al.* 2001).

Definitions and statistical analyses

Fungal species were recorded as either present or absent for each leaf. The number of leaves on which a particular fungal species was found was then designated the ‘occurrence of a fungus’ and was used to calculate the ‘percent occurrence’ of a microfungal species in leaves of one tree species using the following formula (Yanna *et al.* 2002):

$$\begin{array}{lcl} \text{Percent occurrence} & = & \frac{\text{occurrence of taxon A}}{\text{occurrence of all taxa in one tree species}} \times 100 \\ \text{of taxon A} & & \end{array}$$

Fungal species that were observed only on one leaf are termed ‘singletons’.

Species accumulation curves were generated for two datasets: the complete dataset and a second reduced data set from which singletons had been removed (Arnold *et al.* 2001; Paulus *et al.* 2003b). Numerous techniques are available for estimating diversity (e.g. Colwell & Coddington 1994), but nonparametric estimators may be particularly useful for very diverse samples (Hughes *et al.* 2001). These include for example Chao1 (Chao 1984) and Chao2 (Chao 1987), which utilise the frequency of singletons and doubletons to estimate species richness. Chao1 and Chao2 were calculated for abundance data obtained by particle filtration and for presence-absence data from observations of fungal fruiting bodies respectively. Species accumulation curves and diversity estimates were obtained using the software program Wm2s (Turner *et al.* 2000). Individual data and the order of collections were both randomised 100 times to remove habitat heterogeneity and to correct for unequal sample sizes respectively. Sampling completeness (Schnittler *et al.* 2002) was estimated as follows:

$$\text{Estimated sampling completeness} = \frac{\text{observed species numbers} \times 100}{\text{estimated species numbers (Chao1 or 2)}}$$

The overlap and complementarity of microfungi from different leaf species, sites and seasons was calculated as follows (Colwell & Coddington 1994):

$$\text{Overlap (\%)} = \frac{\text{number of taxa shared between A and B} \times 100}{\text{total number of taxa observed in A and B}}$$

$$\text{Complementarity (\%)} = 100 - \text{overlap}$$

where A denotes the number of microfungal species in one leaf species and B the number of microfungal species in another leaf species (or season or site). To facilitate comparisons with previous studies, an additional analysis was undertaken in which singletons were removed from calculations. The similarity between microfungal assemblages on different host substrata was expressed by 1. the percentage of shared species and 2. by Bray-Curtis similarity index (Bray & Curtis 1957), which has been shown to have a robust monotonic and linear relationship with ecological distance (Bray & Curtis 1957; Faith *et al.* 1987). The Bray-Curtis similarity matrix (based on observations of fruiting bodies) was then ordinated using Non-metric Multidimensional Scaling (NMDS), and the goodness-of-fit of the ordination was measured by the stress value in the software program PRIMER (Clarke & Warwick 2001). We also undertook hierarchical cluster analyses based on average linkage (Clarke & Warwick 2001) for data obtained by both methods.

The statistical differences in microfungal assemblages observed on different hosts, sites and in different seasons by the direct method were tested using a Two-Way

Crossed Analysis of Similarities (ANOSIM; Clarke & Warwick 2001). ANOSIM is a simple non-parametric permutation procedure, which was applied to a Bray-Curtis similarity matrix. The R value in ANOSIM is equal to one if there is a complete separation of groups and zero when groups are similar and has measure of statistical significance (*P*) attached to it (Clarke & Warwick, 2001). The factors tested included host species and season in a first analysis, and host species and sites in a second analysis. Pairwise comparisons were undertaken only for differences at the family level, as at the species level, *F. destruens* and *D. ferruginea* were represented by only two individual trees. Nevertheless, this analysis had the potential to discriminate between the four main target trees, which all belong to different families.

Finally, correlations between microfungal species richness and substratum characteristics were examined using Pearson's Correlation Coefficient (SPSS 2001). Data on leaf morphology, texture and chemistry of living leaves was obtained for five of the six study species from an extensive survey of rainforest foliage conducted in 1997 at sites on the Atherton Tablelands. Leaf chemistry data were not available for *O. heterophylla*. Details of collection methods and analyses are given in Kanowski (1999). Data included specific leaf area (SLA, i.e. leaf area/unit dry mass), toughness, thickness, condensed tannins, total phenolics, alkaloids, nitrogen, calcium, potassium, magnesium, phosphorus, sulphur, aluminium, boron, cobalt, chromium, copper, iron, manganese, sodium, nickel, selenium and zinc. Data from sites with different environmental attributes were pooled and mean parameters calculated. To control for differences in sampling effort between tree species (three species were represented by six individuals, and two species by two individuals), the average richness was calculated from all pairwise combinations of individuals for the former three species in correlation analyses.

RESULTS

Climatic and microclimatic conditions

During the study year, rainfall was the lowest since records began with 2143 mm recorded at Topaz and 1756 mm at Millaa Millaa, but the distribution of rainfall was still strongly seasonal (Fig 1). In the 28 days before the wet and dry season collections, the total rainfall recorded was 409 mm and 134 mm, respectively. Mean temperature and relative humidity (average of both sites) differed significantly between season ($P<0.01$), with values of 22.5°C and 79% during the wet season, and 20.6°C and 74% during the dry season. On average, the Millaa Millaa site was slightly cooler (0.6°C) and more humid (7%) than the Topaz site ($P<0.01$). Temperature and relative humidity did not differ significantly ($P>0.1$) among tree species across sites.

Microfungal diversity

Using direct observation, total of 185 species of microfungi were detected on fallen leaves of the six tree species in both seasons. With one exception, anamorph-teleomorph connections were not confirmed; therefore, the actual number of species may be lower. Observations of species richness ranged from 31 species on *D. ferruginea* to 81 on *C. mackinnoniana* and Chao2 species estimates from 57 to 145 (Table 3). Species accumulation curves for raw data did not reach an asymptote when plotted for each leaf type separately (Fig 2.A) but levelled off when singletons were removed (Fig 2.B). During the wet season, 135 species were detected in 753 occurrences, compared with 111 species in 646 occurrences during the dry season.

Overall, 36% of species were recorded only once ('singletons'). Shannon-Wiener's diversity indices for microfungal assemblages on each tree, pooled across seasons, ranged from 2.96 to 3.76 (Table 3).

Data obtained by isolating cultures using particle filtration detected a greater diversity than the direct method. In the wet season 2001, between 111 and 203 morphotypes (419 morphotypes in total) were isolated from leaves of individual tree species while Chao1 species estimates ranged from 163 to 370. Overall, 55% of morphotypes were observed only once. Similar to the results of the direct observation, accumulation curves levelled off when singletons were removed from the data set (not shown).

Microfungal distribution

The microfungal assemblages on each tree species were relatively distinct as evidenced by data from both direct observations and isolations of cultures. Pairwise comparisons show that 70 to 88% of microfungal species were only detected on only one of two hosts (direct method; Table 4). Overall, 60% of microfungal species were recorded only in leaves of one host species. The number of microfungi shared between different tree species varied from 22% in any two host species to 2% in all six host species with similar results obtained by particle filtration (Table 5).

For data obtained by direct observations, 52% species were shared between sites and 35% between seasons (78 and 52% respectively when singletons were removed). The species overlap between sites was smaller in the particle filtration data (31%; 55% without singletons). Average Bray-Curtis similarities of microfungal assemblages appeared to be influenced by the following factors in order of importance: phylogeny of substrata (species > genus > family) > season > site; with

greatest similarities for collections from the same host species and lowest similarities for collections from the host species in different families (0.09; Table 6). The range of Bray-Curtis values observed in each category shows high variability for both methods (Table 6). For particle filtration data, the same comparison was not possible as only data from one season and from a smaller number of tree species were considered. Average Bray-Curtis similarities for microfungi on the same host at different sites was 0.31 (range 0.24-0.36), on different hosts at the same site 0.17 (0.11-0.34) and on different hosts at different sites 0.15 (0.10-0.28).

Ordination of microfungal assemblages observed by the direct method clearly separated the four plant families (Fig 3), and collections within the same tree species clustered closely together irrespective of site or collection time. Microfungal assemblages of the congenics *F. destruens* and *F. pleurocarpa* grouped closely together. The two species belonging to the same family, viz. *D. ferruginea* and *O. heterophylla*, were also closely associated with each other (Fig 3). Hierarchical cluster analyses of data obtained by both the direct method and particle filtration supported the same groupings (Fig 4A & B). The four main clusters of the dendrogram corresponded to the four plant families studied. Subgroups were formed by microfungal collections from *E. angustifolius*, *C. mackinnoniana*, *F. pleurocarpa*, and *F. destruens* (Fig 4A). In contrast, collections from *D. ferruginea* and *O. heterophylla* were interspersed within two clusters, which separated at a greater distance compared to *F. destruens* and *F. pleurocarpa* (Fig 4A). Collections from the same season rather than the same site were more closely associated. Particle filtration data from the same tree species grouped together consistently with site factors taken a secondary role (Fig 4B). The results of ANOSIM showed that the variation between microfungal assemblages observed by the direct method was strongly associated with host family

($R=0.884$; $P=0.001$); all pairwise comparisons between each family also differed significantly ($P=0.001$). Microfungal assemblages also varied significantly between seasons but not between sites ($R=-0.014$; $P=0.68$). The labour-intensive nature of isolating cultures by particle filtration meant that fewer replicates were included compared to the direct method and hence, an ANOSIM was not undertaken.

Correlates with leaf attributes

Of the leaf morphology and chemistry parameters tested, species richness was significantly and negatively correlated with total phenolics ($r^2=0.77$, $P=0.049$). Significant positive correlations were observed between species richness and thickness ($r^2=0.81$, $P=0.036$) and manganese ($r^2=0.83$, $P=0.033$).

DISCUSSION

Microfungal diversity

The present study demonstrated a similarly rich microfungal diversity as reported previously for decaying leaves in tropical rainforests (Table 2 and 3; e.g. Bills & Polishook 1994; Parungao *et al.* 2002) and highlighted the degree to which observed and estimated species richness is dependent on the study approach (Table 3). The potential advantages and limitations of various methods used to detect fungal presence (see Introduction; Booth 1971; Bills & Polishook 1994; Paulus *et al.* 2003b) mean that assessments of fungal species numbers always have a degree of uncertainty attached. Although the actual number of microfungal species present in leaf litter during the present study remains unknown, indicators of sampling adequacy, such as the proportion of singletons, accumulation curves (Fig 2A), and estimated sampling

completeness (Table 3) all suggest that considerably more species remain to be discovered. These are likely to be rarely observed species as accumulation curves approximated an asymptote when singletons were excluded (Fig 2B; Arnold *et al.* 2001). Singletons may include ‘persistent’ species (considered ‘rare’ due to undersampling), ‘transient’ species (common elsewhere) or genuinely rare species (Magurran & Henderson 2003) at unknown proportions. It is, therefore, not clear whether an increased sampling effort could markedly reduce the number of singletons and hence change estimates of sampling completeness. Despite the marked differences in species numbers detected by the two methods, estimates of sampling completeness were remarkably similar (Table 3). The usefulness of this index to describe sampling effort in studies utilising the same sampling and isolation strategies needs to be assessed in further studies.

Microfungal distribution

The present study confirmed the hypothesis that microfungal assemblages in decaying leaves of the tropical tree species studied are strongly influenced by host phylogeny and modulated by seasonal and site factors (Table 2, 5 & 6, Figs 3 & 4 A, B). The distinct effect of host species on microfungal assemblages was most clearly evident in the data from direct observations, which (due to the greater number of replicates) could be analysed using multivariate analyses. Differences in microfungal assemblages were statistically significant at the level of host family, and collections from the same plant family grouped together irrespective of collection site or collection time (Figs 3 and 4 A). Furthermore, microfungal assemblages on the same host species were the most similar, followed by host genus and host family (Table 6). These results support and extend those of previous studies. Quantitative differences of

microfungi in decaying leaves of different hosts have been reported previously for a range of habitats and geographical regions, including terrestrial litter from Nevada (Cowley 1970), from Panama (Cornejo *et al.* 1994) and from Mauritius (Dulymamode *et al.* 2001). Similarly, differential colonisation of leaf species by aquatic hyphomycetes has been reported from Belarus (Gulis 2001), Spain (Chauvet *et al.* 1997) and Australia (Thomas *et al.* 1992).

In the present study, particle filtration data supported the conclusions drawn from direct observations. For example, complementarity (Table 4) and overlap calculated for both isolation methods (Table 5) agreed well, and morphotypes on the same host species were also more similar than those at the same site (Fig 4B). This suggests that the observed affinity of some fungi for one host species was not solely an indication of fruiting preference, but was also expressed in the mycelia colonising leaves.

Although the host species appears to be a major determinant, other factors modulate the distribution of species. Spatial heterogeneity was apparent but not statistically significant in the present study. Direct observations of fruiting bodies suggested a species overlap of 78% between sites while the overlap was lower for particle filtration data (55%). A study from the neotropics, which also utilised a particle filtration protocol, reported a remarkably similar species overlap between two sites (52%; Polishook *et al.* 1996). The reasons for the observed spatial heterogeneity in the present study may be related to the significantly different microclimatic conditions at the two study sites and/or other factors that were not measured in the present study.

In contrast to numerous studies from cold temperate climates (e.g. Hudson 1968; Gessner 1977; Kuter 1986; Widden 1986b), few studies have considered the seasonality of microfungi in tropical forests. In the present study, microfungal

assemblages detected by direct observations differed significantly between the wet and dry season ($R=0.467$; $P= 0.001$; Fig 4A, Table 6). This may be related to the significant differences in rainfall and microclimatic conditions during wet and dry season at the sites. However, the hypothesis of microfungal seasonality would have been strengthened by replication both within the same season and in a second year, but this was not practical due to the labour-intensive nature of isolating and identifying microfungi. Nevertheless, these results support the observations of previous studies. For example, Cornejo *et al.* (1994) noted significantly different abundances for some leaf litter species between irrigated and control plots during the dry season. Similarly, a seasonal effect was also proposed by Hutton & Rasmussen (1970) who found that 35% of the 23 plant species studied in Puerto Rico had no epiphyllous fungi in common between the wet and dry season, and 44% had only one fungus in common. The role of additional factors in shaping microfungal assemblages was not tested in the present study. However, the great variation within average Bray-Curtis similarities (Table 6) may point to the role that chance or additional factors, such as fungal competition, selective grazing by insects, and the biotic history of the leaf (Carroll & Wicklow 1992), may play in shaping microfungal assemblages.

Correlates with leaf chemistry and characteristics

The underlying reasons for host affinity and differences in species richness between tree species were not tested in the present study. However, we correlated available leaf parameters such as surface area, thickness, texture, and chemical composition (Kanowski 1999) with fungal species richness to assess any potential relationships. Of the leaf parameters that were available for testing, the levels of phenolics contained in living leaves were significantly and negatively correlated with species richness of

saprotrophic fungi. The role of phenolic compounds in plant defence against both herbivores and plant pathogens has been well documented (e.g. Stumpf & Conn 1981; Gutschick 1999; Kursar & Coley 2003). Phenolic compounds may persist during the decay process, and it is possible that higher concentrations may depress overall species numbers of saprotrophic microfungi.

Species richness was positively correlated with leaf thickness, which may be related to the greater volume of substratum available for mycelial growth. However, this relationship may only hold for leaf litter, as decaying wood of smaller volumes such as branches support more fungal species than, for example, logs (Heilmann-Clausen & Christensen 2004). The positive correlation between species richness and manganese concentrations require further evaluation. Manganese is an important trace element required for fungal growth (Jennings & Rayner 1984) and as a component of fungal enzymes (e.g. Mester & Field 1998). It has also been shown to stimulate sporulation in *Trichoderma* (Sierota 1982) and to enhance biodegradation in complex ways (Aust 1996).

These correlations are based on a limited number of host species and can only serve to generate hypotheses, which need to be evaluated in more detailed studies. Additional data from other hosts may provide evidence for further significant linear or more complex, non-linear relationships between leaf attributes and species richness. As secondary metabolite concentrations vary considerably within plant species, populations and even within the same plant (Shelton 2000), we recommend that future studies analyse leaf litter attributes and microfungal assemblages from leaf samples of the same study trees.

Host affinity and specialisation

Host affinity was evident in several taxa new to science, but also in some cosmopolitan species (Table 2). For example, four of the nine microfungal species in *F. pleurocarpa* leaves with relative abundances greater than 3%, were new to science and were restricted either to this host species or to the genus *Ficus*. These include a species of *Discostroma*, a *Gaeumannomyces*-like species, a new taxon, which in molecular studies showed an affinity with *Myrothecium* but may represent a new genus (P.W. Crous, personal communication), and an undescribed new genus of Dermataceae (F492; Table 2). The latter species was restricted to petioles and midribs of the two *Ficus* species studied and may be involved in the decomposition of latex contained in petioles and midribs (Paulus *et al.* 2006).

Some other saprotrophic taxa with affinity for one host are also highly specific to certain plant tissues (Monod 1983; Yanna *et al.* 2001, 2002; Kumar & Hyde 2004; Lee *et al.* 2005). For example, fruiting bodies of *Gnomonia queenslandica* were restricted to petioles and midribs of *E. angustifolius* and were present in 72% of all leaf samples (Paulus *et al.* 2003a). Together with *G. elaeocarpa*, which covered a major proportion of the leaf lamina on 86% of all *E. angustifolius* leaf samples (Paulus *et al.* 2003a), this species must have contributed to a major extent to the decomposition of those leaves. Both species of *Gnomonia* thrived on leaves of *E. angustifolius* despite the reported high levels of phenolics (Kanowski 1999). Although the underlying reasons for tissue and host specificity among saprotrophs are unknown, one might hypothesise that adaptation to potentially hostile environments, such as tissues containing latex or phenolic compounds, plays a major role in the development of specificity in some fungal saprotrophs.

Host affinity has also been reported for some fungi with an endophytic (Zhou & Hyde 2002; Hyde *et al.* 2006) or pathogenic (Photita *et al.* 2004) phase in their life cycle. The majority of these taxa may be replaced rapidly by other taxa (Paulus *et al.* 2006) and may, therefore, represent only a small proportion of microfungi in leaf litter. For example, 22% of endophytes were recovered from leaf litter of the same tree species in a neotropical forest, but this represented only 1 % of the total observed diversity of leaf litter fungi (Lodge 1997). Certainly, host affinity is not restricted to species fruiting early in decay; for example, *Dactylaria ficusicola* was detected only in leaves of *F. pleurocarpa* at later stages of decay (Paulus *et al.* 2006).

In addition to apparently restricted taxa, some cosmopolitan, plurivorous taxa also showed an affinity towards one host in the present study. This included, for example, *Chaetopsina fulva*, which was found only in *C. mackinnoniana* leaves (Table 2). Other plurivorous species, such as *Beltraniella portoricensis* and *Beltrania rhombica*, showed distinct quantitative differences between different hosts (Table 2). These observations might support the view that physical and chemical leaf characteristics rather than host phylogeny might influence the distribution of saprotrophic microfungi in leaf litter (Polishook *et al.* 1996). In our study, phylogeny and leaf attributes were confounded. Closely related host taxa could be selected in future studies that differ in leaf texture and chemistry.

Although the extent of host affinity in rarely observed species is difficult to determine, this question is vital for refining estimates of global fungal species numbers. For example, if rarely observed species were able to proliferate on a particular host with the onset of optimal microclimatic or substratum specific conditions, they may include host specific taxa; estimates of fungus to host ratios

would need to be scaled upwards accordingly. Alternatively, some rare species may have colonised a leaf by chance and persist under less than optimal conditions ('transient' species, Magurran & Henderson 2003). These might be drawn from a common pool of leaf litter species or may be adapted to different tree species. In any case, overall lower estimates of host to fungus ratios would be indicated. Further work is required to address these questions.

Future directions

The present study indicated a high degree of host affinity among leaf litter microfungi, possibly suggesting a higher fungus to host ratio for tropical rainforests than the 6:1 ratio reported for the British Isles. However, we refrain from making any predictions at the present time, as further, well-replicated studies are required to fill in the knowledge gaps, in particular for rarer fungal species and for host-fungus relationships at generic or higher taxonomic levels. A useful strategy for future studies may be a centrifugal-phylogenetic approach (Wapshere 1974), where closely related hosts are studied first and then more distantly related plants are included. Due to the high diversity of tree species at all taxonomic levels, the rainforests of the wet tropics of Australia would provide an ideal study site for ongoing research into the host affinity of microfungal species. In addition to field studies, systematic characterisation of plant secondary metabolites in combination with nutritional studies of individual microfungal species are required to elucidate the underlying factors involved in host and tissue specificity.

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Figure captions

Fig 1 Monthly rainfall for Topaz (T) and Millaa Millaa (M) for the year 2002.

Vertical arrows indicate collection dates.

Fig 2 Accumulation curves of fungal species including singletons (A) and excluding singletons (B) for microfungi in leaf samples of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh) pooled over wet and dry seasons detected by the direct method.

Fig 3 Three-dimensional representation of the similarity between microfungal assemblages from decaying leaves of *Cryptocarya mackinnoniana* (Lauraceae), *Elaeocarpus angustifolius* (Elaeocarpaceae), *F. pleurocarpa* (Moraceae) and *Opisthiolepis heterophylla* (Proteaceae). Additional taxa are marked Df (*Darlingia ferruginea*) and Fd (*Ficus destruens*). Relative distances were based on a matrix of Bray-Curtis similarities and ordinated using Non-metric Multidimensional Scaling. Stress = 0.15.

Fig 4 Dendrograms of microfungal assemblages in decaying leaves of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh) collected over two seasons (wet 'w' and dry 'd') and at two sites (Topaz 'T' and Millaa Millaa 'M'). A. Data obtained by direct observation of fruiting bodies and B. data obtained by particle filtration.

Table 1. Leaf attributes of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh)

Tree species	SLA ^a	Thickness	Toughness
<i>C. mackinnoniana</i>	52.50	0.56	4.35
<i>D. ferruginea</i>	73.33	0.35	3.13
<i>E. angustifolius</i>	83.67	0.24	2.53
<i>F. destruens</i>	42.00	0.50	2.90
<i>F. pleurocarpa</i>	53.00	0.46	5.50
<i>O. heterophylla</i>	n.a.	n.a.	n.a.

^a SLA refers to Specific Leaf Area (i.e. leaf area/unit dry mass)

Table 2. Species list and relative abundances of microfungi observed on decaying leaves of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh) during the wet and dry season 2002.

No.	Species name	Cm	Df	Ea	Fd	Fp	Oh
F666	<i>Acremonium</i> sp. 1						0.5
F757	<i>Acremonium</i> sp. 2					0.3	0.5
F895a	<i>Acremonium</i> sp. 3					0.3	
F599	<i>Acremonium</i> sp. 4	0.3	1.2			1.3	1.0
F892b	<i>Acremonium</i> sp. 5			1.8		0.6	1.0
F626	<i>Asterina</i> sp.	1.4					
F768	<i>Auxarthron</i> sp.	0.3					
F803	<i>Bahusutrabeeja</i> <i>bunyensis</i>	3.0					
F864	<i>Beltrania</i> cf. <i>concurvispora</i>				3.9	0.9	0.5
F815a	<i>Beltrania rhombica</i>	1.6		0.6	7.8	1.9	
F815b	<i>Beltrania</i> sp. ^a	0.3					
F807	<i>Beltraniella</i> <i>portoricensis</i>	9.3	3.7	0.3	1.0	1.9	
F858	<i>Botryodiplodia</i> <i>theobromae</i>		1.2			0.3	
F450	<i>Botryosphaeria</i> -like sp.					2.2	
F661	<i>Brooksia tropicalis</i>		1.2			0.6	0.5
F652	<i>Cephalosporiopsis</i> sp.1			0.6			3.5
F892a	<i>Cephalosporopsis</i> sp. 2						0.5
F746	<i>Chaetopsina fulva</i>	0.5					
F845	<i>Chaetospermum</i> sp.	0.3	2.5	0.3	4.9	4.4	6.4
F889	<i>Chalara</i> cf. <i>nigricollis</i>	2.2					0.5

F635	<i>Chalara</i> sp.	1.6	0.3		
F860a	<i>Circinotrichum</i> <i>falcatisporum</i>			0.5	
F860b	<i>Circinotrichum</i> sp. ^a <i>Circinotrichum</i> -like sp.			0.5	1.3
F769	<i>Cladosporium</i> sp. 1	0.3			
F812	<i>Cladosporium</i> sp. 2	0.5	1.2	0.3	0.5
F818	<i>Coccomyces</i> cf. <i>limitatus</i>		13.		3
F645	coelomycete (acervular) A		0.6		
F455	coelomycete (acervular) B		7.8	14.	2
F921	coelomycete (acervular) C		0.3		
F921	coelomycete (pycnidial) A			0.3	
F878	coelomycete (pycnidial) B	1.2			
F592	<i>Colletotrichum</i> sp. 1		1.0		
F922	<i>Colletotrichum</i> sp. 2			0.3	
F835	<i>Colletotrichum</i> sp. 3			0.3	
F813b	<i>Conioscypha</i> sp.	0.3			
F876	<i>Cryptophiale</i> cf. <i>guadalcanensis</i>	14.			8
F613	<i>Cryptophiale</i> <i>kakombensis</i>	0.8	1.2	1.0	
F1006	<i>Curvularia</i> sp.		1.0	0.3	
F848	<i>Cylindrocarpon</i> cf.			0.5	

	<i>ianthothele</i>						
F859	<i>Cylindrocarpon</i> cf.				0.6	2.5	
	<i>orthosporum</i>						
F849	<i>Cylindrocladiella</i> sp.				0.6		0.5
F857	<i>Cylindrocladium</i>		1.2	0.6	1.0	0.9	0.5
	<i>coulhounii</i> var.						
	<i>coulhounii</i>						
F722	<i>Cylindrocladium</i>				0.3		
	<i>floridanum</i>						
F839a	<i>Cylindrosyndrium</i>	1.4			1.0	0.3	
	<i>cryptocaryae</i> sp. nov.						
F1005	<i>Dactylaria belliana</i>						0.5
b	sp.nov.						
F874	<i>Dactylaria ficusicola</i>				1.0		
	sp.nov.						
F853	<i>Dactylaria</i> section	0.3					
	<i>Mirandina</i> sp. 1						
F710b	<i>Dactylaria</i> section	0.3					
	<i>Mirandina</i> sp. 2						
F873	<i>Dendrosporium lobatum</i>				0.3		
F472	Dermataceae gen. nov.				6.9	11.	
	(F472)					9	
F747	<i>Dictyochaeta</i> cf. <i>novae-</i>	0.5			0.6		
	<i>guineensis</i>						
F800	<i>Dictyochaeta simplex</i>	3.3	11.	0.9	2.0	2.2	3.0
			1				
F801	<i>Dictyochaeta</i> sp. 1	2.2			0.3		0.6
F916	<i>Dictyochaeta</i> sp. 2	1.6			0.3	1.0	
F894	<i>Dictyochaeta</i> -like sp.				2.7		

F914	<i>Dictyosporium</i> cf. <i>australiense</i>					0.5
F905	<i>Dinemasporium</i> sp. 1	0.5				
F804	<i>Dinemasporium</i> sp. 2	0.3				
F827	<i>Dischloridium</i> sp.	0.3	4.2	1.0		
F833	<i>Discostroma ficusicola</i> sp. nov.					3.5
F767	<i>Flabellocladia</i> sp.	0.3				
F726	<i>Fusarium</i> sp.					0.3
F899	<i>Gaeumannomyces</i> -like sp.nov.					3.5
F621	<i>Geotrichum</i> sp.	0.3				
F822	<i>Gliocephalotrichum</i> <i>simplex</i>		3.9			
F811	<i>Gliocladiopsis</i> sp.	2.5	1.2	1.0	4.0	
F829	<i>Gliocladiopsis tenuis</i>		0.6	0.3		
F801	<i>Gliocladium</i> sp. 1	1.9	0.3	1.0	1.9	4.0
F601a	<i>Gliocladium</i> sp. 2				0.3	0.5
F843b	<i>Gliomastix luzulae</i>					1.0
F843a	<i>Gliomastix murorum</i>					3.0
F706	<i>Gliomastix</i> sp. 1					0.5
F736a	<i>Gliomastix</i> sp. 2					1.5
F736b	<i>Gliomastix</i> sp. 3 ^a					2.0
F820	<i>Gnomonia elaeocarpa</i> sp. nov.		13.			9
F819	<i>Gnomonia</i> <i>queenslandica</i> sp. nov.		14.			2
F901	<i>Gnomonia</i> sp. 1	1.9				0.5
F883	<i>Gnomonia</i> sp. 2	0.3	3.7			

F863	<i>Goidanichiella</i> sp.				5.9
F821	<i>Guignardia</i> sp.				1.8
F847	<i>Hansfordia pulvinata</i>	0.5	0.3	0.3	1.5
F824a	<i>Harpoglyphium</i> sp. 1				0.6
F875	<i>Harpoglyphium</i> sp. 2				12.
					3
F627	<i>Helicosporium griseum</i>	0.8			0.5
F881	<i>Helicosporium</i> sp.	0.3	4.9		3.5
F596	<i>Hyponectria</i> sp.				1.0
F865	<i>Idriella acerosa</i>		0.3	3.9	0.3 3.0
F873a	<i>Idriella cagnizzari</i>	0.8	3.3	3.9	0.5
F861	<i>Idriella lunata</i>			3.9	0.6 0.5
F708	<i>Ijuhya</i> sp. ^a				0.5
F776	<i>Ijuhya aquifolii</i>		1.2		1.0
F927	<i>Ijuhya leucocarpa</i>				2.0
F817	<i>Iodosphaeria</i> sp.	0.3	0.3		
F906	<i>Isthmolongispora</i>	0.8			
	<i>intermedia</i>				
F877b	<i>Kramasamuha</i> cf. <i>sibika</i>	1.2	0.9		
F884	<i>Lachnum</i> sp. nov.	5.8	3.7		
F862	<i>Lanceispora amphibia</i>				4.9
F917	<i>Lauriomyces</i>				0.3
	<i>helicocephala</i>				
F850	<i>Linocarpon</i> -like sp.	0.3			
F1012	<i>Marasmius</i> sp.	3.3	1.2		0.3 3.0
F464	<i>Meliola</i> sp.				1.9
F802	<i>Menisporopsis</i>	9.9			
	<i>theobromae</i>				
F824b	<i>Microdochium</i> sp.	0.6			0.6

F816	<i>Minimidochium</i>	0.8					
	<i>microsporum</i>						
F743	<i>Minimidochium</i> -like sp.	0.3					
F907	<i>Mollisia</i> sp.	0.8					
F887	<i>Mycena</i> sp. 1	2.5		0.6			
F1001	<i>Mycena</i> sp. 2	0.3		0.3			
F724	<i>Myrothecium</i> sp. 1			0.3			
F924	<i>Myrothecium</i> sp. 2			0.3			
F772	<i>Niesslia</i> sp.			0.3			
F834	<i>Nodulisporium</i> sp.			0.6			
F915	<i>Oidiodendron</i>			0.5			
	<i>tenuissimum</i>						
F513	<i>Ophiognomonia</i>		1.0	6.9			
	<i>elasticae</i>						
F867	<i>Ophiognomonia</i> sp.	0.3	1.2	1.0	2.5		
F1004	<i>Paraceratocladium</i>	0.5					
	sp.nov.						
F837b	<i>Parasymphodiella</i>	0.5	4.9	0.3	1.0	0.3	2.5
	<i>elongata</i>						
F837a	<i>Parasymphodiella laxa</i>			1.0	0.5		
F825	<i>Penicillium</i> sp.	13.		0.5			
		3					
F828	<i>Pestalotiopsis</i> spp.	0.5	4.9	2.1	5.9	8.5	4.5
F918b	<i>Phaeoisaria</i> sp			0.3			
F1010	<i>Phialocephala</i>	0.3					
	<i>bactrospora</i>						
F826	<i>Phoma</i> sp. 1	2.4		0.3	2.5		
F866	<i>Phoma</i> sp. 2		1.0	0.9	2.0		
F699	<i>Phoma</i> sp. 3		2.0				

F789	<i>Phomopsis</i> sp. 1		1.0	0.3
F880	<i>Phomopsis</i> sp. 2	1.2		
F632	<i>Pseudobeltrania</i> sp.	1.6	4.9	
F851b	<i>Pseudomicrodochium</i> <i>antillarum</i>	0.8		
F806	<i>Pyricularia</i> sp. 1	0.3		
F877a	<i>Pyricularia</i> sp. 2	0.3		
F637	<i>Pyricularia</i> sp. 3	0.3		
F873b	<i>Rhinocladiella</i> <i>cristaspora</i>		1.0	
F851a	<i>Rhinocladiella</i> sp.	0.3		
F903	<i>Rhytismataceae</i>	0.5		
F909	<i>Roumeguerilla</i> sp. nov.	2.7		
F742	<i>Scolecobasidium</i> cf. <i>fusiforme</i>	1.5	0.3	
F893	<i>Scolecobasidium</i> sp. ^a	0.9	0.3	
F891	<i>Selenodriella</i> sp.	0.3		
F854a	<i>Selenosporella</i> sp. 1	2.5	0.3	0.3 2.5
F854b	<i>Selenosporella</i> sp. 2	0.3		
F611	<i>Selenosporella</i> sp. 3	1.6	2.5	
F616	<i>Selenosporella</i> sp. 4	0.3		
F1008	<i>Speiropsis</i> sp.			0.5
F698	<i>Sphaeridium pilosum</i>		0.6	
F904	<i>Spiropes</i> sp.	0.3		
F840	<i>Sporidesmium</i> cf. <i>ponapense</i>	1.1		1.5
F449	<i>Sporidesmium</i> sp. nov.		2.2	
F813a	<i>Sporodesmiella</i> <i>garciniae</i>	1.6		

F844a	<i>Stachybotrys</i> cf. <i>parvispora</i>		0.6	6.4
F844b	<i>Stachybotrys</i> sp. ^a			3.0
F823	<i>Stilbella</i> sp. 1	0.3		1.0
F669	<i>Stilbella</i> sp. 2	1.2		
F890	<i>Subulispora procurvata</i>	0.8		
F865	<i>Thozetella boonjiensis</i> sp.nov.	0.8		
F919	<i>Thozetella falcata</i> sp.nov.	2.2		
F885	<i>Thozetella gigantea</i> sp.nov.	1.9	1.2	
F809	<i>Thozetella</i> <i>queenslandica</i> sp.nov.	4.7		0.5
F886a	<i>Thozetella</i> sp.	1.2		
F910	<i>Trichoderma viride</i>	3.0	1.0	0.3
F598	Unidentified synnematal hyphomycete	1.2	1.0	
F601b	unidentified hyphomycete	0.3		
F784	unidentified hyphomycete	0.3		
F839b	unidentified hyphomycete	0.3		
F846	unidentified hyphomycete			0.5
F895b	unidentified hyphomycete			0.5
F923	unidentified	0.3		

	hyphomycete			
F830	unidentified	1.2		
	hyphomycete			
F860c	unidentified	0.3	3.7	
	hyphomyete			
F732	<i>Verticillium</i> sp. 1	0.8		
F838	<i>Verticillium</i> sp. 2		1.0	0.6
F655	<i>Verticillium</i> sp. 3		2.0	1.3 2.5
F928	<i>Verticimonosporium</i>	0.5		
	<i>ellipticum</i>			
F870	<i>Volutella</i> sp.		2.0	
F750	<i>Volutella</i> -like sp.	0.5		
F805	<i>Wiesneriomyces</i>	7.4	2.0	3.0
	<i>javanicus</i>			
F896	<i>Xenogliocladiopsis</i> -like		3.5	2.5
	sp. or gen. nov. ^b			
F900	<i>Zygosporium</i>	1.1	1.6	3.0
	<i>echinosporium</i>			
F929	<i>Zygosporium mansonii</i>		4.4	0.5
F740	? <i>Catenosubulispora</i> sp.	0.3		
F756	? <i>Dactylaria</i> sp.	0.3		
F872	? <i>Hyponectria</i> sp.	2.5	2.9	0.3
F730	? <i>Kylindria</i> sp.	0.3		

^a species delimitations of taxa with congeneric taxa were based on small differences. Due to sparse material, further assessments could not be undertaken. Results of statistical analyses did not vary markedly when these taxa were ‘lumped’ with congeners.

^b DNA sequence analysis is unequivocal with respect to placement of this specimen (P.W. Crous, personal communication). Further systematic work of *Myrothecium* and *Xenogliocladiopsis* by a different research group is currently in progress.

Table 3. Diversity of microfungal assemblages identified from decaying leaves of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh).

Tree species	Occur-ences	Direct observation				Particle filtration				
		S ^a	Chao2 ^b	Comple-teness (%)	H' ^c	Occur-ences	S ^a	Chao1 ^d	H' ^c	Comple-teness (%)
Cm	365	81	137 (85, 189)	59	3.75	513	203	370	4.73	55
Df	81	31	57 (22, 92)	55	3.04	n.a.	n.a.	n.a.	n.a.	n.a
Ea	331	47	70 (43, 97)	67	2.96	356	133	206	4.44	65
Fd	102	41	94 (29, 159)	44	3.40	n.a.	n.a.	n.a.	n.a.	n.a
Fp	318	63	102 (62, 142)	62	3.38	346	111	163	4.11	68
Oh	202	61	145 (48, 242)	42	3.76	360	156	241	4.69	65
Total	1399	185	269 (212, 326)	69	4.50	1575	419	627	5.35	67

^a S refers to species richness

^b species richness estimate according to Chao (1987) based on presence/absence data. Numbers in brackets refer to 95% confidence intervals.

^c H' refers to Shannon-Wiener diversity index

^d species richness estimate according to Chao (1987) based on abundance data of fungal isolates

Table 4. Percent complementarity in pairwise comparisons of microfungal assemblages detected in decaying leaves of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp), and *Opisthiolepis heterophylla* (Oh) by direct observation and by particle filtration; n.a. refers to cells for which no data is available. Singletons were included in analysis.

	Direct method				Particle filtration			
	Sp.1	Overlap	Sp.2	Comple- mentarity	Sp.1	Overlap	Sp.2	Comple- mentarity
<i>Cm-Df</i>	68	17	16	84	n.a.	n.a.	n.a.	n.a.
<i>Cm-Ea</i>	58	14	28	86	51	13	35	86
<i>Cm-Fd</i>	62	16	22	84	n.a.	n.a.	n.a.	n.a.
<i>Cm-Fp</i>	51	14	35	86	56	22	23	78
<i>Cm-Oh</i>	50	16	34	84	48	15	37	85
<i>Df-Ea</i>	33	12	55	88	n.a.	n.a.	n.a.	n.a.
<i>Df-Fd</i>	33	18	49	82	n.a.	n.a.	n.a.	n.a.
<i>Df-Fp</i>	22	16	62	84	n.a.	n.a.	n.a.	n.a.
<i>Df-Oh</i>	23	17	61	84	n.a.	n.a.	n.a.	n.a.
<i>Ea-Fd</i>	45	17	37	82	n.a.	n.a.	n.a.	n.a.
<i>Ea-Fp</i>	30	22	48	78	50	16	34	84
<i>Ea-Oh</i>	33	19	48	81	41	13	46	87
<i>Fd-Fp</i>	21	30	49	70	n.a.	n.a.	n.a.	n.a.
<i>Fd-Oh</i>	29	19	52	81	n.a.	n.a.	n.a.	n.a.
<i>Fp-Oh</i>	37	28	35	72	28	22	50	78
Range		14-30		70-88		13-22		78-87

Table 5. Overlap of microfungi detected in decaying leaves of two to six tree species by both isolation methods. First row indicates number and percentage of species occurring only in one tree species. Values in brackets indicate the overlap of microfungal species after singletons have been removed from data set.

No. of tree species compared (k)	Direct method		Particle filtration	
	No. of fungal species shared between k tree species	Percentage fungal species shared (%)	No. of fungal species shared between k tree species	Percentage fungal species shared (%)
1	110	60 (39)	289	69 (44)
2	41	22 (33)	90	22 (39)
3	15	8 (12)	26	6 (11)
4	12	7 (10)	15	3 (6)
5	3	2 (2)	n.a.	n.a.
6	4	2 (3)	n.a.	n.a.

Table 6. Summary of Bray-Curtis similarity indices for pairwise comparisons of microfungi in decaying leaves of *Cryptocarya mackinnoniana* (Cm), *Darlingia ferruginea* (Df), *Elaeocarpus angustifolius* (Ea), *Ficus destruens* (Fd), *F. pleurocarpa* (Fp) and *Opisthiolepis heterophylla* (Oh) detected by the direct method.

Comparisons of categories	Mean Bray-Curtis similarities (Range)
Same host species – different site – same season	0.53 (0.41-0.67)
Same host species – same site – different season	0.41 (0.15-0.64)
Same host species – different site – different season	0.36 (0.13-0.52)
Congeneric host species (<i>Fd-Fp</i>)	0.29 (0.20-0.35)
Different host genus – same family (<i>Df-Oh</i>)	0.14 (0.04-0.26)
Different host species – different families	0.09 (0.00-0.29)

A

B

A.

B.