DON'T BE AFRAID OF THE F-WORD: PROSPECTS FOR INTEGRATING FUNGI INTO BIODIVERSITY MONITORING

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Fungi are diverse and functionally significant components of ecosystems yet are omitted from current ecological monitoring in Victoria, especially in relation to fire. The taxonomic and morphological diversity of fungi complicates identification but sampling by molecular profiling is now a practical alternative to specialised and time-consuming methods of culturing and fruit-body survey. Suggestions are provided to guide the implementation of ecological monitoring of fungi.

Key Words: fungi; fire; ecological function; biodiversity monitoring; molecular profiling.

FUNGI are highly diverse and essential components of all ecosystems—as symbiotic partners, decomposers and nutrient cyclers and as sources of food for vertebrates and invertebrates. Fire is an integral part of many Australian ecosystems and it significantly affects not only plants and animals, but also fungi, both directly and through their interactions with other organisms.

McMullan-Fisher et al. (2011) recently reviewed research on fire and fungi in Australian ecosystems. They found that there were relatively few rigorous (replicated for all treatments) studies of the effect of fire on fungi. In addition the various studies focussed on different groups of fungi, using different methods of detection and identification. Certainly, some fungi require fire for fruit-body production, others seem to favour long unburnt vegetation, and some species (such as stonemaker fungi in the genus *Laccocephalum* see Fig. 1) are positively or negatively impacted by fire depending on the life cycle stage. The relationship between fungi and fire is also complex due to interactions with substrates (which can be destroyed or created by fire) and symbionts, such as mycorrhizal partners and animal dispersers, which have their own relationships with fire.

In the short term, there is usually a loss of litter and smaller wood, and consequently of the fungi that are dependant on these substrates. In the longer term, fire can promote formation of standing dead wood and coarse woody debris, which support different suites of decomposer fungi. There is a succession of terrestrial macrofungi after fire, with a distinct set of pyrophilous fungi dominating in the first few years after fire (Robinson et al. 2008; Claridge et al. 2009a). Pyrophilous macrofungi have important roles in reducing post-fire erosion (Claridge et al. 2009b). Many pyrophilous discomycetes are ectomycorrhizal (Warcup 1990; Tedersoo et al. 2009) and their presence might assist seedling establishment and survival. However, propagules of arbuscular mycorrhizal fungi may decrease after fire (Brundrett et al. 1996a).

Fire regime is acknowledged as an important factor in managing biodiversity (Gill & Allan 2008) although management based on vegetation will not necessarily be a good predictor of the response of other biota (York 2003; Clarke 2008; New et al. 2010; Driscoll et al. 2010). There are few studies on fire regimes and fungi in Australia. In south-west Western Australia, Wittkuhn et al. (2011) found only weak evidence of compositional difference for macrofungi in relation to different fire intervals, and, in Victoria, Osborn (2007) could not detect an effect on the fungal community of differences in the season or interval of low intensity fuel reduction burns. However, in Queensland, Anderson et al. (2007) and Bastias et al. (2006a) report significant differences in the fungal community between sites in *Eucalyptus pilularis* forest burnt at two- and four-year intervals. For biological soil crusts, lichen cover was greatest with longer fire intervals in mallee vegetation (Eldridge & Bradstock 1994) but with shorter intervals in temperate grassland (O'Bryan et al. 2009).

McMullan-Fisher et al. (2011) pointed out that

Fig. 1. Stonemaker fungus *Laccocephalum sclerotinium*, common after fire (Image R Robinson). .

studies on fungi and fire were concentrated in eucalypt forests of south-eastern Australia, with a lack of studies for many ecosystems, particularly grasslands. In all Australian ecosystems, ecological knowledge of the highly diverse plant parasitic fungi of native plants was also considered particularly poor.

In terms of specific recommendations for managing fire for fungi, McMullan-Fisher et al. (2011) could only provide general guidelines, due to the lack of Australian research. In particular, they advocated the management of substrates (such as coarse woody debris) across host species and decay classes as a surrogate for specific knowledge of the response of substrate-specific fungi to individual fires and to fire regimes. They also highlighted the high diversity of macrofungi in long-unburnt vegetation such as Cool Temperate Rainforest. McMullan-Fisher et al. (2011) concluded that 'there is an urgent need to include fungi in … long-term monitoring programs in relation to the effect of fire across Australia'.

The *2009 Victorian Bushfires Royal Commission* (Teague et al. 2010: 35) recommended 'a long-term program of prescribed burning based on an annual rolling target of 5% minimum of public land' (Recommendation 56). The Commission also made recommendations about the need to assess the effects of prescribed burning on biodiversity, specifically that 'The Department of Sustainability and Environment report annually on prescribed burning outcomes … including … impacts on biodiversity' (Rec. 57) and further recommended that 'The Department … significantly upgrade its program of long-term data collection to monitor and model the effects of its prescribed burning programs and of bushfires on biodiversity in Victoria' (Rec. 58).

Despite the clear requirement to monitor all biodiversity, fungi are not or are barely included in recent and current biodiversity monitoring in Victoria. We briefly summarise the high taxonomic and functional diversity of fungi and the lack of attention currently paid to fungi in conservation and management. Consideration of sampling and identification of fungi in current ecological studies demonstrates that effective monitoring fungi, both financially and scientifically, is feasible using newly emerging molecular techniques. Suggestions

Fig. 2. 'Vulnerable' macrofungus *Hypocreopsis amplectens*, only found in long unburnt woodland. (Image T. May)

are provided for monitoring of fungi in relation to fire that would assist in properly enacting the recommendations of the Royal Commission so that they apply to biodiversity as a whole including fungi.

CONSERVATION AND MANAGEMENT OF FUNGI

Protocols for monitoring flora in relation to fire have been developed by the Victorian Department of Sustainability and Environment (DSE) (Cawson & Muir 2008a, 2008b). These include standard assessments for vital attributes, life-stages, indicator species and all species. Tolerable fire intervals for native vegetation in Victoria have been compiled by Cheal (2010). However, there are no monitoring protocols for fungi and no data on their tolerable fire intervals.

In Victoria, only three fungi are formally listed under the *Flora and Fauna Guarantee Act 1988* (DSE 2010). The macrofungus *Hypocreopsis amplectens* (Fig. 2) is classified as 'vulnerable'. It forms fruit-bodies on senescing *Leptospermum* in long unburnt woodland and fire is considered a threatening process. Fire is also likely to be a threatening process for the alpine and montane lichens *Neuropogon acromelanus* and *Xanthoparmelia suberadicata*. Despite the three species being formally listed between 2001 and 2004, none have Action Statements to guide their management.

The omission of fungi from biodiversity monitoring programs in Victoria is not surprising. Fungi have been omitted or given scant attention in most biodiversity policy documents, management plans and formal conservation schedules throughout Australia (Pouliot & May 2010). The omission of fungi, despite their obvious diversity and functional importance, seems due to (1) lack of awareness compounded by the lack of teaching of mycology at tertiary level and (2) lack of mycologists in government conservation and management agencies and in research institutions. In only one state, Western Australia, is a fungal ecologist

employed by the local Department of Environment and Conservation and consequently macrofungi are included in the ForestCheck biodiversity monitoring system (Robinson & Williams 2011). However, there is increasing attention and involvement of community groups such as Fungimap in monitoring and recording fungi.

There is little published research on fire and fungi in Victoria: McMullan-Fisher et al. (2002) analysed the succession of macrofungi after fire in *Eucalyptus regnans* forest and Launonen et al. (1999) considered the effect of regeneration burns on mycorrhizae of *E. regnans*. There does not appear to be any current long-term monitoring of fungi in Victoria, especially on replicated permanent plots, whether in relation to fire or for testing other factors such as climate change, silvicultural practices or for investigating the effectiveness of using vegetation as a surrogate for specific knowledge of the fungal community. In particular, fungi are not included in the several large scale research programs on fire and biota in Victoria that are part of the Victorian DSE monitoring programs, including 'HawkEye – biodiversity monitoring for improved fire management' (www.dse.vic.gov.au/hawkeye).

FUNCTIONAL DIVERSITY OF FUNGI

All fungi are heterotrophs, but there is considerable functional diversity with nutritional strategies among fungi including: saprotrophism (decomposition), parasitism and the formation of mutualistic partnerships such as mycorrhizas and lichens (May & Simpson 1997). Consequently fungi have a range of substrates, niches, autecological characteristics and interactions with other biota and thus fire will affect different trophic groups of fungi in various ways. Therefore, knowledge of functional diversity is important in making informed decisions on experimental design for monitoring and eventually for developing appropriate management practices.

Saprotrophic fungi grow within substrates such as soil, litter or wood. In addition to their roles as decomposers and in nutrient cycling, they also create nesting hollows for vertebrates. The majority of parasitic fungi are microfungi that live on or in hosts that are plants, animals and even other fungi. In agricultural and silvicultural systems parasitic fungi can cause extensive damage, but in natural ecosystems parasitic fungi are an integral part of the interactions between organisms. Mycorrhizae are a mutually beneficial relationship between specific fungi and the roots of most terrestrial plants in which there is an exchange of nutrients between fungus and plant (Brundrett 2009). Mycorrhizal fungi may also protect plants against pathogens and increase tolerance to stresses such as drought (Tommerup & Bougher 2000). There are several different types of mycorrhizas, such as ectomycorrhizas and arbuscular mycorrhizas, each with characteristic structure and each predominantly formed by particular groups of fungi and plants (Brundrett 2009). Most terrestrial Australian plants form mycorrhizae.

Fungi are food for many animals, both vertebrates and invertebrates (for which fungal fruit-bodies are also habitat) (Hanski 1989; Vernes 2009). In particular, sequestrate (truffle-like) fungi form a substantial proportion of the diet of native mammals such as potoroos and bettongs (Claridge & May 1997). Sequestrate fungi rely on mycophagous mammals for spore dispersal. There is a three-way mutualistic partnership between sequestrate fungi (most of which are mycorrhizal), their plant hosts, and mycophagous animals (Martin 2003).

The trophic mode of particular fungi is established from a combination of field observations of substrate, investigations of enzymatic capability, stable isotope data (indicative of the carbon source) and, for mycorrhizal fungi, *in vitro* synthesis experiments that inoculate plant hosts with fungi. Observations of substrate alone may not be sufficient as some saprotrophs and ectomycorrhizal fungi can fruit either on the ground or on wood (Lilleskov & Bruns 2005). Trophic status is highly correlated with phylogeny, and is generally uniform within genera but can vary considerably within families (Tedersoo et al. 2009).

TAXONOMIC DIVERSITY OF FUNGI

Organisms colloquially referred to as 'fungi' belong in three Kingdoms: most are true Fungi, but there are also fungoid organisms in the Chromista and Protoctista. Within the Fungi, there is considerable morphological and taxonomic diversity across numerous families, of which there is low awareness, even among scientists. The terms macrofungi and microfungi are used for convenience to distinguish fungi with readily visible fruit-bodies from those without. Familiar examples of macrofungi include agarics (mushrooms), puffballs, coral fungi and bracket fungi. Microfungi include mildews, moulds and rust and smut fungi. The variety of fungal substrates across living and dead plant and animal host species coupled with host-specialisation has

given rise to a massive diversity of fungi. It is estimated that there are 11 846 described species of fungi known from Australia, of which 3495 are lichens (Chapman 2009). There are likely to be at least 10 000 macrofungi alone (about twice as many as currently known) and estimates of the number of species of Australian fungi are in the range 50 000–250 000 species. The biology of some fungal pathogens of agricultural crops that are of major economic importance is well studied, but the autecology of native fungi is usually poorly known, including aspects such as spore bank dynamics, dispersal distances and population genetics.

SAMPLING FOR FUNGI IN ECOLOGICAL STUDIES

Sampling for fungi requires an understanding of biology, especially in relation to the life cycles of different groups, and a wide range of sample and isolation techniques has been required for trapping overall fungal diversity (Rossman et al. 1998; Mueller et al. 2004). Typically, fungal spores germinate to produce hyphae, which grow within the substrate (e.g. soil or living or dead tissue of other organisms) forming a diffuse mycelium. Spores may be produced directly on the mycelium or in specialised fruit-bodies. Most fungi are microfungi and are usually only visible in the field when their hosts develop symptoms such as leaf spots. Fruit-bodies of fleshy macrofungi such as mushrooms are relatively short-lived and their production is highly dependent on prevailing weather conditions and can be sporadic from one year to the next. Fungi may therefore be easily overlooked either because they are microscopic or because they are not producing fruit-bodies at the time of sampling.

Morphological methods

Macrofungal communities have traditionally been studied by the relatively time-intensive method of surveying the presence of fruit-bodies over repeat visits (Watling 1995; Peter et al. 2001; Mueller et al. 2004). Most fruit-bodies only last for a few weeks so even fortnightly surveys conducted during peak fruiting periods can miss significant amounts of diversity (Mueller et al. 2004). For ectomycorrhizal macrofungi, additional species may be recovered from root tips as compared to fruit-body sampling (Glen et al. 2008).

Sequestrate fungi have fruit-bodies that are usually hypogeal and consumed by small mammals. Location of fruit-bodies requires intensive survey by raking away leaf litter and upper soil. Alternatively, spores can be identified in the scats of mammals that eat the fungi. Scat analysis can yield a higher diversity compared to fruit-body surveys, but spores can often only be identified to genera or broad groups of species (Vernes et al. 2004).

Microfungi of a particular substrate such as soil, plant tissue or decaying wood can be observed by direct examination but it is more useful to isolate them into pure culture. Culturing is especially used for isolating endophytic fungi from living plants or fungi inhabiting the digestive system of invertebrates. However, many fungi, especially most forming ectomycorrhizas, will not grow in culture (Liesack & Stackebrant 1992). Thus, the diversity of fungi isolated into pure culture may differ from that in nature due to differential survival and growth under artificial conditions. Ectomycorrhizal root tips can be recovered from soil cores by the relatively laborious method of sieving, washing and initial sorting based on morphotypes.

It is possible to carry out gross quantification of fungal biomass by (1) estimation of hypha length from microscopic examination (Sharma et al. 1997; Wallander 2006) which is tedious and subject to observer variability (Stahl and Parkin 1996) or (2) measurement of fungal-specific molecules, such as chitin, ergosterol or phospholipid fatty acids (PLFAs) (Wallander et al. 2001). However, these are crude approaches that do not allow for differential effects on different trophic groups or take into account the great diversity of fungi potentially present, among which there may be positive and negative effects of a given treatment.

Molecular methods

Isolation of small sections of nucleic acid directly from substrates or from fruit-bodies or mycorrhizal root tips is becoming the method of choice for studying fungal communities (Horton & Bruns 2001; Peay et al. 2008). Essentially, a short, taxon specific primer is utilised in combination with the polymerase chain reaction (PCR) to yield sufficient DNA or RNA to be visualised, such as by electrophoresis. Different species have different lengths of amplified nucleic acid.

Anderson & Cairney (2004) list a number of

visualisation methods for fungal community profiling including denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). For example, all target organisms within a handful of soil can be visualised on a DGGE gel as different bands; as long as the primers are specific to the target organisms (such as at Kingdom or Phylum level) and if the region adjacent to the primers varies sufficiently between species. Confirmation of the identity of the bands in DGGE and T-RFLP is possible by PCR and sequencing of individual bands. Problems with community profiling methods include single bands representing more than one species and primers differentially amplifying different groups (Anderson & Cairney 2004).

An impediment to the wider application of molecular methods has been that the isolation and sequencing costs were initially relatively expensive. However, the cost per sample is rapidly diminishing and new high-throughput techniques such as 454 pyrosequencing can generate many thousands of sequences (Hibbett et al. 2009) without the need for visualisation techniques such as DGGE or T-RFLP.

IDENTIFICATION OF FUNGI IN ECOLOGICAL STUDIES

In the 20th century, there were relatively few comprehensive studies on fungal communities using plot-based replicated methods due to the highly specialised knowledge needed for correct identification coupled with the difficulty of identifying the numerous new species that were encountered in surveys.

Even among the comparatively well-known macrofungi, while some species can be recognised in the field, many need detailed examination of microscopic features for identification. Where recent monographs are available such as for *Mycena*, many collections can still not be identified with confidence to known species and numerous 'tag' or 'field' names have been used (Robinson and Tunsell 2007). While field names are consistent within a particular study, there has been no attempt to match them up across studies in different ecosystems.

Microfungi, mostly as pure cultures, have also been traditionally identified by morphology (Stalpers 1978), although some species do not produce spores in culture. For ectomycorrhizal root tips, macro-morphological characters rarely allow identification to species (Anderson and Cairney 2007) but microscopic characters of sections of the ectomycorrhizal fungal sheath do allow identification of most species (Brundrett et al. 1996b). Morphology can be compared against a databank of characteristics of mycorrhizas formed by known fungus+host combinations from nature or *in vitro* synthesis (Agerer 1987-2002) although very few mycorrhizas formed by Australian fungi are so far documented.

Molecular methods

Development of molecular methods in recent years has not only revolutionised fungal ecology in terms of sampling, but also the identification of fungi. Short nucleic acid sequences can be identified that discriminate at the species level. The internal transcribed spacer (ITS) of the ribosomal RNA has recently been confirmed as the 'barcode' region for identification of fungi, and successfully discriminates at species level in 70% of cases (Nilsson et al. 2008). There are often other regions that are more successful at species discrimination, but they lack primers universal for fungi. However, they can be useful when targeting particular groups of fungi.

Despite the more than 147 000 fungal ITS sequences available in International Nucleotide Sequence Databases (INSD) such as GenBank, there remain problems for identification of sequences from ecological studies due to (1) original mis-identification of sequences in INSD, many of which are 'environmental sequences' with no voucher specimens and (2) incomplete sampling of known fungal diversity, which is more pronounced for southern hemisphere regions. Misidentification in INSD has prompted creation of curated sequence banks that are backed up by reputably determined voucher specimens, such as UNITE (Abarenkov et al. 2010).

Sequences, whether from fruit-bodies or environmental samples, can be identified against those in INSD by either matching on overall similarity, such as by using the Basic Local Alignment Search Tool (BLAST) or by including the unknown sequences within a phylogenetic analysis. Numerous cryptic species are being revealed by analysis of molecular data. Reliability of identification will improve as each study adds to the number of sequences available for analysis. Mass sequencing of reference collections, such as currently underway on type specimens at the CBS Fungal Biodiversity Centre in The Netherlands, offers promise of rapidly increasing sequence data on known species

FUNGAL ECOLOGY IN THE 21ST CENTURY

Against the background of high diversity and difficulty of identification, two approaches to fungal community ecology have been used in the last decade.

One approach builds up an extensive reference collection of fruit-bodies (with data on macro- and micro-characters) from a particular ecosystem, allowing identification from fruit-body surveys carried out over multiple years. This is the approach taken in the south-west of Western Australia by researchers in the ForestCheck program and associated investigations on the response of macrofungal communities to fire, silvicultural regimes and dieback in forest habitats (Robinson et al. 2008; Anderson et al. 2010; Robinson & Williams 2011; Wittkuhn et al. 2011). A similar approach has been used in studies of the Warra long term ecological monitoring site and other sites in Tasmania (Gates et al. 2005, 2009, 2011a, 2011b, 2011c).

The other approach uses rapid molecular sampling and profiling techniques to produce a snapshot of total fungal diversity. A series of studies in the Peachester State Forest in Queensland has investigated the response of fungi to different fire regimes (Bastias et al. 2006a, 2006b, 2009; Anderson et al. 2007; Campbell et al. 2008; Artz et al. 2009). Molecular profiling has also been applied in Australia to comparisons between the fungal community of: (i) native forest and *Pinus* plantations (Bastias et al. 2007), (ii) natural and agricultural sites (Midgley et al. 2007), (iii) sites rehabilitated after mining and surrounding forest (Glen et al. 2008), (iv) ectomycorrhizas of different hosts (Tedersoo et al. 2008), (v) native forest, plantations and pastures (Kasel et al. 2008), (vi) remnant *Allocasuarina* trees and surrounding paddocks (Bennett et al. 2009), (vii) pasture and reafforested sites (Carson et al. 2010) and (viii) soil and rhizosphere of *Araucaria* (Curlevski et al. 2010). Worldwide, molecular methods are a significant feature of current research on fungi and fire, both at the community level (Buscardo et al. 2010) and for investigating factors such as susceptibility of propagules to heating (Kipfer et al. 2010).

Ideally, the two approaches of intensive fruit-body sampling and molecular profiling need to be integrated. Molecular analysis of fruit-body reference collections would be useful to test the morphological species concepts adopted and also to add to the databank against which environmental sequences can be identified. Collection of fruit-bodies and isolation of cultures at sites where molecular profiling is carried out would add to the likelihood of species level identification of bands and allow testing that single bands in methods such as DGGE correspond to unique species. Knowledge of the specific identity of bands in community profiling methods would allow assignment of samples to trophic groups.

WHY FUNGI SHOULD BE INCLUDED IN ECOLOGICAL MONITORING

If we consider building a modern biodiversity monitoring system from the ground up, rather than being bound by historical practices, it is important to include representatives across phylogeny and across function. Fungi clearly belong in a modern monitoring scheme because (i) the Fungi are in a separate kingdom and are megadiverse, (ii) fungi have important functional roles, such as decomposition and nutrient cycling, that are not or little duplicated in other kingdoms, and (iii) many fungi form mutualisms (e.g. mycorrhizas are the obligate symbiotic partners of most terrestrial plants) and consideration of one partner in isolation may lead to erroneous conclusions about functional traits and fitness (Cavagnaro et al. 2010; Friesen et al. 2011).

Use of surrogates is often suggested as a way to tackle the omission of organisms such as fungi from monitoring schemes. However, for such surrogates to be effective, some knowledge of the responses of at least a sample of the organisms to be replaced (e.g. fungi) is required before surrogates can be chosen. In fact, once a monitoring system for fungi was established, the cost per species for molecular sampling and identification could turn out to be less than in labour-intensive trapping or collecting of particular species or groups of animals and plants, with subsequent manual identification. Hence, other organisms as surrogates for fungi may not be necessary, although choosing individual fungi as representative of taxonomic and functional classes of fungi would be a practical approach to covering the high diversity of fungi.

EFFECTIVE AND EFFICIENT ECOLOGICAL MONITORING OF FUNGI

Fungi clearly belong in a modern biodiversity monitoring system. Aspects that would contribute to an effective and efficient system of monitoring fungi in relation to fire (or indeed other factors) in Victoria

include the following:

(1) Integration with existing studies. Given the difficulty of setting up studies that have sufficient replicated sites for different fire treatments it makes sense that fungi monitoring should be integrated with existing large scale and long term studies. Integration with existing studies creates additional efficiency because assessments of site characteristics, such as for soil and vegetation, do not have to be done from scratch.

(2) Development of standard sampling and identification protocols. Just as guidelines have been developed for monitoring of flora, standard protocols should be developed for molecular profiling of the fungal community and for fruit-body surveys. Factors that need to be considered include experimental design of initial soil or cores or other substrate samples, amplification conditions, selection of appropriate primers and choice of restriction enzymes in T-RFLP.

(3) Creation of local barcode databank. To support molecular profiling of fungi in soil and other substrates, and facilitate identification of fruit-bodies and mycorrhizal root-tips, a barcode sequence databank is needed, supported by reference collections. Data from monitoring surveys can contribute significantly to this databank.

(4) Selection of readily recognisable macrofungi for survey. Such target species, as used in the Fungimap scheme, should include not only rare species (including all formally listed species) but also common species, so that at least some taxa are encountered on all sites. There is scope for significant community contribution to macrofungal fruit-body surveys. However, for this to be effective, it must be supported by creation of appropriate identification aids and access to training.

(5) Initiation of autecological studies. There is a need for basic autecological information of at least some species in regard to factors such as spore dispersal distance, longevity and survival after fire, and rate of establishment and lifespan of individuals. Survival of fungi in and on mycorrhizal roots under different fire conditions also needs to be investigated. Such knowledge will contribute to the development of vital attribute data for the management of fungi.

(6) Selection of representative target species. Where community sampling or autecological studies are restricted to certain target species, ensure that these species are chosen to represent fungi from different lineages, trophic groups, substrates and hosts.

(7) Co-investigation of symbionts. Where there are strong relationships between biota, both partners should be monitored, such as for mycorrhizal fungi and the orchids that rely on them for germination and growth or for sequestrate mycorrhizal fungi and the mycophagous mammals that rely on them for food.

CONCLUSION

Fungi are megadiverse and carry out ecological roles that often differ from the roles represented by groups that are currently included in fire and other monitoring schemes. This phylogenetic and trophic diversity coupled with a high degree of interconnectedness with other organisms means that fungi should be included in modern biodiversity monitoring systems. Using emerging molecular techniques for community profiling in concert with curated sequence databases, it is feasible to survey for fungi and answer research questions such as concerning the affect of fire and fire regimes on fungi. Fungi can no longer be put in the too-hard-basket as far as the practicality and cost of their inclusion in ecological studies.

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REFERENCES

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U. & Kõljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytologist* 186: 281–285.

Agerer, R. (Ed.), 1987-2002. *Colour Atlas of Ectomycorrhizae*. 1st-12th del, Einhorn-Verlag, Schwäbisch Gmünd.

Anderson, I.C. & Cairney, J.W.G., 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environmental Microbiology* 8: 769–779.

ANDERSON, I.C. & CAIRNEY, J.W.G., 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. FEMS *Microbiology Reviews* 31: 388–406.

ANDERSON, I.C., BASTIAS, B.A., GENNEY, D.R., PARKIN, P.I. & CAIRNEY, J.W.G., 2007. Basidiomycete fungal communities in Australian sclerophyll forest soil are altered by repeated prescribed burning. *Mycological Research* 111: 482–486.

ANDERSON, P., BRUNDRETT, M., GRIERSON, P. & ROBINSON, R., 2010. Impact of severe forest dieback caused by *Phytophthora cinnamomi* on macrofungal diversity in the northern jarrah forest of Western Australia. *Forest Ecology and Management* 259: 1033–1040.

ARTZ, R.R.E., REID, E., ANDERSON, I.C., CAMPBELL, C.D. & CAIRNEY, J.W.G., 2009. Long-term repeated prescribed burning increases evenness in the basidiomycete laccase gene pool in forest soils. *FEMS Microbiology Ecology* 67: 397–410.

BASTIAS, B.A., HUANG, Z.Q., BLUMFIELD, T., XU, Z. & CAIRNEY, J.W.G., 2006a. Influence of repeated prescribed burning on the soil fungal community in an eastern Australian wet sclerophyll forest. *Soil Biology & Biochemistry* 38: 3492–3501.

BASTIAS, B.A., XU, Z.H. & CAIRNEY, J.W.G., 2006b. Influence of long-term repeated prescribed burning on mycelial communities of ectomycorrhizal fungi. *New Phytologist* 172: 149–158.

BASTIAS, B.A., ANDERSON, I.C., XU, Z.H. & CAIRNEY, J.W.G. 2007. RNA- and DNA-based profiling of soil fungal communities in a native Australian eucalypt forest and adjacent *Pinus elliotti* plantation. *Soil Biology & Biochemistry* 39: 3108–3114.

BASTIAS, B., ANDERSON, I., RANGEL-CASTRO, J., PARKIN, P., PROSSER, J. & CAIRNEY, J., 2009. Influence of repeated prescribed burning on incorporation of 13C from cellulose by forest soil fungi as determined by RNA stable isotope probing. *Soil Biology & Biochemistry* 41: 467–447.

BENNETT, L.T., KASEL, S. & TIBBITS, J., 2009. Woodland trees modulate soil resources and conserve fungal diversity in fragmented landscapes. *Soil Biology and Biochemistry* 41: 2162–2169.

BRUNDRETT, M.C., 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* 320: 37–77.

BRUNDRETT, M.C., ASHWATH, N. & JASPER, D.A., 1996a. Mycorrhizas in the Kakadu region of tropical Australia. II Propagules of mycorrhizal fungi in disturbed habitats. *Plant and Soil* 184: 173–184.

BRUNDRETT, M.C., BOUGHER, N., DELL, B., GROVE, T. & MALAJCZUK, N. (Eds.), 1996b. *Working with Mycorrhizas in Forestry and Agriculture*, Australian Centre for International Agricultural Research, Canberra.

BUSCARDO, E., RODRÍGUEZ-ECHEVERRÍA, S., MARTÍN, M.P., DE ANGELIS, P., PEREIRA, J.S. & FREITAS, H., 2010. Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. *Fungal Biology* 114: 628–636.

CAMPBELL, C.D., CAMERON, C.M., BASTIAS, B.A., CHEN, C. & CAIRNEY, J.W.G., 2008. Long term repeated burning in a wet sclerophyll forest reduces fungal and bacterial biomass and responses to carbon substrates. *Soil Biology & Biochemistry* 40: 2246–2252.

CAVAGNARO, T.R., DICKSON, S. & SMITH, F.A., 2010. Arbuscular mycorrhizas modify plant responses to soil zinc addition, *Plant and Soil* 329: 307–313.

CARSON, J.K., GLEESON, D.B., CLIPSON, N. & MURPHY, D.V., 2010. Afforestation alters community structure of soil fungi, *Fungal Biology* 114: 580–584.

CAWSON, J. & MUIR, A., 2008a. *Flora Monitoring Protocols for Planned Burning: A User's Guide*. Fire and Adaptive Management Report No. 74. Department of Sustainability and Environment, East Melbourne, Victoria, Australia.

CAWSON, J. & MUIR, A., 2008b. *Flora Monitoring Protocols for Planned Burning: A Rationale Report.* Fire and Adaptive Management Report No. 75. Department of Sustainability and Environment, East Melbourne, Victoria, Australia.

CHAPMAN, A.D., 2009. *Numbers of living species in Australia and the world*. Australian Biological Resources Study, Canberra. < http://www.environment.gov.au/biodiversity/abrs/publications/other/species-numbers/2009/pubs/nlsaw-2nd-complete.pdf>

CHEAL, D., 2010. *Growth stages and tolerable fire intervals for Victoria's native vegetation data sets*. Fire and Adaptive Management Report No. 84. Department of Sustainability and Environment, East Melbourne, Victoria, Australia.

CLARIDGE, A.W. & MAY, T.W., 1997. Mycophagy

among Australian mammals. *Australian Journal of Ecology* 19: 251–275.

Claridge, A.W., Trappe, J.M., Mills, D.J. & Claridge, D.L., 2009a. Diversity and habitat relationships of hypogeous fungi. III. Factors influencing the occurrence of fire-adapted species. *Mycological Research* 113: 792–801.

Claridge, A.W., Trappe, J.M. & Hansen, K., 2009b. Do fungi have a role as soil stabilizers and remediators after forest fire? *Forest Ecology and Management* 257: 1063–1069.

Clarke, M.F., 2008. Catering for the needs of fauna in fire management: science or just wishful thinking? *Wildlife Research* 35: 385–394.

Curlevski, N., XU, Z., Anderson, I. & Cairney, J., 2010. Diversity of soil and rhizosphere fungi under *Araucaria bidwillii* (Bunya pine) at an Australian tropical montane rainforest site. *Fungal Diversity* 40: 12–22.

Driscoll, D.A., Lindenmayer, D.B., Bennett, A.F., Bode, M., Bradstock, R.A., Cary, G.J., Clarke, M.F., Dexter, N., Fensham, R., Friend, G., Gill, M., James, S., Kay, G., Keith, D.A., Macgregor, C., Russell-Smith, J., Salt, D., Watson, J.E.M., Williams, R.J. & York, A., 2010. Fire management for biodiversity conservation: key research questions and our capacity to answer them. *Biological Conservation* 143: 1928–1939.

DSE, 2010. *Flora and Fauna Guarantee Act 1988 Threatened List October 2010*. Department of Sustainability and Environment, Melbourne.

Eldridge, D. J. & Bradstock, R. 1994. The effect of time since fire on the cover and composition of cryptogamic soil crusts on a eucalypt shrubland soil. *Cunninghamia* 3: 521–527.

Friesen, M.L., Porter, S.S., Stark, S.C., von Wettberg, E.J., Sachs, J.L. & Martinez-Romero, E., 2011. Microbially mediated plant functional traits, *Annual Review of Ecology, Evolution, and Systematics* 42: 23–46.

Gates, G.M., Ratkowsky, D.A. & Grove, S.J., 2005. A comparison of macrofungi in young silvicultural regeneration and mature forest at the Warra LTER site in the southern forests of Tasmania. *Tasforests* 16: 127–152.

Gates, G.M., Ratkowsky, D.A. & Grove, S.J., 2009. Aggregated retention and macrofungi: a case study from the Warra LTER site, Tasmania. *Tasforests* 18: 33–54.

Gates, G.M., Mohammed, C., Wardlaw, T., Ratkowsky, D.A. & Davidson, N.J., 2011a. The ecology and diversity of wood-inhabiting macrofungi in a native *Eucalyptus obliqua* forest of southern Tasmania, Australia. *Fungal Ecology* 4: 56–67.

Gates, G.M., Mohammed, C., Wardlaw, T., Davidson, N.J. & Ratkowsky, D.A., 2011b. Diversity and phenology of the macrofungal assemblages supported by litter in a tall, wet *Eucalyptus obliqua* forest in southern Tasmania, Australia. *Fungal Ecology* 4: 68–75.

Gates, G.M., Mohammed, C., Ratkowsky, D.A., Wardlaw, T. & Davidson, N.J., 2011c. Diversity and ecology of epigeous ectomycorrhizal macrofungal assemblages in a native wet eucalypt forest in Tasmania, Australia. *Fungal Ecology* 4: 290–298.

Gill, A.M. & Allan, G., 2008. Large fires, fire effects and the fire-regime concept. International *Journal of Wildland Fire* 17: 688–695.

Glen, M., Bougher, N.L., Colquhoun, I.J., Vlahos, S., Loneragan, W.A., O'Brien, P.A. & Hardy, G.E.S.J., 2008. Ectomycorrhizal fungal communities of rehabilitated bauxite mines and adjacent, natural jarrah forest in Western Australia. *Forest Ecology and Management* 255: 214–225.

Hanski, I., 1989. *Fungivory: fungi, insects and ecology*. In *Insect-fungus Interactions*. Academic Press, London.

Hibbett, D.S., Ohman, A. & Kirk, P.M., 2009. Fungal ecology catches fire, *New Phytologist* 184: 279–282.

Horton, T.R. & Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10: 1855–1871.

Kasel, S., Bennett, L. & Tibbits, J., 2008. Land use influences soil fungal community composition across central Victoria, south-eastern Australia. *Soil Biology & Biochemistry* 40: 1724–1732.

Kipfer, T., Egli, S., Ghazoul, J., Moser, B. & Wohlgemuth, T., 2010. Susceptibility of ectomycorrhizal fungi to soil heating. *Fungal Biology* 114: 467–472.

Launonen, T.M., Ashton, D.H. & Keane, P.J., 1999. The effect of regeneration burns on the growth, nutrient acquisition and mycorrhizae of *Eucalyptus regnans* F. Muell. (Mountain Ash) seedlings. *Plant and Soil* 210: 273–283.

Liesack, W. & Stackebrandt, E., 1992. Unculturable microbes detected by molecular sequences and probes. *Biodiversity and Conservation* 1:

250–262.

Lilleskov, E.A. & Bruns, T.D., 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, Tomentella sublilacina, via soil food webs. *Mycologia* 97: 762–769.

Martin, B. G., 2003. The role of small ground-foraging mammals in topsoil health and biodiversity: Implications to management and restoration. *Ecological Management & Restoration* 4: 114–119.

May, T.W. & Simpson, J.A., 1997. Fungal diversity and ecology in eucalypt ecosystems. In: *Eucalypt ecology: individuals to ecosystems,* J.E. Williams & J.C.Z. Woinarski, eds, Cambridge University Press, Cambridge.

McMullan-Fisher, S.J.M., May, T.W. & Keane, P.J., 2002. The macrofungal community and fire in a Mountain Ash forest in southern Australia. *Fungal Diversity* 10: 57–76.

McMullan-Fisher, S.J.M., May, T.W., Robinson, R.M., Bell, T.L., Lebel, T., Catcheside, P. & York, A., 2011. Fungi and fire in Australian ecosystems: a review of current knowledge, management implications and future directions. *Australian Journal of Botany* 59: 70–90.

Midgley, D.J., Saleeba, J.A., Stewart, M.I., Simpson, A.E. & McGee, P.A., 2007. Molecular diversity of soil basidiomycete communities in northern-central New South Wales, Australia. *Mycological Research* 111: 370–378

Mueller, G.M., Bills, G.F. & Foster, M.S., 2004. *Biodiversity of fungi - Inventory and monitoring methods*. Elsevier Academic Press, London.

New, T., Yen, A., Sands, D., Greenslade, P., Neville, P., York, A. & Collett, N., 2010. Planned fires and invertebrate conservation in south east Australia. *Journal of Insect Conservation* 14: 567–574.

Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K.-H., 2008. Intraspecific ITS variability in the Kingdom Fungi as expressed in the International Sequence Databases and its implications for molecular species identification. *Evolutionary Bioinformatics* 4: 193–201.

O'Bryan, K., Prober, S., Lunt, I. & Eldridge, D., 2009. Frequent fire promotes diversity and cover of biological soil crusts in a derived temperate grassland. *Oecologia* 159: 827–838.

Osborn, M., 2007. Long-term effects of frequent burning on fungal communities and the role of fungi in fire-prone forests. PhD thesis, University of Melbourne.

Peay, K.G., Kennedy, P.G. & Bruns, T.D., 2008. Fungal community ecology: a hybrid beast with a molecular master. *BioScience* 58: 799–810.

Peter, M., Ayer, F., Egli, S. & Honegger, R., 2001. Above- and below-ground community structure of ectomycorrhizal fungi in three Norway spruce (*Picea abies*) stands in Switzerland. *Canadian Journal of Botany* 79: 1134–1151.

Pouliot, A.M. & May, T.W., 2010. The third 'F' — fungi in Australian biodiversity conservation: actions, issues and initiatives. *Mycologia Balcanica* 7: 41–48.

Robinson, R.M. & Tunsell, V.L., 2007. A list of macrofungi recorded in burnt and unburnt *Eucalyptus diversicolor* regrowth forest in the southwest of Western Australia: 1998–2002. Conservation Science 6: 75–96.

Robinson, R.M. & Williams, M.R., 2011. FOREST-CHECK: the response of epigeous macrofungi to silviculture in jarrah (*Eucalyptus marginata*) forest, Australian Forestry 74: 288–302.

Robinson, R.M., Mellican, A.E. & Smith, R.H., 2008. Epigeous macrofungal succession in the first five years following a wildfire in karri (*Eucalyptus diversicolor*) regrowth forest in Western Australia. Austral Ecology 33: 807–820.

Rossman, A.L., Tulloss, R.E., O'Dell, T.E. & Thron, G., 1998. Protocols for an all taxa biodiversity inventory of fungi in Costa Rican conservation area, Parkway Publishers, Boone, USA.

Sharma, P.D., Fisher, P.J. & Webster, J., 1977. Critique of the chitin assay technique for estimation of fungal biomass. *Transcriptions of the British Mycological Society* 69: 479–483.

Stahl, P.D. & Parkin, B., 1996. Relationship of soil ergosterol concentration and fungal biomass. *Soil Biology & Biochemistry* 28: 847–855.

Stalpers, J.A. ed, 1978. *Identification of wood-inhabiting fungi in pure culture*, Institute of the Royal Netherlands Academy of the Arts and Sciences.

Teague, B., McLeod, R. & Pascoe, S., 2010. *2009 Victorian Bushfires Royal Commission, Final Report, Summary*. Parliament of Victoria, Melbourne.

Tedersoo, L., Jairus, T., Horton, B., Abarenkov, K., Suvi, T., Saar, I. & Kõljalg, U., 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon specific primers. *New*

Phytologist 180: 479–490.

TEDERSOO, L., MAY, T.W. & SMITH, M.E., 2009. Ectomycorrhizal lifestyle in fungi: patterns of evolution and distribution. *Mycorrhiza* 20: 217–263.

TOMMERUP, I.C. & BOUGHER, N.L., 2000. The role of ectomycorrhizal fungi in nutrient cycling in temperate Australian woodlands. In *Temperate Eucalypt Woodlands in Australia,* R.J. Hobbs & C.J. Yates, eds, Surrey Beatty and Sons, Chipping Norton.

VERNES, K., 2009. Mycophagy in a community of macropod species. In *Macropods: The Biology of Kangaroos, Wallabies and Rat-kangaroos,* G.M. Coulson & M.D.B. Eldridge, eds, Surrey Beattie and Sons, Sydney.

VERNES, K., JOHNSON, C.N. & CASTELLANO, M.A., 2004. Fire-related changes in biomass of hypogeous sporocarps at foraging points used by a tropical mycophagous marsupial. *Mycological Research 108: 1438–1446.*

WALLANDER, H., 2006. External mycorrhizal mycelia - the importance of quantification in natural ecosystems. New Phytologist 171: 240–242.

WALLANDER, H., NILSSON, L., HAGERBERG, D. & BÅTH, E., 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* 151: 753–760.

WARCUP, J.H., 1990. Occurrence of ectomycorrhizal and saprophytic discomycetes after a wildfire in a eucalypt forest. *Mycological Research* 9: 1065–1069.

WATLING, R., 1995. Assessment of fungal diversity: macromycetes, the problems. *Canadian Journal of Botany* 73: S15–S24.

WITTKUHN, R.S., MCCAW, W.L., WILLS, A., ROBINSON, R.M., ANDERSEN, A.N., VAN HEURCK, P., FARR, J.D., LIDDELOW, G.L. & CRANFIELD, R., 2011. Variation in fire interval sequences has minimal effects on species richness and composition in fire-prone landscapes of south-west Western Australia. *Forest Ecology and Management* 261: 965–978.

YORK, A., 2003. Invertebrates and fire: Think small, think big. The need to incorporate a major component of our biodiversity into adaptive management. In: *Proceedings of a Conference on Ecologically Sustainable Bushfire Management, Sydney*. Nature Conservation Council of New South Wales Inc, Sydney.